Increased therapeutic efficacy of liposomal glucocorticosteroids in chronic MOG-EAE: clinical, histological and MRI analysis in a model of multiple sclerosis

C. Weller, R.A. Linker, A. Mohr, J. Schmidt, M. Knauth, D. Kehrer, J.M. Metselaar, R. Gold; University of Gottingen (Gottingen, D); University of Utrecht (Utrecht, NL)

High-dose glucocorticosteroids (GS) are the mainstay for treatment of acute relapses in multiple sclerosis (MS), an inflammatory demyelinating disease of the central nervous system (CNS). Even stronger therapeutic effects can be achieved with polyethylene glycol (PEG)-coated, long circulating liposomes encapsulating GS in monophasic experimental autoimmune encephalomyelitis (EAEnice). The goal of our study was to investigate chronic EAEnice and compare liposomal encapsulated steroids of different genomic vs. non-genomic potency. EAEnice in the Dark Agouti rat, a chronic model closely reflecting clinical course and histopathology of MS, was induced by immunization with recombinant myelin oligodendrocyte glycoprotein (MOG). A single injection of prednisolone liposomes (PL) was compared to methylprednisolone (MP) liposomes (MPL), and to three injections of free MP (at 10 mg/kg). Furthermore, therapeutic effects of 10 mg/kg PL (PL 10), were compared to 4mg/kg PL or MPL (PL 4 and MPL 4 respectively) in a dose titration experiment. Clinical rating was supplemented by MRI and histological analyses. Only treatment with liposomal GS substantially ameliorated chronic MOG-EAE. Striking therapeutic effects were especially observed after application of PL 10 after onset of the first relapse with prevention of damage late in the chronic disease phase (mean score 0.4 ± SD 0.9 for PL 10 vs. mean score 7 ± SD 3.5 for MP 10 on day 31 p.i.). MPL 4 displayed slightly superior clinical effects when compared with PL 4. In vivo T2 weighed MRI of rat brain after the first relapse revealed a decrease in lesion load specifically after PL10 treatment. In histological analyses, liposomal GS were superior to free MP thus leading to a 75% decrease in macrophage and T-cell infiltration as well as a restoration of blood-brain-barrier integrity and a reduction of tissue destruction. Ongoing experiments further address superiority of non-genomic MPL effects by comparing treatment with PL 10 to MPL 10. Targeting of GS to phagocytic cells by liposomal encapsulation increases their therapeut-ic efficacy in animal models for MS. GS with enhanced non-genomic potency such as MP may be superior. These findings lay the groundwork for applying liposomal GS in clinical MS trials in the near future. Supported by a grant from the Gemeinnützige Hertie-Stiftung (project no: 1.01.1/04/009).

Sample size estimates based on hierarchical regression models of atrophy rates in relapsing-remitting multiple sclerosis

V.M. Anderson, J.W. Bartlett, L. Fisniku, G.R. Davies, W. Rashid, N.C. Fox, D.H. Miller; University College London (London, UK); London School of Hygiene & Tropical Medicine (London, UK)

Background: Brain atrophy measures are being used increasingly to monitor neuroaxonal degeneration and disease progression in multiple sclerosis (MS). With the evolution of new disease-modifying drugs and neuroprotective agents, brain atrophy may be a good primary outcome measure in trials of new treatments. However it is essential that such trials are of sufficient power to detect treatment effects. Objective: To model and estimate brain atrophy rates in relapsing remitting MS (RRMS) and control subjects, and subsequently perform power calculations to estimate sample sizes required for placebo-controlled trials. Methods: Sixteen control and 27 RRMS subjects, not on disease-modifying treatment, had T1-weighted volumetric MRI at baseline and up to three annual follow-up scans. Brain atrophy was quantified over 1, 2 and 3 years using i) segmented brain volume difference; ii) the registration-based methods of brain boundary shift integral (BBSI) and structural image evaluation, using normalisation, of atrophy (SIENA). Hierarchical regression models were used to estimate mean (SD) atrophy rates in controls and RRMS using each method. Sample sizes for a placebo-controlled trial were calculated for varying lengths of follow-up and effect sizes, allowing for normal aging effects. Results: Over two years estimated mean (SD) atrophy rates in controls were −0.18%/year (0.45%) and −1.53%/year (0.88%) respectively. Sample sizes required to detect a given proportional reduction in atrophy rate were smaller using BBSI and SIENA than volume difference. For a treatment causing a 30% decrease in atrophy rate, the numbers of patients required in each trial arm were 454, 170 and 108 for volume difference, BBSI and SIENA respectively (two year follow-up, 90% power, 5% significance level). For BBSI and SIENA it was found that longer follow-up (three years) did not decrease group size greatly (144 and 98 subjects in each arm), whilst shorter follow-up (one year) increased group sizes to 309 and 158 subjects. Conclusion: Sample size estimates based on a modest slowing of brain atrophy rates measured by registration-based methods appear to be comparable to, or less than those based on disability progression, for a two year placebo-controlled trial. Registration-based atrophy measures may be sensitive enough to show significant treatment effects after one year.

Disability status and the relationship between the T2-MRI abnormalities in CIS patients and the long-term outcome in a 20-year follow-up


Background: Clinically isolated syndrome (CIS) such as optic neuritis, brainstem or spinal cord syndromes are usually the first clinical presentation of multiple sclerosis (MS). MS natural history studies have reported a variable prognosis for disability after 15 years from onset. Previous follow-up studies have shown that T2-weighted brain magnetic resonance imaging (MRI) abnormalities are associated with an increased risk of MS and (to an extent) disability. Aim: To report the disability status and to evaluate whether the previously observed longitudinal relationships between the MRI and clinical evaluation are maintained over a uniquely long period of 20 years. Methods: CIS patients were initially recruited between 1984 and 1987 and all had clinical assessment and brain MRI performed. We followed up 87 patients after a mean of 19.5 years (range, 17.6 to 21.7). Clinically definite MS (CDMS) was diagnosed by using Poser criteria and disability was measured with the use of Kurtzke’s EDSS (69 by examination and 13 by telephone; 3 who died from severe complications of MS were assigned an EDSS of 10). 2 patients had died from non MS related causes. Spearman rank-correlation coefficient was used to evaluate the relationship between the volume of T2
lesions on brain MRI and EDSS. **Results:** CDMS developed overall in 6/30 (20 percent) with a normal MRI scan and in 49/57 (86 percent) with an abnormal MRI scan. 33 had relapsing remitting MS, 22 (40 percent) could be considered as “benign” (EDSS ≤ 3), 22 had secondary progressive MS (median EDSS 7.5; including 3 who died from severe disease). T2 lesion volume at 0, 5, 10, 14 and 20 years was moderately correlated with 20 year EDSS (r values 0.50–0.65, p < 0.001). Changes in T2 lesion volume was correlated with changes in EDSS in years 0–5 (r = 0.59, p < 0.001), in years 5–10 (r = 0.39, p = 0.004), and years 10–14 (r = 0.36, p = 0.021) but not for years 14–20 (r = 0.18, p = 0.23). **Comment:** Although the study is limited by the loss of some subjects to clinical and/or MRI examination and by changes in MRI technology a long-term relationship of lesion volume and EDSS was observed.

7

**No evidence for increased glial cell activity in the normal appearing white matter of patients with clinically isolated syndromes suggestive of multiple sclerosis using high field MRS**

M.P. Wattjes, M. Harzheim, G. Lutterby, L. Klotz, H. Schild, F. Traber; University Hospital of Bonn (Bonn, D)

**Background:** Proton MR spectroscopy (1H-MRS) is a well established method for the in vivo investigation of the normal-appearing white matter (NAWM) in patients with multiple sclerosis (MS). NAWM metabolic changes are of special interest in patients with clinically isolated syndromes suggestive of MS (CIS) regarding prognostic classifications. **Objective:** To investigate NAWM metabolic alterations in CIS patients using high field 1H-MRS and to compare the results to healthy controls and to patients with an early course of MS. **Methods:** 36 patients (26 female, 10 male) presenting with a CIS (median age 35 years, median disease duration 30 days, median EDSS 1.5) 12 patients (10 female, 2 male) with an early course of MS (median age 29 years, median disease duration 132 days, median EDSS 0.5) and 20 healthy volunteers (11 female, 9 male, median age 29 years) were included. Single voxel 1H-MRS (PRESS localization, repetition time 2000 ms, echo time 38 ms and 140ms) of the parietal NAWM was performed at a 3.0T whole body MR system in all participants. Absolute concentrations of N-acetyl-aspartate (tNAA), myo-inositol (Ins), choline (Cho), and total creatine (tCr) were measured in comparison to the control group, both MN and IN number were reduced in MS cases at the UC (MN p = 0.0038; IN p = 0.0431) and UT (MN p = 0.020; IN p = 0.0179) levels. In MS cases, IN and MN loss predominately occurred within demyelinated plaques, although there was also evidence of MN loss within myelinated areas, particularly at the UT level (p = 0.0282). There is some evidence of a size-related susceptibility to MN loss, the cell body and the nuclei of remaining MNs were larger in MS cases, suggesting a preferential loss of smaller MNs. In comparison to controls, there was a reduction of IN size in MS cases at all cord levels (UC p = 0.0000; UT p = 0.0002; Lum p = 0.0415). This neuronal shrinkage was most marked within MS plaques, but also occurred within myelinated areas. **Conclusion:** In this report, currently the largest autopsy study of neuronal pathology in MS, we demonstrate substantial neuronal loss and shrinkage within the spinal cord. Neuronal loss appears to be predominantly related to grey matter plaques.

9

**Cortical lesions appear early, are frequent and clinically relevant in multiple sclerosis**

M. Calabrese, V. Bernardi, M. Atzori, L. Rinaldi, A. Morra, C. Romualdi, M. McAuliffe, L. Barachino, P. Perini, B. Fischl, P. Gallo; Multiple Sclerosis Center (Padua, I); Euganea Medica (Padua, I); CRIBI (Padua, I); Biomedical Imaging Research Services Section NIH (Bethesda, USA); Artificial Intelligence Laboratory MIT (Cambridge, USA)

**Background:** Increasing evidence suggests that cortical pathology may play a relevant patho-physiological role in Multiple Sclerosis (MS). The demonstration of cortical lesions (CLs) in vivo may give informations for a better understanding MS pathology and disability as well as to achieve an earlier and more correct diagnosis. **Material and Methods:** 125 patients were clinically classified as followed: 16 clinically isolated syndrome (CIS), 40 possible MS (pMS), 54 relapsing remitting MS (RRMS) and 15 secondary progressive MS (SPMS). 40 age and gender matched healthy volunteers (HV) constitute the reference population. All patients and HV underwent MR (1.5 Tesla, Philips Achieva) and accurate neurological examination. The following sets of images were achieved: Double Inversion recovery (DIR), 3D Fast Field Echo (3DFFE), Fluid Attenuated Inversion Recovery (FLAIR), conventional turbo-Spin Echo (DP/T2) and postcontrast spin echo T1. Pure intracortical lesions, brain parenchyma fraction (BPF), grey matter fraction (GMF), white matter fraction (WMF), T2 lesion volume (T2LV) and global cortical thickness were analyzed. Disability was measured by Expanded Disability Status Scale (EDSS). Cerebrospinal fluid (CSF) examination for intrathecaly synthesized oligoclonal IgG bands (IgGOB) was performed in all the patients included in this study. **Results:** CLs...
were early and frequently detected in MS patients throughout the course of the disease. Up to 44.7% of pMS had CLs versus 64.3% of RRMS and 66.7% of SPMS. 90% of patients with CLs had IgGOB in the CSF versus 60% of patients without CLs, and the difference was highly significant (p < 0.001). The presence of CLs was found to correlate with T2LV and WMF while no correlation was observed with CTh and GMF. Finally, CLs nicely correlated with EDSS score.

Discussion: We demonstrate that CLs appear early and are a relevant pathological phenomenon in MS. They correlate with white matter pathology, with disability and with the presence of IgGOB in the CSF. Moreover their demonstration may help in achieving the criterion of dissemination in space of lesions.

Young Researchers’ Session II

10 Anti-IFNβ NABs mediated abolition of IFNβ stimulation is characterised by a concomitant increase in IFNβ/b receptor 2 transmembrane isoforms expression and a decrease in IFNa/b receptor 2 soluble isoform expression, in lymphocytes

F. Gilli, P. Valentino, A. Sala, M. Caldano, M. Capobianco, S. Malucchi, F. Marnetto, A. Bertolotto; ASO S. Luigi (Orbassano, I)

Background: IFNα/b receptor 2 (IFNAR2) exists in 3 mRNA splice variants, resulting in 2 transmembrane (IFNAR2.1 and IFNAR2.2) and 1 soluble (IFNAR2.3) isoforms that are generated by an alternative RNA processing of the hulIFNAR2 gene. Information on the molecular mechanism of expression and regulation of hulIFNAR2 could provide insights on the function of each isoform, and how each contributes to IFNβ therapeutic efficacy. Objectives: to 1) investigate the expression of IFNAR2 isoforms in normal controls and multiple sclerosis (MS) untreated patients; and 2) to study the regulation of IFNAR during IFNβ therapy in patients with and without neutralising antibodies (NABs). Methods: quantitative PCR measurements of IFNAR1, IFNAR2.1, IFNAR2.2, and IFNAR2.3 were performed in mononuclear cells from 61 MS patients and 32 healthy controls. Blood samples were taken before and after (+6 and +12 months) treatment with IFNβ. Patients were also regularly screened for NABs using a cytopathic effect assay (CPE). Results: basal expression of IFNAR2.1 and IFNAR2.2 was shown to be lower in untreated MS patients than in controls (all p < 0.0163). Upon prolonged treatment with IFNβ, patients displayed a state of decreased IFNAR2.1 and IFNAR2.2 expression (p = 0.0083 and p = 0.011), while levels of IFNAR2.3 were slightly increased (p = 0.0656). The presence of NABs reverted these effects, i.e. led to reduced IFNAR2.3 (p = 0.0023) and increased IFNAR2.1 and IFNAR2.2 (p < 0.0001 and p = 0.0006) expression levels. In contrast, expression of IFNAR1 remained unaffected by IFNβ therapy and the presence of NABs. Interestingly, in NAB+ patients basal expression of IFNAR2.1 and IFNAR2.2 were found to be lower than in NAB- patients (p = 0.012 and p = 0.0033). Conclusions: this study is the first to evaluate IFNAR2 isoforms expression in MS. Untreated patients present a significantly lower expression of IFNAR2 transmembrane isoforms than healthy controls. In NAB- patients, prolonged stimulations with IFNβ establish a feedback loop, in which IFNAR2 transmembrane isoforms expression is down-regulated and IFNAR2 soluble expression is up-regulated. On the contrary, abolition of IFNβ stimulation due to NABs, is characterised by a concomitant increase in IFNAR2 transmembrane isoforms expression and a decrease in IFNAR2 soluble isoform expression. Taken together these findings show the existence of specific mechanisms of adaptation to chronic stimulation, as well as to the abolition of the receptorial stimulus.

11 Characterisation of multiple sclerosis in the early stage by means of MR-morphometric and neuropsychological techniques


Background and Objectives: Cognitive impairment has been recognised as considerable feature in multiple sclerosis (MS). However, heterogeneous results exist concerning the evolution of cognitive deficits in early-onset MS and on how to sensitively identify their morphological substrate. This study aims at the neuropsychological and morphological assessment of early-onset MS and is conducted as a longitudinal design over 2 years. Design and Methods: 32 MS patients (mean disease duration 2.48 years, mean EDSS 2.36) and 32 matched healthy controls were included. Extensive neuropsychological tests addressing different cognitive domains were carried out every 6 and 12 months, respectively. Subsequently to any neuropsychological test session, parametric magnetic resonance images (MRI) (T2Z)-weighted, Magnetisation Transfer Imaging, Diffusion Tensor Imaging, single-voxel MR Spectroscopy (MRS)) have been acquired. The MR-data of the patients were compared with the data of 12 healthy young (25 ± 3 years) and 12 healthy elderly (65 ± 7 years) controls. Results: In the initial test session, significant differences were found in tests of attention and information processing. Additionally, patients were significantly impaired in tests of working memory (Paced Auditory Serial Addition Test, N-back). Further, patients performed worse in immediate and delayed recall in the logical memory test although their results were mostly within normal range. Verbal fluency (‘S’) was also reduced. Parametric MRI data revealed significant differences between patients and controls in both normal-appearing white matter (NAWM) and normal-appearing grey matter (NAGM). Individual region-specific histograms suggested significant differences between young controls and both other groups. Interestingly, histograms for the patient group showed a high degree of correspondence with the results for the elderly population. For MRS, significant metabolic differences included increased N-acetylaspartylglutamate and reduced glutamate, glutamate and glutamine (Glx), as well as myoinositol and, as a trend below significance, reduced total choline in the patients compared to young controls. Conclusion: Cognitive deficits occur in early MS, particularly in working memory and speed of information processing. Parametric MRI data revealed a similarity between elderly controls and patients which suggests an effect of accelerated aging primarily in NAWM (tentatively also in NAGM) secondary to disease progression.

12 JNK inhibition as potential treatment for multiple sclerosis


The c-Jun N-terminal Kinase (JNKs) pathway is involved in gene expression, cellular survival, proliferation in response to cytokines and can be induced in activated T cells. These events are commonly associated to the pathogenesis of autoimmune diseases, including multiple sclerosis (MS). To understand the potential role of JNK pathway in MS we first analyzed the expression of JNK isoforms in PBMC of various form of MS patients. JNK2 was the only isoform upregulated in RR-MS patients. To further evaluate the involvement of JNK pathway, we assessed the cellular activity of a JNK non isoform specific inhibitor, in a set of in vitro assays using healthy volunteers PBMC stimulated with CD3/CD28. This stimulation significantly increased JNK 1 and 2 isoforms mRNA expression, inflammatory cytokine production, c-Jun phosphorylation and cell...
proliferation. The JNK inhibitor did not have a significant impact on cytokine release and JNK isoform expression. However it significantly reduced cell proliferation by inducing T cell apoptosis and C-Jun dephosphorylation. These results suggest a specific target activity of the compound. On the basis of this potential mode of action, we administered the JNK inhibitor in a mouse chronic EAE model both in preventive and curative regimens. Daily oral dosing significantly reduced the severity of the pathology and contributed to the delay in the onset of the disease. This was paralleled by decreased in CNS inflammatory infiltrates and an improvement in neuronal function. These results support the concept that the inhibition of JNK pathway could be beneficial in the treatment of RR-MS.

### NMO-IgG: a French experience

R. Marignier, J. De Seze, F. Durand-Dubief, S. Vukusic, H. Zephir, P. Vermersch, P. Cabre, G. Cavillon, J. Honnorat, C. Confavreux; Hopital Neurologique (Lyon, F); Hopital Civil de Strasbourg (Strasbourg, F); CHRU de Lille (Lille, F); CHU Fort de France (Fort de France, F); Inserm U433 Neurologique (Lyon, F)

Devic’s disease (neuromyelitis optica: NMO) is an inflammatory demyelinating disease restricted to optic nerves and spinal cord which has been frequently assimilated to multiple sclerosis (MS). However, recent works concerning clinical, epidemiological, pathophysiological and immunological aspects lead to consider that MS and NMO are distinct entities. NMO-IgG is a serum autoantibody biomarker that has been recently described (Lennon et al.; Lancet, 2004) in patients with definite NMO and so called “high risk” patients for NMO. Its specificity allows to discriminate between NMO and classic MS. Our objectives were to assess the diagnostic yield of this novel serum biomarker for NMO, and to confirm its usefulness in clinical practice. Indirect immunofluorescence with a substrate of adult rat cerebellum and midbrain was used in order to identify the distinctive NMO-IgG staining pattern described by Lennon et al. We tested masked sera from 20 patients with NMO (group 1), 18 patients with idiopathic acute transverse myelitis (group 2), 18 patients with bilateral or recurrent idiopathic optic neuritis (group 3), 52 patients with classic MS (group 4), 36 patients with HTLV-1 infection (group 5) and a miscellaneous group (n = 7) (group 6). The NMO-IgG staining pattern outlined microvessels in the white and grey matter of the cerebellum and midbrain, with a prominent staining of the granular and molecular layers. This particular staining was observed in 11/20 samples in group 1, 5/18 in group 2 (positive results only in the longitudinally extensive transverse myelitis subgroup: 5/11), 3/18 in group 3, 5/52 in group 4, 0/36 in group 5, 0/7 in group 6. Sensitivity of the test was 55% considering detection of definite NMO’s patients only (group 1), and specificity was 94% considering groups 4, 5 and 6, only. Detection of NMO-IgG was therefore technically reproducible in our laboratory. We confirm its interest in the distinction between NMO and MS. Our data underline that this test should allow earlier diagnosis and appropriated therapy. Further samples from different countries are currently analysed. Results will be presented at the meeting.

### The role of susceptibility loci in benign, aggressive and primary progressive multiple sclerosis in Northern Ireland

O.M. Gray, H. Abdeen, G.V. McDonnell, C. Graham, S.A. Hawkins; Royal Victoria Hospital (Belfast, UK); Belfast City Hospital (Belfast, UK)

**Objective:** To establish the prognostic value of susceptibility loci in benign, aggressive and primary progressive multiple sclerosis in Northern Ireland. **Background:** The Genetic Analysis of Multiple Sclerosis in Europeans (GAMES) initiative and follow up refined analysis identified 15 candidate susceptibility loci within the Northern Irish population for Multiple Sclerosis (MS). We aimed to establish the role of the 12 most significant markers in benign, aggressive and primary progressive cohorts. **Methods:** Cases with probable or definite MS according to the Poser criteria were included and classified as benign (Kurtzke EDSS ≤ 3.0 at 10 years), aggressive (Kurtzke EDSS ≥ 6.0 by 10 years) or primary progressive MS (progressive clinical history from onset of first symptoms, with no clear evidence of relapses or remissions). All cases were Caucasian of Northern Irish origin. DNA was extracted from venous blood and microsatellite markers were amplified using multiplex PCR. The products were electrophoresed and allele sizes identified. Allele frequencies of each marker in the three groups of patients were compared statistically using a chi-squared test for 3 x k tables using SPSS 11.5. P-values were deemed significant at the level < 0.001 (0.05/3). **Results:** The allele frequencies of five microsatellite markers were significant at the 0.017 level. They included D3S1278 (Chr 3q13, p < 0.001), D4S432 (Chr 4p16, p = 0.001), D2S347 (Chr 2q14, p = 0.003), D19S903 (Chr 19q13, p = 0.003) and TNFa (Chr 6p21, p < 0.001). A statistically significant difference in allele frequencies between the benign and aggressive cohorts was identified in four of the microsatellite markers: D4S432, p = 0.002; D2S347, p = 0.004; D19S903, p = 0.003; TNFa, p < 0.001. **Conclusions:** Four candidate susceptibility loci showed statistically significant differences in allele frequencies between groups of patients with benign and aggressive MS in the Northern Irish population. This would support the utility of these loci for potential future testing to discriminate between patients who will follow a benign or aggressive clinical course.

### Design of clinical trials

**20**

**Ethics of placebo use in the era of disease modifying drugs**

L. Kappos; University Hospital (Basel, CH)

Based on the results of well designed placebo controlled clinical trials, the first disease modifying drugs for multiple sclerosis were approved and are now widely used. An intriguing finding of these placebo controlled studies was that placebo treated patients experienced significant “improvements” in clinical outcomes and even in MRI measures. On the other hand, long-term observational studies suggest that even after several years patients initially randomized to placebo, retained disadvantages as compared to those treated with active drug. In designing future studies, keeping the fine balance (equipoise) between the interest of the individual with MS in obtaining the best available treatment and the interest of the society in improvement of insufficient treatment options, is becoming more and more difficult. Recruitment of patients for placebo controlled trials would be expected to result in more and more skewed, most probably more benign populations with unpredictable negative impact on power assumptions. Interestingly up to now comparison of baseline characteristics of placebo controlled and comparative trials does not fully support this notion. Comparative trials of new against established therapy only superficially solve ethical problems with placebo: By inflating the numbers of patients needed to ascertain sufficient power, they do not only create problems of feasibility and costs, but do also expose more participants to risk, as long as it is possible that the new treatment might be less efficient or more dangerous than its comparator. This also applies to add-on treatment designs, because combinations may harbour dangers of negative interactions. Improving sensitivity and reliability of outcome measures, developing more sophisticated predictive algorithms for better selection of informative trial participants as well as innovative designs that help in minimizing the numbers of placebo treated patients, will be challenges to take in the development of further treatment options in MS.
Improving the design of clinical trials
C.H. Polman; VUMC (Amsterdam, NL)

Due to the availability of a variety of drugs that have been approved for the therapy of multiple sclerosis (MS), the conduct of future clinical trials has become increasingly problematic. In this lecture I will discuss possible new strategies for the design of trials, especially addressing alternative trial designs, new endpoint definitions (time to event studies), new outcome measures (clinical and MRI), and alternative statistical analytic methods. Special focus will be placed on how to overcome the ‘placebo-problem’. In addition, some preliminary strategies will be discussed to address the issue of how to measure the effect of ‘neuroprotection’ and ‘neurorepair’ strategies.

Is the current trial definition of confirmed disability progression in multiple sclerosis an accurate measure of permanent disease progression?

Introduction: Trials of MS therapeutics usually report two clinical primary outcome measures: the relapse rate and the rate of confirmed disability progression. Ideally, “confirmed disability progression” as a meaningful relapse-independent endpoint should equate with “permanent” disability progression. Aim: We assessed whether the trial definition of “confirmed disability progression” equates with “long-term sustained” EDSS progression, using the prospectively acquired global MSBase dataset. Background: The most common trial definition of “confirmed disability progression” is an increase in the EDSS score of at least 1 point compared to baseline, sustained on the next evaluation at least three months later. For patients with a baseline EDSS of 0, the required EDSS increase is 1.5 points. Analysis is usually presented as a survival curve. Consequently patients whose EDSS score improves at later evaluation are still considered as “confirmed disability progression”.

Methods: This study analysed all patient records from the web-based MSBase international registry. At present, 32 MS clinics in 14 countries contribute anonymised data to MSBase (www.msbase.org). Quality assurance is maintained with inbuilt data quality checking in the local record system. Competency in EDSS assessments is maintained by Neurostatus testing for all members. Results: The global dataset contained 28,815 EDSS evaluations from 3796 patients and 2642 patients had at least 3 (average 10) EDSS evaluations at least 3 months apart. 1114 of these patients had at least one recorded 1-point increase in EDSS compared to baseline and 508 (45%) fulfilled the trial definition of “confirmed disability progression” 3 months after the initial 1-point EDSS increase. On subsequent EDSS evaluations, 25% of these 508 patients improved, and 33% continued to worsen. Approximately 75% of the patients with confirmed progression were in the relapsing-remitting phase of MS. Conclusion: In a mixed population of relapsing and progressive MS patients attending specialised MS clinics, at least 25% of patients who fulfil the current trial definition of “confirmed disability progression” subsequently improve. In a very active relapsing-remitting population, typical for therapeutic trials, this percentage is likely to be higher. We caution that the current definition of “confirmed disability progression” does not reliably identify “permanent” disability progression and question its usefulness in therapeutic trials.

Short-term changes in gadolinium enhancing lesion activity in relapsing-remitting multiple sclerosis: implications for clinical trial design
Y.S. Zhao, A. Traboulsee, A.J. Petkau, D. Li; University of British Columbia (Vancouver, CAN)

Background: MRI activity in terms of gadolinium enhancing lesions (EL) is a common outcome measure in multiple sclerosis (MS) clinical trials. Some trials restrict enrollment to patients with pretreatment EL to reduce required sample sizes. Others use a crossover design, comparing MRI activity pre and post treatment. There is a concern regarding the possibility of decreasing MRI activity over time independent of treatment. Objectives: To examine the short term changes in monthly gadolinium EL activity in patients with different initial activity levels. Methods: Post hoc analysis was performed on 65 patients with clinically definite relapsing-remitting MS randomized to placebo in a double blind placebo-controlled trial of interferon beta-1a (PRISMS). Patients were screened for relapses but not MRI activity. Eleven monthly MRI scans were taken at screening (month – 1), baseline (month 0) and follow-up (months 1 – 9). Two radiologists assessed the scans by consensus for new EL. Patients were classified into “no” (0 EL), “low” (1 – 3 EL) and “high” (>3 EL) activity groups at screening. Monthly new EL rates were examined at baseline, months 1 – 3, 4 – 6 and 7 – 9 using mixed effects Poisson regression. Results: 32, 19, 14 patients were classified as “no”, “low”, and “high” activity. Over 9 months, 24/32 (75%) “no” activity patients developed at least 1 EL, while all “low” and “high” activity patients developed at least 2 EL. Monthly activity (95% confidence interval) of the “no” group remained stable: 0.69 (0.18,1.21), 0.49 (0.19,0.78), 0.54 (0.20,0.87) and 0.63 (0.25,1.01) at baseline, months 1 – 3, 4 – 6 and 7 – 9, respectively. The corresponding rates for the “low” group were: 1.67 (0.80,2.54), 1.59 (0.85,2.33), 1.18 (0.63,1.74), 0.86 (0.45,1.27) and for the “high” group: 4.67 (2.10,7.25), 4.45 (2.24,6.66), 3.32 (1.66,4.98) and 2.41 (1.18,3.64). For the “low” and “high” groups, activity decreased 4% (~32%, 33%), 29% (0%, 50%) and 48% (27%, 64%) at months 1 – 3, 4 – 6 and 7 – 9 compared to the baseline scan, roughly a linearly decreasing trend over time on the log scale. Conclusion: The majority of patients with no EL at screening will develop new activity over a 9 month trial. Since including such patients enhances recruitment, there may be no advantage to excluding them from studies. As with clinical relapses, there is regression to the mean of gadolinium ELs over time in untreated patients with initial activity. This needs to be taken into account in designing clinical trials.

Multiple sclerosis in children

Diagnosis of multiple sclerosis in children
B. Banwell; Hospital for Sick Children (Toronto, CAN)

The onset of Multiple Sclerosis (MS) in childhood and adolescence is being increasingly recognized [1–18,18–25]. Diagnosis requires familiarity with the clinical features of MS in children, and with disorders considered in the differential. A particular challenge relates to differentiation of the chronic demyelinating condition that defines MS from monophasic or transiently multiphasic demyelinating disorders. Recently, an international working group (under the auspices of the National Multiple Sclerosis Society) have proposed working clinical definitions for acquired demyelinating disorders in children (Neurology, in press). These definitions will be presented in the context of clinical cases. In addition to the clinical features, MRI imaging provides invaluable insights into the appearance of MS in children. The MRI appearance, however, may differ from the classic appearance of MS in adults, and MRI criteria optimized in adult-onset...
**Abstracts**

MS may not be equally applicable in children [23,26]. MRI images of children with MS, as well as other acquired demyelinating syndromes will be presented, and the current literature on MRI in pediatric demyelination will be reviewed [23,26,27].

References


**26 Clinical features of paediatric multiple sclerosis**

A. Ghezzi; Centro Studi Sclerosi Multipla (Gallarate, I)

The relative frequency of multiple sclerosis (MS) before 16 years of age (early onset MS – EOMS-) is approximately 3 – 5% of the whole MS population. It is rare before 10 years of age, accounting for about 0.2 – 1.6%. A peculiar finding of EOMS is the preponderance of females affected, in particular during puberty, with a female/male ratio of about 4. Clinical symptoms at presentation are not different from those of the adult form, but the onset can sometimes be hyperacute, with encephalopathic symptoms. The most frequent clinical course is the relapsing one, in about 3/4 of cases, with an annualised the relapse rate of 0.9 – 1.2, a figure slightly higher than that of adults. An open question is whether EOMS has a different prognosis with respect to the adult form: recent studies have shown that subjects with EOMS reach both mild (EDSS 3 – 4) and severe (EDSS 6) disability after a longer interval but at a younger age compared to adults MS cases. The presence of a short inter-attack interval, of an high relapse rate, of a relapsing-progressive course, of sequelae or high disability in the first year are related to a negative prognosis. At its first presentation, EOMS can be difficult to differentiate from ADEM: oligoclonal bands can be found in ADEM too and MRI cannot discriminate the two diseases. The final diagnosis is given by the follow-up, is the criteria of dissemination in space or time are fulfilled. It is not clear if CSF and MRI findings in EOMS have the same sensitivity and specificity of the adult form.

References


**27 Disease modifying drugs in multiple sclerosis of childhood**

S.N. Tenenbaum; Gattuhan (Buenos Aires, RA)

Disease modifying drugs (DMD) have widely demonstrated positive outcomes in adult patients with relapsing forms of Multiple Sclerosis (MS), by reducing the frequency of clinical relapses and disease activity seen on brain MRI, with a well-established safety and tolerability profile. Current DMD for MS modulate or suppress the immune system. These therapies are now initiated soon after diagnosis based on several clinical controlled trials performed in adult patients with MS, showing substantial benefit from early treatment. Pediatric-onset MS is being increasingly recognized. Retrospective studies comparing clinical course and prognosis of MS in pediatric and adult patients indicate that although the time to reach persistent physical disability or conversion to secondary progressive MS was longer in children, the age at which this outcome occurs is younger in...
children. As all the pivotal trials for DMD have enrolled only patients older than 18 years, these therapies have not been officially approved in the pediatric age group. Nevertheless, many young patients are already receiving off-label treatment with DMD approved only for adult patients with MS. Guidelines for the use of these MS therapies in children do not exist. In addition, it is not clear what frequency and dosage should be used in children because of weight and body surface differences. Available literature on the use of DMD in children with MS is limited, and most of published papers are based on comprehensive patient registries with regular systematic follow-up of children. There are no controlled clinical studies in the pediatric population. This presentation will review current knowledge on DMD in children with MS based on some recently published studies. Most of them are retrospective studies in pediatric cohorts with relapsing forms of MS, and are consistent with Class III evidence. Relevant data on safety and tolerability from these studies will be outlined, showing that DMD appear safe and well tolerated in children with MS. Conclusions about overall efficacy are difficult to obtain in view of the lack of controlled studies, in addition to the poorly predictive nature of long-term disease progress in children. Nevertheless, some beneficial data on effectiveness could be found using clinical outcome measures (relapse rate, relapse-free status) on patients serving as their own controls. Additional research is needed and new tools should be developed to assure the success and applicability of controlled clinical trials in children. It should be considered that pediatric studies of medications proven effective in adults will face ethical challenges, like the appropriateness of placebo-controlled trials in pediatric patients with the same disease. Multicenter and multinational prospective studies of the impact of these therapies on clinical and MRI outcome should be designed in children and adolescents with MS.

28

Prognostic factors after a first demyelinating attack in children
R.F. Neuteboom, C.E. Catsman, J.S.H. Vles, M. Boon, R.H. Goossens, I.J. Rotteveel, H. Stroink, E.A.J. Poeters, R.Q. Hintzen; Enansmus MC (Rotterdam, NL); AZM (Maastricht, NL); AZG (Groningen, NL); UMC Utrecht (Utrecht, NL); UMC Nijmegen (Nijmegen, NL); Elisabethbeth Ziekenhuis (Tilburg, NL); Juliana Kinderziekenhuis (Den Haag, NL)

Objective: To identify risk factors for development of clinically definite multiple sclerosis (CD-MS) after a first attack of inflammatory demyelination in the central nervous system. Study design: A retrospective multi centre study in the Netherlands of children with a first demyelinating attack with an age of onset under 16 years. Methods: Clinical and laboratory data were collected from patient records. Brain MRI’s were re-evaluated. Results: Out of 54 patients 33 remained monofocal within a median time of follow-up of 12 months and 13.5 months for respectively a monofocal attack and a multifocal ADEM-like attack. The remaining 21 developed CD-MS within a median time to the second attack of 7 months. Progression to CD-MS after an initial multifocal ADEM-like attack was observed in 7 out of 24 patients (29%). Monophasic ADEM was characterized by an earlier age of onset (mean: 6.7 years vs. 10.8 years in the MS patients). Seizures, encephalopathy and meningism were seen significantly more often in the group with monophasic ADEM, as was a preceding infection. Large lesions (>2 cm) on MRI but not thalamic or basal ganglia lesions were also significantly associated with monophasic ADEM. Cerebrospinal fluid analysis showed no significant predictive value of the presence of oligoclonal bands or elevated IgG-index. Presence of at least 3 out of 4 Barkhof MRI criteria was significantly predictive of developing CD-MS However this was seen in only in 45% of the CD-MS patients. Furthermore, small lesions (<2 cm), corpus callosum perpendiclar lesions and the presence of well-defined lesions were significantly predictive of developing multiple sclerosis. The combination of the latter two (Barkhof or KIDMUS criteria) was seen in only 35% of our MS patients. Conclusion: 1) Children with an initial multifocal ADEM-like attack developed CD-MS in 29%.

29

Applicability of the International Pediatric MS Groups Consensus definitions of acute disseminated encephalomyelitis and subsequent clinical outcomes
N. McLinskey, M. Milazzo, W. MacAllister, D. Madigan, T. Chitnis, A. Belman, L. Krupp; SUNY Stony Brook (Stony Brook, USA); Massachusetts General (Boston, USA)

Background: Acute disseminated encephalomyelitis (ADEM) has been variably defined in the literature. To advance research and standardize terminology, the International Pediatric MS Study Group proposed consensus definitions for ADEM and multiphasic ADEM. Goal: To examine the applicability of the Study Group’s definitions for ADEM among patients referred to the National Pediatric MS Center and report the patients’ subsequent clinical course. Methods: A chart review was performed on 22 patients referred for ADEM. The Study Group’s criteria for ADEM were applied: a first clinical attack affecting multifocal areas of the CNS with encephalopathy which includes behavioral change or altered consciousness. Typical MRI findings were defined as multifocal or unifocal large (>1 cm) lesions predominantly affecting the white matter. Criteria for multiphasic ADEM require a subsequent neurological event associated with encephalopathy and involving new CNS areas. Clinical follow-up for the reviewed cases ranged from 1 to 8 years. Results: Age of presentation ranged from 2-15 years. All patients had typical MRI features of ADEM. 95% of patients had initial events consistent with ADEM criteria with encephalopathy defined as (irritability n=2, lethargy/excessive sleepiness n=11, confusion n=2, coma n=3, and seizures n=3. Oligoclonal bands (OCB) tested in 15 patients were absent in 93%. Patients were classified into subgroups by subsequent course. Group A had no further events (n=5). Group B had one or more events WITHOUT encephalopathy (n=10), Group C had one or more events WITH encephalopathy, hence meeting multiphasic ADEM criteria (n=7). MRI findings improved or fully resolved in all Group A and B patients. In Group C, MRI abnormalities either fluctuated or worsened subsequent to the initial ADEM, yet lacked the typical appearance of MS. In Group B, isolated optic neuritis (ON) was the most common subsequent event (90%). Group C patients continued to have subsequent neurological events which frequently included ON. One patient each from Groups B and C ultimately met criteria for NMO. Conclusions: Of patients referred for ADEM to a Pediatric MS Center, the initial event meets consensus definition for ADEM in 95% of cases. Almost all referred cases have events following the ADEM episode which frequently include optic neuritis. Of the group with multiphasic ADEM, subsequent events occurred suggesting that ultimately alternative diagnoses will be made.

Update in immunology

33

CD4+ and mechanisms of CNS damage
N. Goebels; University Hospital (Zurich, CH)

In the last decades, CD4 T cells have been in the center of interest for many multiple sclerosis researchers, for good reasons: Multiple sclerosis susceptibility is genetically linked to certain MHC class II loci; experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis, can be transferred between syngeneic animals by myelin specific CD4 T cells; and techniques to isolate and
characterize myelin specific CD4 T cells from humans became available more than a decade ago, leading to a wealth of literature on this cell population. Recently, however, the exclusive importance of CD4 T cells in the pathogenesis of MS has been questioned, when the ablation of CD4 T cells failed to show beneficial effects on relapse rate or inflammatory MRI activity in MS patients. On the contrary, a number of contemporary studies support the role of CD8 T cells in MS pathogenesis. CD8 T cells outnumber CD4 T cells in MS lesions; clonal expansion-indicating an antigen driven immune response-and enrichment of memory cells is preferentially seen in the CD8 T cell subset in the brain and cerebrospinal fluid of MS patients. New animal models, which employ myelin specific CD8 T cells to induce central nervous system autoimmunity, resemble some aspects of MS more closely than animal models mediated by CD4 T cells. At the level of the “immunological synapse” it has been demonstrated that neurons can be induced to express MHC class I mediated by CD4 T cells. At the level of the “immunological synapse” it has been demonstrated that neurons can be induced to express MHC class I in vitro and in vivo, a prerequisite for the interaction with CD8 T cells. In vitro, CD8 T cells can exhibit direct cytotoxic effects on neurons and axons. In MS lesions, CD8 T cells were observed in close proximity to demyelinated neurites and some researchers found a correlation between the extent of axonal damage in MS and the number of CD8 T cells. This lecture will focus on current understanding of the role of CD8 T cells in MS and new approaches to investigate the pathogenic relevance of this cell population.

An animal model for cortical pathology of multiple sclerosis
R. Weissert, M. Albert, M. Aytemur, T. Olsson, H. Laassmann, M.K. Storch; University of Tubingen (Tubingen, D); Karolinska Hospital (Stockholm, S); University of Vienna (Vienna, A); University of Graz (Graz, A)

Recently it has been demonstrated that cortical pathology is present in multiple sclerosis. So far it is not well understood how cortical lesions develop and to what extent they contribute to the disease manifestation. Potentially they are the primary correlate for progressive disability and cognitive impairment in multiple sclerosis. We have developed an animal model of cortical pathology in MHC congenic LEW.1AR1 rats. These show after active immunization with the extracellular domain of myelin-oligodendrocyte-glycoprotein all types of cortical lesions that have been shown to be characteristic in chronic multiple sclerosis. In this model we are presently evaluating the mechanisms which lead to this type of pathology which is dependent on a specific MHC haplotype. This finding underscores that also in multiple sclerosis development of cortical pathology may be genetically controlled. We explore this model regarding pathogenetic mechanisms leading to cortical lesions by histopathology, immunology and molecular biology and evaluate the efficacy of preclinical therapeutic interventions which may be used for treatment of multiple sclerosis. In summary we have established an animal model in rats for cortical pathology of multiple sclerosis which will be explored in multiple ways.

Altered reaction of plasmacytoid dendritic cells to toll-like receptor 9 stimulation in relapsing-remitting multiple sclerosis
A. Bayas, M. Stasiolak, N. Kruse, K.V. Toyka, K. Schmaj, R. Gold; University of Wurzburg Klinikum Augsburg (Augsburg, D); Medical University of Lodz (Lodz, PL); University of Göttingen (Göttingen, D); University of Wurzburg (Wurzburg, D)

Background: Plasmacytoid dendritic cells (pDCs) represent a DC-subtype, which exerts divergent functions in innate and adaptive immunity including the reaction to microbial factors and the induction of immunoregulatory responses. We could show that pDCs have an impaired maturation and altered regulatory function in multiple sclerosis (MS) (Brain 2006, 129: 1293–305). In this study we assessed the influence of toll like receptor (TLR) 9 ligation on regulatory function of pDCs and TLR expression in pDCs.

Methods: Clinically stable MS patients with relapsing-remitting MS were compared with healthy controls. To obtain sufficient cells for co-culture assays, peripheral blood mononuclear cells (PBMCs) had to be obtained by leukapheresis from MS patients (n = 4) and controls (n = 3). PDCs were first cultured with autologous naïve T-cells and unmethylated cytosine-phosphate-guanosine oligodeoxynucleotides (CpG-ODN), #2006, a TLR9 ligand. After 7 days cells were co-cultured with allogeneic irradiated PBMCs with or without autologous naïve T-cells as responders. For TLR analysis PBMCs were obtained from MS patients (n = 7) and healthy controls (n = 12) and cultured over 3 days with CpG-ODN 2006 and 2216, another TLR 9 ligand. Production of IFN-gamma in supernatants was assessed by ELISA, TLR 1–10 expression by RT-PCR.

Results: In healthy controls pDCs co-cultured with autologous naïve T-cells and CpG-ODN 2006 induced significantly higher interferon gamma expression in the allogene co-culture assay in co-cultures with responders than in those without responders. In MS patients however there was no significant difference. In PBMCs from MS patients stimulated with CpG-ODN 2006 and 2116 TLR expression was significantly higher at day 1 for TLR 1, 2, 4, 5 and 8 after CpG-ODN 2216 stimulation than in controls, but only for TLR1 after CpG-ODN 2006 stimulation.

Conclusions: These findings suggest the existence of an altered reaction of pDCs and PBMCs to certain TLR ligands in MS, which might be one of the factors influencing the disordered immune balance in this disease. Supported by a binational grant from Ministerstwo Nauki i Informatyzacji, Poland, Bundesministerium für Bildung und Forschung, Germany (01GZ0303), the University Research Fund, Wuerzburg, and the German Multiple Sclerosis Society (to NK and RG). We thank Prof. Hermann Wagner, TU Munich, for support.

Mesenchymal stem cells treat CNS autoimmunity through a dual effect on inflammation and tissue damage
E. Gerdoni, B. Gallo, S. Casazza, S. Musio, E. Pedonomonte, G.L. Mancardi, R. Pedotti, A. Uccelli; University of Genoa (Genoa, I); National Neurological Institute C. Besta (Milan, I)

Mesenchymal stem cells (MSC), a subset of adult stem cells derived from the bone marrow stroma, have generated much enthusiasm as possible cell source for tissue repair including the nervous system. Recent studies have shown that MSC can also modulate T and B cell responses thus providing a rationale for their use as therapy for experimental autoimmune encephalomyelitis (EAE). In this study we show that i.v. injected MSC can ameliorate both relapsing-remitting (PLP-induced) and chronic progressive (MOG-induced) EAE before and after disease onset. In vivo T cell response and antibodies production against the immunizing antigens and mitogens was significantly decreased in MSC treated mice. The adoptive transfer of encephalitogenic PLP- sensitized, preconditioned with MSC, induced a very mild disease compared to controls thus suggesting the induction of peripheral tolerance. Upon i.v injection, Green Fluorescent Protein labeled MSC were detected inside the lymph nodes early upon injection and inside the inflamed CNS at later stage. MSC injection resulted in a decreased number of inflammatory infiltrates, reduced demyelination, axonal loss and, in contrast, sparing of neurons, astrocytes and oligodendrocytes. We observed no evidence of GFP labeled transdifferentiation into neural cells. Overall, we propose that MSC may block the autoimmune attack on CNS and protect neural cells from death and therefore may be effective for the treatment of MS, an autoimmune disease of the CNS where degeneration of neural cells follows inflammation.
Mechanisms of damage to axons and myelin

Glutamate toxicity and experimental myelin injury
C. Matute; Universidad del Pais Vasco (Leioa, E)

Glutamate is the principal excitatory neurotransmitter in the central nervous system (CNS), but it is also a potent neurotoxin that can kill nerve cells. Glutamate damages nerve cells in at least two ways: by excitotoxicity, which is caused by sustained activation of ionotropic GluRs, and by receptor-independent mechanisms, which are secondary to glutamate uptake. In neurons, NMDA receptors, which are highly permeable to calcium and distributed widely on CNS neurons, are the major initiators of excitotoxicity. In recent years, it has been shown that glutamate can also be toxic to white matter oligodendrocytes and to myelin by both mechanisms. In particular, glutamate receptor-mediated injury to these cells is mediated by AMPA, kainate and NMDA glutamate receptor types. Thus, these receptor classes, and the intermediaries of the signal cascades they activate, are potential targets for drug development to treat white matter damage in acute and chronic diseases. Alterations in glutamate homeostasis in white matter can determine glutamate injury to oligodendrocytes and myelin. Astrocytes are responsible for most glutamate uptake in synaptic and non-synaptic areas and consequently, are the major regulators of glutamate homeostasis. Activated microglia in turn may secrete cytokines and generate radical oxygen species, which impair glutamate uptake and reduce the expression of glutamate transporters. Finally, oligodendrocytes, also contribute to glutamate homeostasis. My presentation aims at summarizing the current knowledge about the mechanisms leading to oligodendrocyte cell death and demyelination as a consequence of alterations in glutamate signalling, and their clinical relevance to multiple sclerosis. In addition to direct glutamate toxicity, this neurotransmitter, at sublethal concentrations, can also sensitize alterations in glutamate signalling, and their clinical relevance to multiple sclerosis. In addition to direct glutamate toxicity, this neurotransmitter, at sublethal concentrations, can also sensitize oligodendrocytes to complement attack, an effect which is mediated by activated microglia in turn may secrete cytokines and generate radical oxygen species, which impair glutamate uptake and reduce the expression of glutamate transporters. Finally, oligodendrocytes, also contribute to glutamate homeostasis. My presentation aims at summarizing the current knowledge about the mechanisms leading to oligodendrocyte cell death and demyelination as a consequence of alterations in glutamate signalling, and their clinical relevance to multiple sclerosis. In addition to direct glutamate toxicity, this neurotransmitter, at sublethal concentrations, can also sensitize oligodendrocytes to complement attack, an effect which is mediated by oxidative stress and can be attenuated by antioxidants. Finally, I will provide evidence that ATP can also injure oligodendrocytes and myelin both in vitro and in vivo, and that blockade of ATP receptors ameliorates chronic EAE. A thorough understanding of how oligodendrocytes and myelin are damaged by excitotoxicity will generate knowledge that can lead to better therapeutic strategies to treat multiple sclerosis.

References

Multiple sclerosis pathology after autologous stem cell transplantation: ongoing demyelination and neurodegeneration despite suppressed inflammation
I. Metz, C.P. Lucchinetti, H. Openshaw, A. Garcia-Merino, H. Lassmann, M. Freedman, R. Azzarelli, O.J. Kolar, H.L. Atkins, W. Brück; Georg-August University (Göttingen, D); Mayo Clinic (Rochester, USA); City of Hope National Medical Center (Duarte, USA); Clínica Puerta de Hierro (Madrid, E); Medical University of Vienna (Vienna, A); University of Ottawa (Ottawa, CAN); Indiana University School of Medicine (Indianapolis, USA)

Autopsy material from five multiple sclerosis patients who had received autologous stem cell transplantation was analysed. A total of 53 white matter lesions were investigated using routine and immunohistochemical stainings to characterize the demyelinating activity, inflammatory infiltrates, acutely damaged axons and macrophages/microglial cells. We found active demyelination in all of the five patients. The inflammatory infiltrate within the lesions showed a tenfold reduction compared to literature data from chronic MS autopsy lesions and CD8+ cytotoxic T cells dominated the T cell population. High numbers of acutely damaged axons were found in active lesion areas. Tissue injury was associated with activated macrophages/microglial cells. The present results indicate an ongoing demyelination and axonal degeneration despite pronounced immunosuppression. Our data parallel results from clinical phase I/II studies showing continued clinical disease progression in MS patients with high expanded disability system scores despite autologous stem cell transplantation.

Upregulated expression of N-type voltage dependent calcium channels (Cav2.2) in autoimmune optic neuritis detected by magnetic resonance imaging and immunohistochemistry
I. Gadjanski, S. Boretius, T. Michaelis, J. Frahm, M. Bähr, R. Diem; University of Göttingen (Göttingen, D); MPI for Biophysical Chemistry (Göttingen, D)

Optic neuritis is one of the most common clinical manifestations of multiple sclerosis (MS), a chronic inflammatory disease of the central nervous system (CNS). Myelin oligodendrocyte glycoprotein (MOG) – induced experimental autoimmune encephalomyelitis (EAE) in female Brown Norway (BN) rats affects the optic nerve (ON) in more than 90% of immunized animals, leading to inflammation, demyelination and degeneration of the axons. The precise mechanisms of this ON damage are not fully understood, but likely to involve excess accumulation of calcium (Ca2+) ions into axons. One of the possible routes of entry of Ca2+ ions under pathological conditions is via voltage dependent Ca2+ channels (VDCC). Since manganese (Mn2+) ions also enter cells via VDCCs and cause signal enhancement in T1-weighted images (MRI), Mn2+ (0.05 mmol/kg) was calculated and compared between these groups as well as to a control group, which comprised of immunized animals not treated with any blocker. Statistical significance was assessed using one-way ANOVA followed by Dunnett’s test. A statistically significant decrease of Mn2+ induced signal enhancement within the ONs in T1-weighted MR images, was detected after IV application of cothoxin GVIA, a specific blocker of N-type VDCCs. Furthermore, an increased expression of N-type VDCCs in demyelinated parts of the ONs was established by immunohistochemistry for alpha1B, the pore-forming subunit of N-type VDCCs. Based on these results, we hypothesize that the upregulated expression of N-type (Cav2.2) VDCCs in inflamed ON leads to an increased calcium influx during MOG-induced optic neuritis. The increase of intracellular Ca2+ concentration activates different mechanisms of neurodegeneration.
Semaphorin (Sema) 3A and 3F are secreted molecules which guide the movement of axons and oligodendrocytes in development. Sema3A acts as a repulsive signal, and Sema3F an attractive signal, through their receptors Neuruplin 1 and Neuruplin 2 respectively. In MS, some demyelinated plaques spontaneously remyelinate, whereas others do not, and a glial scar forms. For remyelination to occur, oligodendrocyte precursor cells (OPCs) must first be attracted to and repopulate the lesion. We hypothesised that Sema3A and 3F may control the OPC repopulation of demyelinated plaques, and hence remyelination, reiterating development. We performed in situ hybridisation (to detect the semaphorins) and immunohistochemistry (to identify the cells expressing these molecules) on post-mortem MS brain in comparison to control ‘normal’ brain. MS plaques were classed as active, chronic active, or chronic, according to the International Classification of Diseases of the Nervous System. There was clear expression of both Sema3A and Sema3F around and within active MS plaques, mostly in astrocytes and microglia. This expression was absent in chronic plaques, NAWM and control brain white matter. The expression varied with the activity of the plaque. Chronic active plaques had less semaphorin expression around the lesion than active plaques, but with a higher proportion of Sema3A expression, repulsive to OPCs. Active plaques had more expression around the lesion, but with proportionally more Sema3F, attractive to OPCs. In addition to this expression around active plaques, there was a two-fold increase in both Sema3A and 3F expressed by neurons of the grey matter in all types of MS tissue compared to control tissue. The differential expression of Sema3A and 3F in active and chronic active plaques may reflect their likelihood of becoming either remyelinated or chronically demyelinated. The up-regulation of Sema3A and 3F in neurones suggests that demyelinated axons may control their own remyelination by secreting semaphorins to influence OPC migration. We are now using experimental models to further investigate the influence of semaphorins on myelin repair. We recently showed that, as in MS, Sema3A and 3F are up-regulated in rat cortical neurones after a demyelinating cortical lesion, in comparison to a contralateral traumatic lesion. In addition, we are analysing the effect of manipulating the levels of semaphorin expression in vitro and in vivo, using lentiviruses.

Evidence of white matter plasticity in early RRMS patients. A diffusion tensor tractography study

J. Ranjeva, B. Audoin, M. Au doung, I. Malikova, S. Confort-Gouny, P. Cazzone, J. Pelletier; CRBRM UMR CNRS 6612 (Marseille, F); Timone Hospital (Marseilles, F)

Objectives: In multiple sclerosis, the impact of diffuse white matter injury was shown to induce deficits in working memory capacity, totally or partially masked by functional compensatory mechanisms. We hypothesise that such functional adaptive processes are concomitant to structural WM plasticity, supposed to play a relevant role to compensate for connectivity disturbance. Structure and density of fiber tracts involved in the executive system of working memory were assessed by diffusion tensor tractography.

Methods: Eighteen patients with early MS were compared to 15 healthy controls matched for age, sex and educational level. DTI acquisition was performed using a transverse single-shot diffusion-weighted SE-EPI sequence. Anatomical regions of interest (ROI) corresponding to the grey matter areas involved in the executive system of verbal working memory were delineated onto a normalized T1 template provided by the SPM2 software. DTI parameters (mean diffusivity, fractional anisotropy) and tractography were computed using the BrainVISA software (SHFJ, Orsay, France). Physical DTI parameters of WM connections defined in the model were assessed by only extracting characteristics (MD, FA, numbers of detected fascicles) of the parts of WM bundles linking the ROIs. Results: Several WM connections of MS patients were less structured than those of controls: This was the case especially for WM connections linking the left Brodmann Area (BA) 45/46 to the left BA9, the right BA45/46 to the right BA9, the right BA9 to the left BA9 and the right thalamus to the left thalamus. No significant decreases in the numbers of WM fascicles were detected in patients relative to controls whatever the connection. In contrast, the numbers of detected WM fascicles were higher in early MS patients relative to controls for the connection linking the right BA45/46 and the right BA9. These increased numbers of WM fascicles correlated with the disease duration and with the extent of tissue injury in several bundles of the executive system. Interpretation: Structure was affected in patients in accordance to the existence of diffuse myelin injury. Concurrently, we observed an increased number of WM bundles connecting the right prefrontal regions, where functional compensatory mechanisms have been previously shown using fMRI in the same population. This study evidences for the first time the existence of structural WM plasticity concomitant to functional plasticity in patients with early MS.

Plenary session 2 - ECTRIMS Lecture

Perspective of multiple sclerosis research in Europe

G. Comi; IRCCS San Raffaele (Milan, I)

Major advancements have been achieved in the last decade in the pathogenesis and pathophysiology of nervous damage in multiple sclerosis (MS), however some key aspect such as the primary cause(s) of the disease, the relationship between demyelination, axonal damage and inflammation and the basis of the chronic progressive phase of the disease is still largely unknown. The “unity” of multiple sclerosis is debated and further researches exploring the correlation between genetic factors, environmental factors, clinical forms, pathological findings should contribute to define if the disease subtypes are the results of a complex interaction of these factors or, if on the contrary they are different diseases. Magnetic resonance techniques characterised by a better pathological specificity are quite promising for progresses in these research areas and some initial positive results in studies combining clinical, pathological and imaging findings support this view. Important progresses have also been produced in treatment, as a consequence of both the recent pathogenetic acquisitions and of theameliorated methodologies for clinical trials. New drugs demonstrated the possibility to reduce the disease activity by two third and to positively influence the progression of disability, nevertheless we have to face major methodological, ethical and practical issues in organizing and performing clinical trials. A very new area of research is the restorative medicine. The ability of the brain to repair itself by neurogenesis and by mechanisms of plasticity are a very recent acquisition and may have a great impact in a more clever rehabilitation approach. Further resources should be devoted to basic and applied researches in this area. The experience of this last decade clearly demonstrated that significant progresses in MS research have been obtained with working groups operating under the umbrella of the European Community research initiatives or as a result of spontaneous collaborations. ECTRIMS should play a more active role in promoting and facilitate these initiatives.
Combination therapy

60

Combination therapy in multiple sclerosis
C. Confavreux; Hôpital Neurologique Pierre Wertheimer (Lyon, F)

Since 1993, the field of multiple sclerosis (MS) therapeutics has changed dramatically with the approval of interferons beta-1b and glatiramer acetate. Although they clearly are beneficial, a sizable proportion of patients have continued relapses or worsening disability despite therapy. Thus, there is a clear need for more effective and better tolerated therapies. Several approaches can be considered.

Combination therapy utilizing disease modifying agents is one of them, with the hope of additive or synergistic efficacy. Several lines of evidence suggest that this approach will be useful in MS. Multiple environmental and genetic factors, and chronic immune dysregulation play a role in the destructive process of MS. Besides, to cover the full scope of MS pathology, strategies for brain repair through protection and regeneration of axons and myelin are to be envisioned. The first question is which therapies to include in the combination. Candidate drugs for combination therapy each should have demonstrated efficacy and should have a mechanism of action different from the other anticipated therapies. In the case of MS, drugs could be directed at different therapeutic domains such as tissue destruction and tissue repair. But the concept also holds within a given therapeutic domain. For example, presently available therapies act through modulating the immune system, but at different levels.

Theoretically, experimental data could direct the choice among several candidate therapies. Unfortunately, results from such experiments do not necessarily predict results in MS patients. The safety and tolerability of the candidate treatments also must be taken into account. The next question is how to test combination therapy in MS patients. Phase II studies should be conducted first for: 1) checking that the combination is acceptably safe and well tolerated; 2) assessing the impact of the combination on the pharmacokinetics of each individual drug in the combination; 3) searching for hints of efficacy or, conversely, adverse interaction by using relatively sensitive clinical criteria such as relapses or surrogate markers such as cranial MRI activity. The third question is how to design the Phase III pivotal efficacy trial. Ideally, a full factorial 2 x 2 design is preferable, with the use of a placebo for each single agent. It allows for comparing the combination to each single agent and to no active treatment by analyzing the four treatment groups individually. These considerations concern combination therapies in which agents are used in parallel. For combination strategies of the sequential type, e.g. an induction phase followed by a maintenance phase, a conventional parallel design is appropriate. A sizable number of trials of combination therapy have been completed, are in progress, or are planned. They will be reviewed during this lecture.

61

Efficacy results from a randomised, double-blind, placebo-controlled study of intramuscular interferon beta-1a, azathioprine, and corticosteroid combination therapy in patients with relapsing-remitting multiple sclerosis
E. Havrdova, R. Zivadinov, J. Krasensky, M.G. Dwyer, I. Novakova, A. Dokezal, V. Tichy, A. Svorobnik, Z. Seidl, E. Houzavickova, D. Horakova; Charles University (Prague, CZ); State University of New York at Buffalo (Buffalo, USA); Masaryk University (Brno, CZ)

Background: Intramuscular interferon beta-1a (IFN-beta-1a) reduces relapse rates by approximately 30% and delays disease progression in patients with multiple sclerosis (MS). Initial combination therapy with other immunosuppressive agents that have varying mechanisms of action, such as azathioprine (AZA) and corticosteroids, is being explored to improve treatment efficacy. Objective: The purpose of this randomised, double-blind, placebo-controlled study was to compare the efficacy of IM IFN-beta-1a plus AZA alone or in combination with low-dose corticosteroids, with that of IM IFN-beta-1a monotherapy as initial therapy in patients with relapsing-remitting MS over 5 years. Methods: Patients with a confirmed MS diagnosis, an Expanded Disability Status Scale score ≤3.5 on the day of screening, and either 2 relapses in the last 12 months or 3 relapses in the last 24 months were included (annualised relapse rate 1.8, disease duration 5.5 years). A total of 181 patients were equally randomised into 1 of 3 treatment groups: Group 1, IFN-beta-1a 30 mcg IM once weekly plus placebo AZA orally (PO) once daily (QD) plus placebo corticosteroid PO every other day (QOD); Group 2, IFN-beta-1a 30 mcg IM once weekly plus AZA 50 mg PO QD plus placebo corticosteroid PO QOD; or Group 3, IFN-beta-1a 30 mcg IM once weekly plus AZA 50 mg PO QD plus prednisone 10 mg PO QOD. The primary study end point was the rate of clinical relapses at 2 years. Sustained disability progression, MRI measures, and safety and tolerability also were assessed. Results: Over 2 years, the annualized relapse rate was 1.19 for Group 1, 1.06 for Group 2, and 0.80 for Group 3 (p = NS). The cumulative proportion of patients with sustained disability progression at 2 years was 16.8% in Group 1, 20.7% in Group 2, and 17.5% in Group 3 (p = NS). At 2 years, the triple combination treatment favored lower accumulation of T2 lesion volume when compared with monotherapy (p = 0.01). In general, combination treatment with AZA was safe and well tolerated. The rate of infection was similar among treatment groups: 81.7% of patients in Group 1, 82.8% in Group 2, and 84.1% in Group 3 reported an on-study infection. Five-year data also will be presented. Conclusions: Overall, the results of this study suggest that while AZA in combination with IM IFN-beta-1a or IM IFN-beta-1a plus prednisone is safe, its use as initial therapy does not provide added clinical benefit to IM IFN-beta-1a monotherapy.

62

Short-term induction with mitoxantrone preceding treatment with glatiramer acetate offers early and pronounced effects on MRI-disease activity in patients with relapsing forms of multiple sclerosis
T. Vollmer, H. Panitch, M.S. Freedman, S.K. Gasda, A. Bar-Or, D.L. Arnold; Barrow Neurological Institute (Phoenix, USA); University of Vermont (Burlington, USA); University of Ottawa Ontario (Ottawa, CAN); Montreal Neurological Institute (Montreal, CAN)

Induction therapy followed by maintenance therapy is used in treating some autoimmune diseases, but rarely used for MS. We hypothesized that treatment with glatiramer acetate after depletion of the autoaggressive T cell population with mitoxantrone might prevent or delay the regrowth of the autoaggressive T cell population and enhance the induction of regulatory GA-specific Tcells, possibly inducing a long-term remission. Objectives: To ascertain whether short-term induction of immunosuppression using mitoxantrone in patients with MS accelerates the onset and enhances the efficacy of long-term glatiramer acetate treatment. Methods: RMS patients age 18 and 55 years with a gadolinium enhancing lesion on screening MRI and EDSS score 0–6.5 were randomized to receive either IV infusions of mitoxantrone monthly for three month followed by sc injections of GA 20 mg/d two weeks after the last infusion for a total treatment period of 15 months (M-GA, n = 21), or GA 20 mg/d for 15 months (GA, n = 19). Brain MRIs were performed at screening, and months 6, 9, 12, and 15. Suspected on-trial relapses were confirmed at an unscheduled visit. Primary outcomes were tolerability and safety as measured by laboratory assessments and the incidence of adverse events. Secondary efficacy measures were the number of Gd-enhanced lesions, relapse rate and changes in EDSS. Results: Of 93 subjects screened, 43 were randomized, 3 discontinued prior to ever receiving study drug, leaving 40 (62.5% female) eligible. The majority of screen failures (54%) did not meet MRI inclusion criteria. Baseline age (mean ±SD) 37.2 ±10.7 years), disease duration (3.5 ±4.8 years), EDSS (2.4 ±1.2), and number of Gd-enhancing lesions (3.75 ± 3.95), were well-matched in the two arms. M-GA induction produced an...
Abstracts

89% greater reduction (relative risk = 0.11, 95% CI = 0.04 - 0.36, p = 0.0001) in the total number of Gd-enhancing lesions at months 6 and 9, compared to GA alone. The efficacy of M-GA induction was sustained at 15 months, while that of GA increased progressively over the 15 months: Gd-enhancing lesion frequency was decreased by 47% at 9 months and 87% at 15 months. Mean relapse rate during the study period was 0.16 in the M-GA group and 0.32 in the GA group. Both treatments were safe. We found that short-term induction with mitoxantrone followed by daily injections GA 20 mg/d for 15 months is safe and very effective, producing an 89% decrease in MRI-disease activity that occurs early and is sustained.

63
Assessing efficacy in combination trials
F.D. Lublin; Mount Sinai School of Medicine (New York, USA)

The next phase of MS therapeutics will undoubtedly utilize combination therapies. None of the current therapies or single agents under study is able to completely control the illness. The advantage of combination therapy is the ability to take advantage of different and complementary mechanisms of action. This could include use of multiple immunomodulatory agents or a combination of an immunomodulator and a neuroprotective agent or a repair agent. Use of combinations can be concurrent or sequential. If sequential, they can depend on either a time to approach or on lack of initial therapeutic response. Also, induction therapies are under consideration, utilizing an aggressive approach early for a short period and then a less intense therapy as maintenance. Evaluation of combination strategies will need careful attention to safety, avoidance of interfering mechanisms and sufficient improved efficacy (at least additive) to justify the increased efforts and costs of combination treatment. This can be done through a multi-staged process starting with a safety trial to determine if the agents of interest conflict with one another or produce a safety issue, not seen with either alone. At this stage one also can determine aspects of proof of principle. Following this, a pivotal trial can be designed and performed using the usual markers of clinical efficacy as well as MRI metrics. For combination trials with immunomodulatory agents, relapse rate reduction and disability progression remain appropriate clinical targets. For trials with neuroprotective agents or repair/restoration agents, the clinical markers will center on change in disability, but may need to be modified to show improvement, rather than slowed accrual of disability. Similarly, MRI metrics will need to be adjusted to measure changes in neuronal/axonal integrity and in myelin repair. Additional benefit from combination trials can be obtained through inclusion of biomarker studies to determine the nature of those that respond to each agent and that of those who fail to respond. The COMBIRX Phase III trial, currently enrolling in North America, will be used as an example of how combination trials can be designed and implemented.

64
Additive effects of interferon beta-1b and glatiramer acetate on the immune response to myelin basic protein: implications for combined immunotherapy of multiple sclerosis
R. Milo, H. Panitch; Barzilai Medical Center (Ashkelon, IL); University of Vermont College of Medicine (Burlington, USA)

Disease modifying drugs for MS such as interferon beta (IFN-b) or glatiramer acetate (GA) are only partially effective. Theoretically, the combination of such drugs with distinct immuno-modulatory properties may mutually enhance their effects on immune responses relevant to MS, such as those directed at myelin antigens, and increase clinical efficacy. We investigated the combined in-vitro effects of IFN-b and GA on the immune response to myelin basic protein (MBP). Long-term antigen-specific Th1 cell lines were generated from healthy individuals with different HLA phenotypes. IFN-b non-specifically inhibited the proliferative response of all T-cell lines specific for MBP, tuberculin (PPD) and tetanus toxoid (TT) to their respective antigens, while GA reduced the proliferation of MBP-specific lines only. When combined together, IFN-b and GA had an additive suppressive effect on MBP-specific T-cell lines, with 70–100% inhibition of proliferation, depending on the concentration of the antigen. The combined effect on PPD and TT-specific lines was similar to the inhibitory effect of IFN-b alone. This additive pattern of inhibition was observed when either autologous peripheral blood mononuclear cells (MNC) or autologous Epstein-Barr virus (EBV) transformed B-cells were used as antigen presenting cells (APC). The same additive pattern of inhibition was observed when APC were pretreated with IFN-b, GA or both, then washed and incubated with the various T-cell lines without the immunomodulator, indicating an effect on APC function. Pretreatment of APC with IFN-b resulted in a dose-dependent reduction in HLA-DR and HLA-DQ expression, which paralleled inhibition of TT-cell line proliferation, and was partially reversed by pretreatment with IFN-g. In contrast, pretreatment of APC with GA had no effect on the proliferation of this cell line, or on MHC class-II expression. The production of the Th1 cytokines IFN-g and IL-2 by MBP specific lines was also inhibited additively by up to 100%. These results demonstrate that proliferation and pro-inflammatory cytokine production by MBP-reactive Th1-cells, which are relevant to the pathogenesis of MS, are additively inhibited by the combination of IFN-b and GA. These effects may be mediated through complementary effects on antigen presentation, with IFN-b down-regulating the expression of MHC class-II molecules on APC, and GA competing with MBP for antigen binding sites on the remaining MHC molecules.

Cannabinoids, immune modulation and the CNS

65
Protection of axonal damage in experimental autoimmune encephalomylitis
D. Baker; Institute of Neurology, University College London (London, UK)

In response to patient claims that cannabis could alleviate symptoms in multiple sclerosis (MS), we began to investigate this in experimental autoimmune encephalomyelitis (EAE) models of MS. We were able to provide objective evidence for the role of the cannabinoids in the tonic control of signs of MS and this appears to be supported by studies in MS. However, cannabinoids may also have the potential to influence progressive MS by protecting axons from damage. Axonal pathology in MS occurs in response to inflammatory attack and also by autoimmune-independent mechanisms that can underlie the cause of progressive disability. Therefore neuroprotection secondary an anti-inflammatory effect may be one route towards axonal sparing. Indeed in EAE it was shown that cannabinoids in cannabis and synthetic cannabinoid receptor agonists could inhibit the immune response in the periphery. This is associated with an inhibition of mononuclear cell invasion of the central nervous system (CNS) and clinical disease that is usually associated with nerve loss. Surprisingly, this was shown to be largely CB1 receptor dependent rather than via CB2 on leukocytes and was found to secondary to stimulation of receptors within the CNS causing the release of immunosuppressive molecules that down regulate, pro-inflammatory Th1 responses, rather than via a direct effect on the immune system. However, cannabinoids may also contribute to protecting axons via a direct action with the CNS. Animals deficient in cannabinoid receptors exhibited neurodegeneration and poorly tolerated inflammatory insults such that they showed exaggerated nerve loss in response to auto-immune attack. Through receptor stimulation it was possible to slow neurodegeneration, independent of mediating inhibition of auto-
immunity in animal models of both MS, uveitis and amyotrophic lateral sclerosis, suggesting that cannabis and the cannabinoid system may have the potential to slow progression of disease by preventing nerve loss in addition to symptom control, as may be suggested from recent clinical studies.

66
Cannabinoid system and neuroinflammation: implications for multiple sclerosis
C. Guaza; Neuroimmunology Group Instituto Cajal (Madrid, E)

The cannabinoid signaling system is composed of cannabinoid receptors, their endogenous ligands, the endocannabinoids, and the enzymes that produce and inactivate them. Endocannabinoids are released after brain injury and neuroinflammation and are believed to attenuate neuronal damage. Growing evidence suggests an important role of the cannabinoid signaling system to modulate CNS inflammation. Advances in the understanding of the physiology and pharmacology of cannabinoid system have increased the interest of studies evaluating potential actions of cannabinoids on pathologies such as multiple sclerosis. Effects of cannabinoids on immune reactivity, neuroprotection and glial cell function will be presented within the context of multiple sclerosis model of Théler’s virus induced demyelinating disease (TMEV-IDD). Data about the beneficial effects of cannabinoids on TMEV-IDD, in terms of motor function improvement, down-regulation of inflammation, remyelination and reduction of axonal pathology points to the prospect of cannabinoid system as a target for development of therapeutic strategies for chronic inflammatory diseases like multiple sclerosis.

References

67
Cannabinoids in multiple sclerosis; an update
J. Killestein; VU Medical Center (Amsterdam, NL)

This paper reviews and critically evaluates the most recent evidence available on the potential of cannabinoid drugs to relieve symptoms like muscle spasticity, bladder dysfunction, tremor, fatigue and pain in multiple sclerosis (MS) patients. Interest in the field has been strengthened by the identification and cloning of cannabinoid receptors located in the central nervous system and the peripheral immune organs, and by the discovery of the endogenous cannabinoid ligands. Cannabinoids have been efficacious in animal models of MS and some believe that their anti-inflammatory and neuroprotective properties may even play a beneficial role in MS relapses and disease progression. However, researchers encounter a number of difficulties in designing sound clinical studies for the use of cannabinoids. Most studies have substantial limitations with respect to dosing, masking, outcome measures and interpretability of the data. Of the more than 15 trials reporting the clinical use of cannabinoids in MS so far, the somewhat better designed studies failed to demonstrate objective improvement. Today, over 1000 MS patients have been participating in clinical trials using different cannabinoid products and routes of administration. Despite the rather high number of reported trial patients who have been using these products, convincing evidence that cannabinoids are effective in MS is still lacking. Nevertheless, experimental evidence supports the therapeutic rationale for the application of both plant-derived cannabinoids and synthetic cannabinoid receptor agonists in MS, and several new efficacy trials, using promising cannabinoid products, are currently underway.

68
Meta-analysis of the effects of Sativex® on spasticity associated with multiple sclerosis
C. Collin, P. Duncombe; Royal Berkshire Hospital (Reading, UK); GW Pharma Ltd (Salisbury, UK)

Background: Symptoms of spasticity are common in patients with Multiple Sclerosis (MS). The objective of this meta-analysis was to estimate the effect of standardised whole plant cannabis medicine (Sativex®) in alleviating symptoms of spasticity by assessing data from two similar studies. Methods: Raw data from two double-blind, randomised, placebo-controlled studies were interrogated in the meta-analysis. A total of 189 patients were randomised in the first study (6 weeks in length) and 337 in the second (14 weeks in length). In all, 291 patients were randomised to Sativex and 235 to placebo. The two studies were similar in age and gender distribution and also spasticity at baseline which was assessed on an NRS scale. The endpoints common to both studies were spasticity as assessed on daily diary cards, Ashworth Scale, global impression of change and responder analysis based on spasticity assessment. Results: The primary analysis showed a statistically significant difference in favour of Sativex compared to placebo for spasticity scores (−0.34, 95% CI: −0.64, −0.04; p = 0.027). A modified ITT population, excluding major inclusion criteria violation, gave a statistically stronger result (−0.40, 95% CI: −0.67, −0.13; p = 0.0084). There was an improvement in spasticity scores for both Sativex and placebo groups during the first week. Whilst scores in the placebo group then remained relatively constant from day 10, the Sativex group showed continued improvement up to day 16 and this was maintained thereafter. The analysis of global impression of change was in favour of Sativex in the ITT population with an odds ratio of 1.55 (95% CI: 1.07, 2.25; p = 0.027) and 1.61 in the Modified ITT population (95% CI: 1.10, 2.35; p = 0.017). No statistically significant treatment differences were seen in the Ashworth Score. In the Sativex group 35% of patients were responders at the 30% level compared to 24% in the placebo group (odds ratio 1.63). This difference was statistically significant (p = 0.019). The sensitivity and robustness of conclusions were explored further using a week 6 endpoint for both studies and also a meta-analysis directly combining the treatment effects from the studies, both confirmed all the findings from the primary analysis of spasticity. Conclusions: This meta-analysis demonstrated clear evidence of statistically significant benefit in the Sativex group compared to placebo for spasticity, global impression of change and proportion of responders to treatment.
The effects of cannabinoids on murine neural stem cell survival and differentiation
Y. Mao-Draayer, F. Eckenstein, H. Panitch; University of Vermont (Burlington, USA)

**Background:** Mouse neural stem cells (MNSCs) may promote remyelination in an experimental model of demyelinating disease. Proliferation of MNSCs requires growth factors such as EGF and FGF, and differentiation into the three major cell lineages of the nervous system (neuron, astrocytes, and oligodendrocytes) occurs only after withdrawal of these factors. As suggested by some basic science and clinical studies, cannabinoids are a potential therapy to impact progression or reverse damage caused by chronic inflammation in multiple sclerosis. However, cannabinoids have also been shown to inhibit neuronal progenitor cell differentiation. Anandamide (arachidonyl ethanolamine or AEA) is a non-specific endogenous agonist that activates cannabinoid receptors. The effects of AEA on MNSC proliferation and oligodendrocyte differentiation from MNSC are largely unknown.

**Methods:** MNSCs were allowed to proliferate in the presence of EGF and FGF (100ng/ml) in Neurobasal media with B27 and L-Glutamine for 4 to 5 days. Upon reaching 70–80% confluence, EGF and FGF were withdrawn to allow cells to differentiate. The cultures were supplemented with AEA in concentrations of 0, 1, 2, 5, 8, 10, 25, 50, and 100 micro Molar at both proliferation and differentiation phases. Cells were counted and cell death was quantified by lactate dehydrogenase (LDH) assay. Oligodendrocyte progenitors were quantified based on immunocytochemical staining with mouse anti-A2B5 monoclonal antibodies. Different cell lineages were detected by real time RT-PCR for the expression of oligoden-

**Results:** The presence of CB1 and CB2 receptors on MNSC was detected by PCR. AEA treatment at concentrations above 25 micro Molar decreased the proliferation of MNSCs. Withdrawal of EGF and FGF resulted in death of a proportion of MNSCs, which was further increased in the presence of 25 micro Molar AEA. In the presence of lower AEA concentrations (<10 micro Molar), MNSC cultures still allowed the appearance of A2B5 positive cells as well as the expression of oligodendrocyte markers NG2 and PDGFRalpha. However, the degree of oligodendrocyte differentiation was greatly lessened in the presence of AEA.

**Conclusions:** MNSC proliferation and differentiation were inhibited by AEA at concentrations above 25 micro Molar. The inhibitory effect was mediated via CB1 and CB2 receptors. We now intend to determine the downstream signaling pathway that mediated the effect.

---

**Intrathecal polyspecific immune response against neurotropic viruses discriminates between multiple sclerosis and other demyelinating diseases of the CNS**
S. Jarius, D. Franciotta, R. Bergamaschi, E. Marchioni, K.-P. Wandinger, J. Sustr-Garriga, X. Montalban; Vall d’Hebron University Hospital (Barcelona, E)

**Background:** Neuromyelitis optica (NMO, Devic’s syndrome), acute demyelinating encephalomyelitis (ADEM), paraneoplastic neurological syndromes (PNS), and chronic neuroborreliosis (NB), are rare inflammatory demyelinating disorders of the central nervous system (CNS). Multiple sclerosis (MS) may mimic these disorders with regard to clinical presentation, time course, MRI and routine laboratory findings. Mild pleocytosis, CSF-restricted oligoclonal bands, an increased IgG index, and mild alterations of the blood-CSF barrier may be present in either of these conditions, not allowing for alternative to exclusively MRI based data. Moreover, CSF analysis may improve the pathologic and prognostic specificity of the lesions found on MRI, which would be particularly relevant in patients presenting with clinically isolated syndromes (CIS). The CSF study may also give information on CIS and MS prognosis as well. Different molecules such as 14-3-3, tau protein or oligoclonal bands of IgM predict conversion of CIS to MS. Oligoclonal IgM against myelin lipids identify a subgroup of MS patients with an aggressive disease course eligible for an early treatment. In summary, CSF study offers valuable data on MS physiopathology, and prognosis, adding accuracy in its diagnosis.
Methods: capable of discriminating between MS NMO, ADEM, and PNS. systematically evaluated in other demyelinating autoimmune dis-
orders of the CNS so far. Objectives: To assess whether MRZ is capable of discriminating between MS NMO, ADEM, and PNS. 
motivated diagnostic testing and follow-up in patients with clinically isolated syndrome (n = 40); RR MS (n = 42) and SP MS (n = 29); non-inflammatory neurological controls (n = 41) and inflammatory neurological controls (n = 40). Results: In agreement with our previous findings, CSF NAA levels were decreased in SP MS patients compared to RR MS patients. The median CSF levels in CIS patients were similar to those in RR MS patients. The median CSF NAA levels in non-inflammatory neurological controls were lower compared to the inflammatory controls or RR MS patients. NAA levels in three patients with headache complaints were similar to levels in CIS and RR MS patients. A negative correlation with EDSS was observed and disease duration in MS patients.

Conclusions: The data suggest that CSF NAA levels decrease during the MS disease course. Decreased CSF NAA levels likely are a reflection of axonal loss in various neurological diseases.

Acknowledgements: Dutch Brain Foundation

The role of CSF in the diagnostic criteria

J. Wolinsky; University of Texas (Houston, USA)

The evaluation of cerebrospinal fluid (CSF) constituents remains central in the diagnostic process and in some instances also for the management of neurologic diseases. However, recently promulgated and revised criteria for the diagnosis of multiple sclerosis (MS) have somewhat de-emphasized the importance of CSF testing in favor of highly characteristic but in isolation, non-diagnostic patterns seen on cross-sectional or serial magnetic resonance imaging (MRI). Clearly MRI and CSF testing provide independent and unique information, and their relative value depends on the context in which the diagnosis is being made or unexpected alterations in the anticipated clinical course of the patient dictate reassessment of the diagnosis. Both have limitations of sensitivity and specificity. The International Panel views Imaging as more sensitive and specific in making an MS diagnosis in most situations, but allows that certain CSF abnormalities as the presence of distinct oligoclonal bands and/or an elevated immuno-
globulin (IgG) index add unique information, particularly when the clinical picture is unusual or the imaging criteria are insufficient for diagnosis. Even when the clinical and imaging criteria suffice for diagnosis, classic CSF findings add to the clinician’s confidence, and unexpected results may dictate more vigilant follow-up or additional testing. Nevertheless, most studies of the value of the original International Panel diagnostic criteria applied to well characterized if somewhat biased patient cohorts suggest that routine testing of CSF is often not necessary. The presence of oligoclonal bands or an elevated IgG index is not obligatory for identification of relapsing or primary progressive MS; the latter leading to a recent specific revision in the International Panel diagnostic criteria.

Cerebrospinal fluid proteome profile in patients with multi-
ple sclerosis

H. Tunani, S. Sässmuth, G. Tauscher, J. Brettschneider, S. Felk, F. Gillardon, V. Lehmensiek; University of Ulm (Ulm, D)

Background: Cerebrospinal fluid (CSF) proteomes may provide important information about the pathomechanisms present in multiple sclerosis (MS). Although diagnostic criteria for early MS are available, there is still a need for biomarkers predicting disease subtype and progression. Therefore, we intended to analyze the CSF proteome profile of MS patients. Methods: CSF samples from 12 patients with relapsing remitting MS (RRMS, mean age 40.4 years; median disease duration 24 months) and from 12 patients with clinically isolated syndrome (CIS, mean age 33.8 years; median disease duration 3 weeks) were analyzed and compared to age matched normal controls; all MS and CIS patients received a lumbar puncture during an acute relapse. CSF samples were analyzed by 2-dimensional difference in-gel electrophoresis (2-D-DIGE) technology. Only those protein spots that showed more than 2-fold difference between both groups were selected for further analysis with MALDI-TOF mass spectrometry. Results: In RRMS, 11 different spots were detected. Only one of them was up-regulated which was identified as Ig kappa chain NIG precursor protein; on the other hand, 10 out of 11 proteins were down-regulated which were identified as transferrin, some serine proteinase inhibitors, alpha-2-HS-glycoprotein, apolipo-
protein E and transthyretin. In contrast, 14 different spots were detected in the CSF of CIS patients. Two of the different proteins were up-regulated which were identified as IgG kappa chain and proapo-A-1-protein. Down-regulated proteins included serum albumin precursor, serum albumine, complement factor 3, some serine proteinase inhibitors, vitamin D-binding protein, trans-
lation-initiation factor eIF-4-gamma, apolipoprotein E precursor and transthyretin. Some of these proteins, serine proteinase inhibitors, alpha-2-HS-glycoprotein and translation-initiation factor eIF-4-gamma, have not been reported in CSF of MS patients yet. Conclusion: Our preliminary results show clear differences in the proteome profile of patients with RRMS and CIS as compared to normal controls. Though the pathophysiological role of these proteins still remain to be elucidated in detail and further validation of the

N-acetylaspartate as a CSF marker for axonal damage in multi-
ple sclerosis patients and controls

C. Teunissen, E. Jacobson, M. Khademi, L. Brundin, N. Verhoeven, C. Jacobs, C. Dijkstra; VUMC Amsterdam (Amsterdam, NL); Karolinska Institutet (Stockholm, S)

Background: N-acetylaspartate (NAA) has been known as a MRI marker for axonal loss in MS. In a previous study, we investigated CSF levels of NAA. We observed lower levels in SP MS patients compared to RR MS patients in the Amsterdam MS cohort. Furthermore, the correlation of CSF NAA levels with EDSS scores and atrophy suggested that CSF NAA levels could be a marker for disease progression in MS. So far, it is not known whether CSF NAA levels are changed early during the disease course of MS and CIS as compared to normal controls. The objective was to repeat the previous findings in a different and larger population of MS patients (Swedish cohort). Methods: CSF NAA levels were determined by a previously described gas-chromatography/mass spectrometry method. The following groups were included: patients with clinically isolated syndrome (n = 40); RR MS (n = 42) and SP MS (n = 29); non-inflammatory neurological controls (n = 41) and inflammatory neurological controls (n = 40). Results: In agreement with our previous findings, CSF NAA levels were decreased in SP MS patients compared to RR MS patients. The median CSF levels in CIS patients were similar to those in RR MS patients. Median CSF NAA levels in non-inflammatory neurological controls were lower compared to the inflammatory controls or RR MS patients. NAA levels in three patients with headache complaints were similar to levels in CIS and RR MS patients. A negative correlation with EDSS was observed and disease duration in MS patients.

Conclusions: The data suggest that CSF NAA levels decrease during the MS disease course. Decreased CSF NAA levels likely are a reflection of axonal loss in various neurological diseases.

Acknowledgements: Dutch Brain Foundation
findings is needed, this non-hypothesis driven approach may have a relevant impact on the identification of disease-specific markers.

Free communications

79

The relationship between neuromyelitis optica and systemic autoimmune disease
B. Weinshenken, S. Pittcock, J. de Seze, P. Vermeersch, D. Wingerchuk, H. Zephir, H. Homburger, C.F. Lucchinetti, V. Lemon; Mayo Clinic College of Medicine (Rochester, USA); Hopital Universitaire de Strasbourg (Strasbourg, F); University of Lille (Lille, F); Mayo Clinic College of Medicine (Scottsdale, USA)

Objective: To study the relationship between neuromyelitis optica (NMO) and systemic autoimmune diseases. Background: NMO differs from multiple sclerosis in prognosis and treatment. A specific biomarker, NMO-IgG, exists for NMO. Clinical or serological markers of Sjögren’s syndrome (SS) or systemic lupus erythematosus (SLE) are found in approximately half of NMO patients, but NMO-IgG is restricted to NMO, recurrent optic neuritis (ON) and recurrent longitudinally extensive transverse myelitis (LETM). ON and LETM may be vasculitic complications of SS or SLE (e.g. “lupus myelitis”). Given their overlapping autoantibody profiles, we hypothesized that LETM and ON in the setting of SS or SLE may be hitherto unrecognized manifestations of NMO. Design/Methods: We tested serum samples from patients from two datasets: 1) USA: definite NMO (n = 78); rLETM (n = 44); controls with either SS (n = 19) or SS (n = 14) but without symptoms of NMO; 2) France: uncomplicated NMO (n = 8); NMO with SS or SLE (n = 6); isolated and/or recurrent ON or myelitis with SS or SLE (n = 8); and SS with neurological disorders aside from ON or myelitis (n = 6); SS (n = 5) and SLE (n = 5) without neurological manifestations. Results: USA: 78.2% (61 of 78) of NMO patients were seropositive for NMO-IgG, 52.6% (41 of 78) for ANA and 16.7% (13 of 78) for ENA; the corresponding seropositivity rates in rLETM patients were 70.4% (31 of 44), 40.9% (18 of 44) and 18.2% (8 of 44). Only 3% (4 of 122) patients with NMO or rLETM fulfilled international diagnostic criteria for SS (n = 2) or SS (n = 2); all 4 patients were seropositive for NMO-IgG. NMO-IgG was not detected in SS or SLE controls. France: 25% (2 of 8) with uncomplicated NMO (50% (2 of 4) with LETM), 50% (3 of 6) with NMO and concomitant SS or SLE and 25% (2 of 8) with isolated and/or recurrent ON or LETM with SS or SLE were seropositive for NMO-IgG. NMO-IgG was not detected in any patient without ON or myelitis, including those with other neurological syndromes. NMO-IgG was associated with NMO or NMO partial syndromes (p = 0.01) but not with systemic autoimmunity (p = 0.58).

Conclusions/Relevance: Patients with NMO and coexisting SS or SLE are seropositive for NMO-IgG as frequently as those with uncomplicated NMO; the pathogenesis of ON and LETM may be the same in these groups.

81

The value of multimodal evoked potential scores in monitoring the clinical disease course in early relapsing-remitting multiple sclerosis: a two-year follow-up study
P. Jung, A. Beyerle, P. Gelnke, T. Richter, U. Ziemann; J.W. Goethe University (Frankfurt am Main, D)

Objective: To evaluate the relationship between abnormalities of multimodal evoked potential scores (mePS) and clinical disability in the first two years of relapsing-remitting multiple sclerosis (RRMS). Methods: Thirty-seven patients with newly diagnosed definite RRMS were prospectively examined. Multimodal evoked potentials (VEP, BAEP, median nerve SEP, tibial nerve SEP, upper limb MEP, lower limb MEP) and the expanded disability status scale (EDSS) were measured at entry and after 3, 6, 12 and 24 months. Each investigation was performed in a clinically stable phase, at least 6 weeks after the last relapse. An mePS was designed, ranging from 0 to 70, with a sensitive EP measure, followed by VEP (62.2%), tibial nerve SEP (42.3%), median nerve SEP (39.2%) and upper limb MEP (34.3%). The mean EDSS (+ 0.3 ± 0.1, p = 0.02, Wilcoxon test) and the mePS (+ 2.6 ± 1.0, p < 0.02) deteriorated slightly but significantly over the two-year follow-up period. The EDSS and the mePS were not correlated at entry (Spearman’s rho = 0.28, n.s.) whereas there was significant correlation between the EDSS after 24 months and the mePS at entry (rho = 0.37, p = 0.03), indicating a moderate prognostic value of the mePS. Moreover, the changes of the EDSS and the mePS during the two-year follow-up period were highly significant (rho = 0.67, p < 0.0001). After two years, RRMS patients with an EDSS progression ≥ 1.0 or ≥ 1.5 if the baseline EDSS was 0.0 (n = 7) showed no significant differences of the EDSS and mePS at entry compared to patients with no relevant change in the EDSS. In contrast, the worsening of the mePS was much more pronounced in the group of patients with relevant EDSS progression (rho = 1.31 vs. 1.1 ± 0.8, p < 0.005, Mann Whitney test).

Conclusions: Serial mePS represent a highly valuable and objective marker of clinical disease progression in early RRMS. Thus, in addition to MRI and clinical tests, mePS should
be used routinely to monitor disease progression and therapeutic effects, at least in the early stages of RRMS.

82

Cognitive impairment and MRI features in early relapsing-remitting multiple sclerosis patients: results of an Italian multicentre study (COGIMUS)

F. Patti, M.P. Amato, M.R. Tola, M. Trojano, P. Frenazza, F. Lijoi, S. Bastianello on behalf of the COGIMUS study group

Background: Cognitive impairment has a 40-65% prevalence in the course of MS and can be detected since the early stages of the disease. Mixed methods were performed through the Rao's Neuropsychological battery; patients also underwent a standardized brain MRI examination to calculate brain T2-w, T1-w lesion load and brain volume with the SIENAX software.

Results: We analyzed the baseline findings of 430 patients, 282 women and 148 men, aged between 18 and 50 years (34.3 ± 8.2 yrs) with a middle high education level (12.5 ± 3.3 yrs), low disability with an EDSS score below 4.0 (1.7 ± 0.9) and with a very short disease duration (1.9 ± 1.4). About one third (33.3%) of patients exhibited cognitive impairment in 2 tests or more of the Rao’s battery (1 SD below the mean Italian normative values). We also found that spatial recall test (1036 SRT) were significantly associated with both brain MRI T2 and T1 lesion load (p = 0.049; p = 0.008 respectively): likely symbol digit modalities test (SDM) was significantly associated with both brain MRI T2 and T1 lesion load and brain atrophy (p = 0.005; p = 0.004; p = 0.04 respectively); paced auditory serial test (PASAT3) was significantly associated with both brain MRI T2 and T1 lesion load (p = 0.02; p = 0.007 respectively). The selective reminding test-consistent long term retrieval (SRT-CLTR) was also significantly associated with both brain MRI T2 and T1 lesion load and brain atrophy (p = 0.01). On the contrary, T1 lesion load was significantly associated with the long term storage (SRT-STS; p = 0.03). Finally, the multivariate logistic regression showed that age, disease duration and T2 lesion load were significant predictors of cognitive impairment in this cohort of patients. Conclusion: This study confirms that cognitive impairment may occur since the early stages of the disease in disability-free patients and is associated with specific MRI parameters. In everyday practice, cognitive assessment should become a factor in clinical decision-making.

83

Prognostic relevance of antomyelin antibodies for progression to multiple sclerosis after a first demyelinating event: results from the BENEFIT study

J. Kuhlke, L. Kappos, C. Pohl, M. Mehling, G. Edan, M. Freedman, H.P. Hartung, D.H. Miller, X. Montalban, C. Polman, F. Barkhof, L. Bauer, S. Dubois, R.L.P. Lindberg, R. Sandbrink; University Hospital (Basel, CH); Schering AG (Berlin, D); Clinique Neurologique (Rennes, F); The Ottawa Hospital (Ottawa, CAN); Heinrich-Heine-Universitaet (Dusseldorf, D); The National Hospital for Neurology & Neurosurgery (London, UK); Hospital Vall d’Hebron (Barcelona, E); Vrije Universiteit Medical Center (Amsterdam, NL)

Patients with a clinically isolated syndrome (CIS) have a high risk of progression to clinically definite multiple sclerosis (CDMS). However, individual prognosis is unpredictable. A significantly increased risk of conversion to CDMS in patients with IgM antibodies against myelin oligodendrocyte glycoprotein (MOG) and myelin basic protein (MBP) was reported (New Engl J Med 2003; 349: 139–145). In the BEtaferon® in Newly Emerging multiple Sclerosis for Initial Treatment study (BENEFIT), a total of 468 patients with a CIS and at least two clinically silent brain MRI lesions were investigated. In this multicentre, double-blind trial, patients were randomised to interferon beta-1b (IFNB-1b, n = 292) or placebo (n = 176) and treated for 24 months or until CDMS was diagnosed. Regular visits were scheduled for the collection of data on neurological disability and MRI before treatment and at study month 3, 6, 9, 12, 18 and 24. We measured serum anti-MOG and anti-MBP antibodies (IgM and IgG) at baseline by Western blot in 462 of 468 patients. With respect to IgM antibody status, 76 (16%) patients were seropositive (+) for both anti-MOG and anti-MBP antibodies, 119 (26%) patients were seropositive only for anti-MOG antibodies, 46 (10%) were seropositive only for anti-MBP antibodies, 221 (48%) were seronegative (−) for both anti-MOG and anti-MBP antibodies. Group sizes with respect to IgG antibody status were: 33 (7%) anti-MOG+ and anti-MBP−; 68 (15%) anti-MOG+ and anti-MBP−; 63 (14%) anti-MOG− and anti-MBP+; 298 (65%) anti-MOG− and anti-MBP−. Cox proportional-hazards regression showed that the risk of CDMS and the risk of McDonald MS over the observation period of two years were not significantly increased in any of the patient groups with positive IgM or IgG antibody findings. Hazard ratios were all close to or even below 1.0. Testing for antibodies against MBP and MOG by Western blot does not allow for a better prediction of conversion to CDMS and McDonald MS in CIS patients. Study supported by Schering AG and the Swiss MS Society.

84

Results of clinical and magnetic resonance imaging analyses following cessation of natalizumab dosing in patients with multiple sclerosis


Natalizumab is a selective α4-integrin antagonist that significantly reduced annualized relapse rate and magnetic resonance imaging (MRI)-detectable brain lesion development in patients with relapsing multiple sclerosis (MS) during phase 3 studies. Dosing of natalizumab was voluntarily suspended when 3 cases (2 in MS and 1 in Crohn’s disease) of progressive multifocal leukoencephalopathy (PML) were reported during natalizumab therapy. This paper reports annualized relapse rate and the number of gadolinium-enhancing (Gd++) lesions over time following suspension of natalizumab dosing. Patients who completed AFFIRM, SENTINEL, or GLANCE studies were eligible to participate in an open-label extension study that evaluated the long-term safety and tolerability of natalizumab. SENTINEL and the open-label extension study were terminated early due the voluntary suspension of natalizumab. All patients who received natalizumab were examined by physicians at a dose-suspension safety evaluation; at this evaluation, patients were also assessed for adverse events and had a cranial MRI scan. In addition, patients who participated in the safety-extension study continued with follow-up visits every 3 months for up to 12 months. At these visits, and at unscheduled visits during this time, relapses were assessed. The annualized relapse rate was 0.175 (n = 1866; 95% CI: 0.114, 0.259) during the 1st month post dosing, 0.262 (n = 1853; 95% CI: 0.184, 0.361) during the 2nd month post dosing, and 0.270 (n = 1839; 95% CI: 0.191, 0.387) during the 3rd month post dosing, peaking at 0.64 (n = 1756; 95% CI: 0.51, 0.79) during the 7th month post dosing; at no time did it exceed the placebo/Avonex control relapse rate during the 2nd year of the AFFIRM and SENTINEL studies (0.67). The mean number (±SD) of Gd++ lesions was 0.1 (±0.4) at 0–3 months post dosing (n = 147), 0.4 (±1.1) at >3–4 months post dosing (n = 65), and 1.0 (±3.2) at >4–6 months post dosing (n = 70); the number of Gd++ lesions was lower than the baseline (pre-dose) number of Gd++ lesions (1.5 ± 4.2; n = 339) for 6 months post dosing. These findings are consistent with pharmacodynamic data showing that both α4-integrin saturation levels and lymphocyte counts return to baseline within 12 to 16 weeks. Consistent with the results of a phase 2 study of natalizumab in MS, which was designed to
Natalizumab in relapsing multiple sclerosis: appropriate patient selection, management guidelines, and a diagnostic algorithm for the differential diagnosis of new neurologic signs or symptoms

L. Kappos, C.H. Polman, D. Bates, J. King, P.W. O’Connor, R.A. Rudick, H.L. Weiner, S.L. Hauser, T.A. Youssy, E.W. Radue, D. Clifford; University Hospital Basel (Basel, CH); Free University Hospital (Amsterdam, NL); Royal Victoria Infirmary (Newcastle-upon-Tyne, UK); Royal Melbourne Hospital (Melbourne, AUS); St. Michael’s Hospital (Toronto, CAN); Cleveland Clinic Foundation (Cleveland, USA); Brigham and Women’s Hospital (Boston, USA); UCSF Multiple Sclerosis Center (San Francisco, USA); Institute of Neurology (London, UK); Washington University (St. Louis, USA)

In a phase 3 clinical trial (the AFFIRM study), natalizumab monotherapy was shown to reduce disability progression by 42% and annualized relapse rate by 68% over 2 years in patients with relapsing MS. Although natalizumab was well tolerated, three cases (2 in MS and 1 in Crohn’s disease) of progressive multifocal leukoencephalopathy (PML), a rare and often fatal infection of the central nervous system, have been associated with natalizumab use (estimated incidence of 1/1,000; 95% CI, 0.2–2.8). The PML literature and features of the three confirmed cases were reviewed and a panel of experts in neurology, neuroradiology, and PML was consulted. In addition an extensive safety evaluation was conducted to assess patients for PML and to determine the utility of various diagnostic tools. The goals of the effort were to establish criteria for patient selection and on-treatment management that may lead to improved benefit-risk ratio for natalizumab in patients with MS. From this, guidelines for appropriate patient selection and management during natalizumab treatment were established. The guidelines emphasize the importance of confirming the diagnosis of MS both clinically and with cranial MRI and the evaluation of patients for factors that may independently place them at risk for PML (e.g., immunocompromise as a result of comorbidities or immunosuppressant use). The guidelines further emphasize use of a three-step diagnostic algorithm which highlights the importance of clinical vigilance for the early detection and treatment of changes in neurologic status that may present in natalizumab-treated MS patients. Thorough clinical monitoring and early dose suspension whenever PML is suspected may be the best means of improving outcome. The details of the diagnostic algorithm along with the use of cranial MRI and cerebrospinal fluid as diagnostic tools will be discussed. In addition, MRI features that help distinguish between MS and PML will be described.

The relationship between disease severity and health-related quality of life in patients with relapsing multiple sclerosis


Two randomised, double-blind, placebo-controlled phase 3 clinical trials evaluated the efficacy and safety of natalizumab (300 mg administered intravenously) as monotherapy (the AFFIRM study) and as add-on therapy to interferon beta-1a (the SENTINEL study) in patients with relapsing multiple sclerosis (MS). In both studies, Quality of life (QoL) was measured using the Multiple Sclerosis Quality of Life Inventory (MSQOL) and well-being using a visual analogue scale (VAS). The MSQOL is an MS-specific health-related QoL instrument that includes a widely used generic measure, the Medical Outcomes Study Short Form-36. In AFFIRM, natalizumab monotherapy significantly improved scores on both the physical (p = 0.003) and mental (p = 0.011) components of the SF-36, and well-being as measured by the VAS (p = 0.007) over 2 years compared with placebo; similar results were observed in SENTINEL. Hence, these studies provide Class I evidence that natalizumab can improve health-related QoL in patients with MS. Analyses were conducted to determine the relationship between disease severity and QOL. In AFFIRM, patients with no disability progression showed improved scores on the physical component (mean change from baseline = 0.78 vs. −3.05; p < 0.001) and mental component of the SF-36 (1.69 vs. −0.93; p = 0.039), and the VAS (0 vs. −9.4; p < 0.001) at 2 years compared to patients with disability progression. Similarly, patients with no relapses showed improved scores on the physical (mean change from baseline = 0.76 vs. −2.38; p < 0.0001) and mental components of the SF-36 (1.56 vs. −0.08; p = 0.032), and the VAS (0.2 vs. −7.7; p < 0.0001) at 2 years compared to patients with ≥2 relapses. In both studies, 2-year change in MSFC was significantly correlated with change in either component of the SF-36 and with change in the VAS. Results of multivariate analyses that explore the influence of disease severity on the effect of natalizumab on QoL, as well as results from SENTINEL, will be reported. The present analyses demonstrate a clear relationship between neurologist assessment of disease severity and patient reported QoL. They further show that patients with less disease severity determined by the neurologist have better scores on measures of health-related QoL.

Natural history

Predictive factors in placebo and natural history data

M. Daumer; Sylvia Lawry Centre for Multiple (Munich, D)

We review the results about predictive factors in placebo and natural history data that have been produced in the first five years at the Sylvia Lawry Centre in Munich and discuss similarities and differences to published knowledge. For example, using multivariate models we could show that the strongest predictor for on-study relapses is the pre-study relapse rate. This result is certainly useful for optimising the entry criteria for future trials with the aim to enrich the trial with informative patients. Standard MRI variables such as the presence of Gadolinium enhancing lesions and T2 lesion volume showed little correlation with clinical scales, in particular if clinical variables were taken into account. We discuss the role of Gadolinium enhancing lesions as a predictor and potential surrogate for relapse rate and of T2 lesion volume as a potential predictor for disability. Our analysis of the widely used outcome measure “time to sustained progression” shows a remarkably large presence of noise in the data, in particular for patients with relapsing remitting disease. We also discuss the considerable heterogeneity between different trials, questions about the generalisability of the results and diversity of opinions about the need for validation.

Analysis of biases in natural history studies

G. Ebers; Radcliffe Infirmary (Oxford, UK)

There are limitations as to what can be established and characterised as the natural history of multiple sclerosis. Ideally, an inception cohort would be identified at onset and followed through until death but this duration exceeds the professional lifespan of the average investigator, much less the domiciliary constancy. Furthermore, the recent widespread use of treatments which might have some impact on natural history has further confounded the chronic problems of...
the disease ascertainment. Biases which are problematic in MS also include the same old ones, familiar to all students of chronic disease, compounded by the length of the MS process and the frequent opting for therapies. These include a variety of ascertainment biases which can occur at several levels. Given the long-term course of the disease and its overall impact this is completely understandable. Our own studies in London, Ontario have been carried over the last three decades. It has been possible to now collect information on the 30-year outcome in the original cohort of 1,044 MS patients. These studies have been used to identify the probability of transition from any given disability level to the next or any subsequent higher level and have the potential to generate a virtual placebo group for the trial planning and execution and for the evaluation of long-term effects therapy. However, the possibility that the natural history of the disease has changed cannot be dismissed. Evidence is strengthening, if not overwhelming, that the disease has increased in incidence and prevalence and there is no a priori reason to discount this change as an earmark of alternative pathogenic processes in a disease already suspected of heterogeneity.

96

Assessment of the predictive role of MRI measures of brain damage in patients at presentation with clinically isolated syndromes suggestive of multiple sclerosis: a multiparametric, large-scale, multicentre study

M.A. Rocca, F. Agosta, P. Tortorella, K.T.M. Fernando, D.H. Miller, A.J. Thompson, M. Tintore, A. Rovira, X. Montalban, T. Korteweg, C.H. Polman, F. Barkhof, M. Filippi; Scientific Institute San Raffaele (Milan, I); Institute of Neurology (London, UK); Hospital Vall d’Hebron (Barcelona, E); VU Medical Centre (Amsterdam, NL)

Background: Previous longitudinal magnetic resonance imaging (MRI) studies of patients at presentation with clinically isolated syndromes (CIS) suggestive of multiple sclerosis (MS) have identified several factors associated to an increased risk of subsequent evolution to clinically definite MS (CDMS). However, the potential of non-conventional MRI metrics as prognostic factors of disease evolution in CIS patients remains a matter of debate, since previous studies using such metrics were based on relatively small samples of patients.

Objectives: To define the relative contributions of lesion burden, normal-appearing white matter (NAWM) damage, and gray matter (GM) damage to tissue loss in patients with CIS and to identify non-conventional MR prognostic factors with the potential to predict CDMS.

Methods: Brain axial conventional and magnetization transfer images (MRI) were obtained from 163 CIS patients and 55 age- and sex-matched controls, recruited in 4 European Centers. Patients were assessed clinically at the time of MRI and after a median period of 3.2 years. Images were analysed centrally and the following measures derived: T2, T1 and gadolinium (Gd)-enhancing lesion volumes (LV), normalized brain volume (NBV), and NAWM and GM MTR histogram-derived metrics. A multivariate Cox model, adjusted for center, was used to define the predictive value MRI-derived metrics for time to CDMS.

Results: During the follow-up, 65 patients converted to CDMS (40%). At baseline, a significant inter-center heterogeneity was detected for Gd LV, the mean values of NBV and those of MTR histogram-derived metrics. Pooled average MTR and histogram peak height values did not differ between CIS patients and controls for NAWM and GM, although there was significant MTR abnormality in both NAWM and GM in the CIS cohort from the largest contributing centre. The final multivariable model retained baseline T2 LV (p = 0.001, odd ratio [OR] = 1.05, 95% confidence interval [CI] = 1.02–1.08), and Gd activity (p = 0.05, OR = 2.19, 95%CI = 1.29–3.72), as independent predictors of conversion to CDMS.

Conclusions: In patients at presentation with CIS, macroscopic focal lesions rather than “diffuse” tissue damage were related to subsequent development of CDMS. Evaluation of any relationship with disability will require longer follow-up. The study is limited by the retrospective post hoc analysis of MTR data which was acquired using multiple sequences and the inter-center heterogeneity of several MR measures.

97

A 50-year follow-up of survival and cause of death of multiple sclerosis in Hordaland county, Norway

N. Grytten, J. Aarseth, S.A. Lie, H. Nyland, K. Myhr; Haukeland University Hospital (Bergen, N); University Research (Bergen, N); University of Bergen (Bergen, N)

Background: Longitudinal follow-up of time trends in survival of MS from a geographical defined area may increase the power of identifying prognostic factors. Knowledge on the cause of death may alert clinicians to further improve treatment and survival.

Objectives: To analyse time trends in the survival of multiple sclerosis (MS) from 1953 to 2003, and to describe the underlying causes of death in MS patients in Hordaland County, Western Norway.

Methods: MS cases were identified by the patient files at the Departments of Neurology and Ophthalmology, Haukeland University Hospital, Bergen, Norway. The study population comprises all patients with onset in Hordaland County during 1953–2003 classified as definite and probable MS according to the Poser criteria. The end of follow-up was set to 1 January 2005 for cause-specific death according to the Norwegian Cause of Death Registry. Results: A total of 878 patients had onset in Hordaland County during 1953–2003. Of these, 198 (17.3%) were dead at follow-up date. The median survival time from onset was 43 years in women, and 36 years in men (p = 0.025). Among relapsing remitting MS patients median survival time was 43 years, and 26 years among primary progressive MS patients (p = 0.001). Comparing the periods 1953–1967, 1968–1982 and 1983–2002 a statistical significant improvement in 10-years survival after onset was observed in the latest period (p = 0.001). Major cause of death according to death certificates was MS (60.5%), cardiovascular diseases (14.1%), cancer (11.4%), infections and respiratory diseases (5.4%), accidents and suicide (2.7%), and other diseases (5.9%). Conclusions: The results indicate that MS patients may have longer survival of MS in Hordaland County, Western Norway than we expected from other studies [1]. Survival was significantly lower in patients with onset during 1953–1967 until it seemed to stabilise for patients with onset after 1968 (p = 0.001). The mortality rate for MS, cancer and infectious disease and cardiovascular diseases confirmed results from the literature [1], whereas the rate for accidents and suicide was lower. Results may reflect improvement in treatment and follow-up of MS and in better case finding due to improved diagnostic techniques.

Reference

98

Familial effects on the clinical course of multiple sclerosis

A.E. Hensiek, S.R. Seaman, L.F. Barcellos, A. Ottarat, M. Ekrakos, E. Cocco, L. Vesci, G. Steward, B. Dubois, J. Bellmann-Strobl, M. Leone, O. Andersen, K. Bencsik, D. Booth, E. Cellius, H.F. Harbo, S.L. Hauser, R. Heard, J. Hillert, K. Myhr, M.G. Marrosu, J. Oksenberg, C. Rajkai, S.J. Sawyer, P.S. Sørensen, F. Zipp, D.A.S. Compston; Cambridge University Clinical School (Cambridge, UK); University of London (London, UK); University of California (Berkley, USA); University of Copenhagen (Copenhagen, DK); University of Istanbul (Istanbul, TR); University of Cagliari (Sardinia, I); University of Szeged (Szeged, HUN); Westnord Millennium Institute (Sidney, AUS); University of Leuven (Leuven, B); Universitàetsklinikum Charite (Berlin, D); Ospedale Maggiore della Carità (Novara, I); Göteborg University Hospital (Göteborg, S); Umeå University Hospital (Olo, N); University of California at San Francisco (San Francisco, USA); Karolinska University Hospital (Stockholm, S); Haukeland University Hospital (Bergen, N)

Background: Familial factors influence susceptibility to multiple sclerosis but it is unknown whether there are additional effects on

www.sagepub.co.uk

Multiple Sclerosis 2006; 12: 51–528
the natural history of the disease. Method: We evaluated 1083 families with ≥2 first degree relatives affected by multiple sclerosis for concordance of age at onset, clinical course and disease severity and investigated transmission patterns of these clinical features in affected parent-child pairs. Results: There is concordance for age at onset for all families (correlation coefficient 0.14; p < 0.001), as well as for affected siblings (correlation coefficient 0.15; p < 0.001), and affected parent-child pairs (correlation coefficient 0.12; p = 0.03) when each is evaluated separately. Concordance for year of onset is present amongst affected siblings (correlation coefficient 0.18; p < 0.001) but not the parent-child group (correlation coefficient 0.08; p = 0.15). The clinical course is similar between siblings (kappa 0.12; p < 0.001) but not affected parents and their children (kappa 0.04; p = 0.09). This influence on the natural history is present in the clinical subgroups of relapsing-remitting, and primary and secondary progressive multiple sclerosis, reflecting a familial effect on episodic and progressive phases of the disease. There is no concordance for disease severity within any of the considered family groups (correlation coefficients: all families analyzed together, 0.02, p = 0.53; affected sibling group, 0.02, p = 0.61; affected parent-child group, 0.02, p = 0.69). Furthermore, there are no apparent transmission patterns of defined clinical features in affected parent C child pairs and no evidence for anticipation or effects of genetic loading. Conclusion: Familial factors do not affect eventual disease severity, but increase the probability of a progressive clinical course, either from onset or after a phase of relapsing remitting disease. The familial effect is more likely to reflect genetic than environmental conditions. The results are of relevance for the counselling of patients and have implications for the design of studies seeking to identify factors that influence the natural history of the disease.

Cross-linking pathological, radiological and clinical subgroups in multiple sclerosis: building a genetic bridge
T. Hooper-van Veen, H. Berkhof, Z. Bochdanovits, J.M. Nielsen, P Houthik, F. Barkhof, C. Verweij, L. Bo, I. Huizinga, C.H. Polman, B.M.J. Uitdehaag; VUMC (Amsterdam, NL); Netherlands Institute of Neurosciences (Amsterdam, NL)

Pathological, radiological and clinical characteristics in MS are highly variable between patients. We hypothesised that this may be associated with the genetic background of patients: different genetic profiles are associated with different pathological characteristics. These in turn will lead to different MRI features and are related to different clinical phenotypes. A total of 549 demyelinated white matter lesions in post-mortem brain samples of 98 patients with MS were scored for activity and perivascular infiltration (PVI), and 251 lesions were scored for axon density. Of the variation in these parameters, approximately 50% was within patients, and 50% between-patients. Using a cluster analysis, we observed three distinct patterns of lesions based on stage, PVI and axon density. Pattern 1 lesions had only 0–50% axons remaining, whereas pattern 2 lesions had more than 50% remaining axons. Pattern 3 lesions were active or chronic active and had PVI. Even though most patients had more than one pattern, these patterns were associated with onset type and with severity of disease, as measured by time to reach EDSS 6.0, age at EDSS 6.0, age at death, disease duration at death (Chiz 2, 3df, p < 0.01). In a genetic association study, we observed significant associations of CCR5 and CCL5 alleles with pathological (remyelination, PVI, and axon density in post-mortem samples from 92 deceased MS patients), radiological (T1 and T2 MRI lesion volumes in 192 living patients), and clinical (disease severity in 637 living patients) characteristics. In another study, we have developed a method to identify genetic subgroups of patients with MS using a Bayesian cluster analysis. In that study, genes involved in the immune response were analysed. Several of these genes were also associated with the lesion patterns described here (i.e. CCL5, IL1RN, IL12p40, FAS, APOE). We are now evaluating genes involved in inflammation and neurodegeneration. In a pathway that is key to survival of axons and oligodendrocytes in inflammatory lesions, we selected 8 genes and 48 SNPs to detect at least 85% of the common haplotypes. At the meeting, we will present the effect of these genes on pathological, MRI, and neurological measures of MS. With this approach that includes pathological, radiological and clinical data in the analysis of genetic factors, we hope to build a bridge to link these elements into a more integral view on the disease.

Clinical aspects: diagnosis and differential diagnosis - Part I

P107
A comparison of clinical outcomes of subjects receiving placebo in two similarly designed relapsing-remitting multiple sclerosis pivotal trials
M. Tulipan; Columbia University (New York, USA)

Background: Because there is paucity of randomized, prospective, blind, head-to-head data comparing the MS disease-modifying drugs (DMDs), there is a tendency to compare results across trials. If one were to compare the 2-year relapse rate reduction data across the natalizumab, interferon (IFN), and glatiramer acetate (GA) pivotal trials, one might conclude that natalizumab is at least twice as effective as the other agents. Similarly, when considering the 1-year relapse data, GA and IFN beta-1b and beta-1a (the Rebif® form) seem to have about 3–4 times the effect of IFN beta-1a (the Avonex® form), but only about half the effect of natalizumab. However, comparing across studies is problematic. Differences in study design, baseline characteristics, and the definition of outcome measures are some of the factors that should be considered. Objective: The GA and Rebif® RR MS pivotal trials were 2-year studies with similar design and inclusion criteria and provide an opportunity compare the results of subjects that received placebo. Methods: Demographic and baseline characteristics, relapse rate, and disability progression in subjects receiving placebo were compared across the GA and Rebif® pivotal trials. Results: Demographic and baseline characteristics in the 2 trials were similar (GA vs. Rebif®: female 76.2 vs. 75, mean number of relapses in the previous 2 years 2.9 vs. 3.0, mean baseline EDSS 2.4 vs. 2.4, age 34.3 (reported as a mean) vs. 34.6 (reported as a median)). The mean disease duration was 6.6 and 4.3 years for the placebo subjects in the GA and Rebif trials, respectively. Subjects receiving placebo in the GA trial had a mean of 1.68 relapses during the study while those receiving placebo in the Rebif® trial had 2.56 relapses. However, symptoms had to be present for at least 48 hours to be considered a relapse in the GA trial and only 24 hours in the Rebif® trial. Although sustained disability progression was defined the same way in the 2 studies, placebo subjects in the GA trial were less likely to have sustained disability progression compared with those that received placebo in the Rebif® study (24.6% vs. 38%; 35% relative reduction). Conclusion: Comparing well-matched subjects that received placebo in the GA and Rebif® RR MS pivotal trials revealed very different outcomes and highlights some of the problems of comparing results across studies. Well-designed, head-to-head studies are necessary to compare the efficacy of the MS DMDs.
P108
Outcomes of children with acute disseminated encephalomyelitis followed by recurrent optic neuritis
N. McLinskey, M. Milazzo, P. Sibony, W. MacAllister, D. Madigan, A. Belman, L. Krupp; SUNY Stony Brook (Stony Brook, USA)

Objective: To characterize clinical outcomes of children with acute disseminated encephalomyelitis (ADEM) followed by recurrent optic neuritis (ON). Methods: 7 children seen at the National Pediatric MS Center are described with the following criteria: 1 or more ADEM episodes followed by recurrent ON independent of the ADEM, or any other neurological event, and ≥ 1 month after steroid completion. ADEM and multiphasic ADEM met diagnostic criteria developed by the International pediatric MS study Group. Neurological and ophthalmologic findings were obtained from co-authors or from medical records from subsequent visits by other physicians. Longitudinal follow-up period was a median of 4.5 years (range 3 – 8).

Results: Monophasic ADEM developed in children at as young as 2 ½ and as old as 11 years. (median = 4). ADEM was monophasic (n = 6); or multiphasic (n = 1). Recurrent ON developed a median of 3 times (range 2 – 4); including bilateral episodes (n = 6); and unilateral episodes (n = 1). Only two patients met subsequent diagnostic criteria for neuromyelitis optica (NMO). Of the other 5 cases, no other neurological features have developed to date. Four of 7 patients have had good visual outcomes bilaterally, while 3 patients have had loss of light perception unilaterally. All patients maintain vision of 20/50 or better in at least one eye. MRI lesions associated with ADEM have resolved in all but one patient, who is now considered to have NMO. CSF testing was negative for OCBs in 6 of the 7 patients. NMO IgG antibody was positive in 1 out of 4 patients tested.

Conclusions: There are children with ADEM followed by discrete episodes of recurrent ON who do well and do not develop MS. Children with ADEM followed by recurrent ON should be tested for NMO-IgG antibody.

P109
A new classification system for multiple sclerosis
J. Herbert; NYU Hospital for Joint Diseases (New York, USA)

Background: Multiple sclerosis (MS) is generally categorized according to clinical phenotype, level of disability or pathological subtype. These classifications are of limited therapeutic utility because they do not accurately reflect disease severity. Identification of subpopulations based on disease severity would enable more homogeneous stratification for purposes of treatment and clinical trial design.

Objective: To develop a classification system for MS based on disease severity. Method: Global Multiple Sclerosis Severity Score (MSSS) is an algorithm described recently by Boxburgh et al. (Neurology, 2005; 64: 1144) relating Expanded Disability Status Scale (EDSS) scores to a distribution of disability in a reference patient population with comparable disease duration. Patients are ranked on a decile scale; MSSS scores thus represent prevalence of patients with particular disability/duration (D/D) coordinates in the reference population. For MS patient subpopulations, single point MSSS scores based on cross-sectional EDSS measurements after one year are representative of overall disease severity over time. To define MS subpopulations of varying severity, various D/D coordinates were applied to the MSSS algorithm.

Results: Six subpopulations were defined: Grade 1 (designated “Mild MS”)-MSSS below 17th percentile (1.7th decile); Grade 2 – MSSS between 17th – 50th percentile; Grade 3 – MSSS between 51st – 83rd percentile; Grade 4 (“Aggressive MS”) above the 83rd percentile. Grade 2 was further subdivided into 2A (“Moderate MS”) and 2B (“Intermediate MS”) at the 34th percentile, and Grade 3 into 3A (“Advanced MS”) and 3B (“Accelerated MS”) at the 67th percentile, thereby creating 6 equally distributed subgroups. Percentile boundaries represent D/D coordinates: 17th – EDSS<3.5/30 yrs; 34th – EDSS 3.0/20 yrs; 50th – EDSS 6.0/20 yrs; 67th – EDSS 6.0/11 yrs; 83rd – EDSS 6.0/7 yrs. Conclusion: A new MS clinical classification system is described, based on disease severity. The system utilizes the MSSS D/D algorithm based on single point EDSS measurements at least one year after symptom onset. Subtype boundaries correspond to recognizable D/D coordinates. Six subpopulations are defined, of equal prevalence in the reference MS population. This classification should contribute greatly to development of MS treatment guidelines and clinical trial design.

P110
Age, number of symptoms and relapse duration predict sequelae after the first relapses in relapsing-remitting multiple sclerosis
M.A. Leone, L. Collimedaglia, F. Tesser, S. Bonisisoni, S. Calzoni, P. Nahli, F. Monaco; Ospedale Maggiore della Carità (Novara, I); Università del Piemonte Orientale (Novara, I)

Background: Although relapse is the pivotal feature of relapsing-remitting multiple sclerosis (RR-MS), only few studies have outlined its clinical characteristics. Objective: To evaluate the factors predictive of non-recovery (sequelae) after the first relapses early in the natural history of RR-MS. Patients and methods: We recruited 71 consecutive patients from January 2001 to December 2003 with clinically isolated syndrome (CIS) or first diagnosis of RR-MS (McDonald criteria). Patients were clinically evaluated every 6 months and at every relapse, up to August 31, 2005. A relapse was defined according to modified Schumacher criteria. Relapses were divided according to the completeness of recovery (with or without sequelae). Symptoms were grouped to agree with the Kurtzke Functional System (FS). Relapse duration was calculated from onset to the date of maximum improvement. Other predictive factors (age, sex, season, speed of onset and of recovery, annualized relapse rate, infection in the preceding month, oligoclonal bands, Link index, number of lesions at MRI, therapy) were operationally defined and collected on a ad hoc prepared form. Results: During follow-up there were 208 relapses: 72 first, 52 second, 30 third, and 54 followings. Sequelae were observed in 71 relapses (34%). Duration was ≤ 30 days for 66 relapses (32%), 31 – 60 days for 77 (37%) and >60 days for 65 (31%). The probability of sequelae was higher for patients with age > 40 yrs (26/52, 50% vs. 45/156, 29%; p < 0.01), with 2 or more affected FSs (46/92, 50% vs. 25/116, 22%; p < 0.01), and with duration > 60 days (35/65, 54% vs. 36/143, 25%; p < 0.01). None of the other factors influenced the probability of complete recovery. Conclusion: Recognition of factors that predict the probability of non-recovery (sequelae) in the first relapses of MS may help to understand the prognosis of the disease and may drive the choice of treatment.

P111
Complex partial status epilepticus as presenting symptoms of multiple sclerosis
A. Toscano, M.C. Fazio, R. Di leo, V. Dattola, A. Blanolino, C. Messina, F. Girlanda; University of Messina (Messina, I)

It is well known that the prevalence of epilepsy in MS patients is higher than in the general population. Complex partial status epilepticus may cause confusional episodes or of impairment of memory or of cognitive functions. This clinical condition has never been reported as presenting feature of MS. We report a case 43-years-old man, admitted to our department because of recurrent episodes of confusional state with anterograde amnesia and retrograde memory failure for personal events. Two months earlier, he developed subtle behavioral changes with tendency to apathy and indifference and a progressive cognitive impairment, including a relevant deficit of memory. At neurological examination he appeared confused, disoriented with a slurred speech and evidence of Epstein’s sign. On the Mini-Mental State Examination he scored 15/30. He also underwent neuropsychological evaluation, which demonstrated...
a marked impairment of all examined cognitive domains: general dilapitation of cognitive processes including language, attention, concentration, cognitive flexibility and abstraction. Blood tests exploring liver and thyroid function, B12 and folate levels, serologic tests for syphilis and HIV did not reveal any significant alteration. Molecular analysis for CADASIL and Prion-Related-Protein were negative. Electrocencephalogram (EEG) recording showed almost continuous sharp waves localized on the bilateral posterior temporal regions with mild left side predominance; the electric activity was also altered for presence of theta waves on all derivations, slightly prevailing on left temporal derivations. Brain MRI revealed a pattern of lesion consistent with MS, according to Barkhof criteria. Cerebral spinal fluid examination showed presence of oligoclonal bands. Visual evoked potentials showed prolonged P100 latency bilaterally, indicating subclinical optic nerve lesions. The patient received the diagnosis of Multiple Sclerosis. He was treated with i.v. diazepam that interrupted the continuous EEG abnormal activity. After 36 hours therapy with carbazepine (400 mg TID), topiramate (150 mg BID) and levetiracetam (1000 mg TID) was started with progressive normalisation of EEG and marked improvement of neuropsychological aspects. To our knowledge, this is the first reported case of Complex partial status epilepticus in a patient with MS.

**P112**

Relapsing encephalopathy in a multiple sclerosis patient with unfavourable outcome (another aspect of Hashimoto encephalopathy?)

N. Yuceyar, I. Aydogdu, A. Akgun, A. Kocaman; Ege University Medical School (Izmir, TR)

Hashimoto’s encephalopathy (HE) is an underdiagnosed immune mediated, steroid-responsive encephalopathy presenting with subacute onset of episodic confusional states, with focal or generalized seizure, myoclonus, tremor and ataxia in patients usually with euthyroidism or subclinical hypothyroidism. We here report a 61-year-old female patient with HE who has been followed up with the diagnosis MS for twenty years. Episodes of focal and generalized seizure preceded or followed by confusion, psychosis with visual hallucinations, paranoid ideations was ultimately diagnosed as HE on the basis of high antithyroid antibody in cerebrospinal fluid (CSF) and sera, low thyroid hormone levels, steroid responsiveness. No cause for encephalopathy was found after extensive investigations, repeated during each relapse, for vascular, toxic, infectious and paraneoplastic origin. Ultrasonographic and scintigraphic findings were also compatible with HT. On her neurological exam, disorientation, agitation, postural tremor in hands, myoclonic jerks were also observed beside her sequela findings attributed to MS. Serial EEG revealed focal or generalized slowing. Although cranial MRI with gadolinium enhancement showed no new lesion other than nonehancing chronic multiple sclerosis plaques, brain SPECT revealed hypoperfusion on bilateral frontal and left frontaloparietal region. She showed gradually improvement after each episode by either pulse methylprednisolone or intravenous immunoglobulin (IVIG) concomitant with EEG recovering. During her follow-up period of two years duration, she had at least five relapses although she was on prednisone, azathioprine, levothyroxine and antiepileptic treatment. Between the attacks, she was free of symptom or seizure except for some cognitive decline. On her last relapse, although her seizure was ceased, her comatose status could not be resolved with plasmapheresis followed by IVIG and we lost her. HE can be seen in association with other autoimmune disorders like rheumatoid arthritis and systemic lupus erythematosus. This is the first MS case with the diagnosis of HE except for a patient who developed HE on interferon therapy. Our aim is to favor the awareness of clinicians on this underdiagnosed steroid responsive encephalopathy in MS. Although it is a treatable condition, our case’s unfavorable outcome can be another aspect of HE.

**P113**

Differential diagnosis of multiple sclerosis and connective tissue diseases according to serological tests and cerebrospinal fluid analysis

C. Jacobi, M. Korporal, B. Wildemann; University of Heidelberg (Heidelberg, D)

Multiple sclerosis (MS) is the most common autoimmune disease of the central nervous system (CNS). Nervous system manifestations including demyelinating syndromes can occur in various connective tissue diseases (CTD), such as systemic lupus erythematoses and Sjogren Syndrome. In these diseases the serological detection of pathological antinuclear antibody titers (ANA) is a powerful tool, however, around 25% of MS patients are also ANA positive. In this study we aimed to optimize the differential diagnosis of MS and CTD by combined assessment of ANA, extractable nuclear antibodies (ENA) and antibodies against double stranded-DNA (ds-DNA abs). We additionally determined the oligoclonal polyspecific immune response and measured intrathecally synthesized antibodies against measles (M), rubella (R), Varizella-Zoster-Virus (Z). The prevalence of the MRZ-reaction is around 90% in MS and unknown in CTD. ANA in serum were measured by immunofluorescence assay (IFA) and ENA (SSA, SSB, Scl-70, Sm, mRNP) by immunoblot. ds-DNA abs were determined by ELISA. Routine CSF analysis included cell count, cytology, albumin, IgG, IgA, IgM and oligoclonal bands. MRZ-antibodies were measured by ELISA and an antibody index (AI) > 1.4 indicated intrathecal synthesis. Serological tests (ANA, ENA, ds-DNA abs) were performed in 60 patients with definite MS before treatment and in 26 patients suffering from CTD with CNS-symptoms. The oligoclonal polyspecific immune response was tested in 20 MS and 25 CTD patients. Serological ANA titer was pathological (> 1:80) in 23% of MS patients and 96% of CTD patients. With an ANA-cut off > 1:160 only two MS patients remained positive. The dual detection of ANA and ENA or ANA and ds-DNA abs was successful in 73% of CTD patients and in none of the MS patients. 17 of 20 MS patients (85%) and 7 of 25 CTD patients (28%) had a positive MRZ-reaction. These findings demonstrate that in MS patients ANA titers are low and ENA reactivities are usually not detectable, whereas high ANA titers with positive ENA or anti-ds-DNA abs are common in CTD with CNS symptoms. The MRZ-reaction is more frequent in MS, but does not appear to discriminate unequivocally the two disorders.

**P114**

Two cases with clinically isolated conus medullaris syndrome

S. Ozkbas, E. Idiman, O. Ozkan; Dokuz Eylul University (Izmir, TR)

Although sacral cord or conus medullaris lesions and bowel and bladder disturbances are not rare, demyelinating lesions that responsible for these lesions are rare. We presented two definite MS patients who have clinical signs of isolated conus medullaris involvement. First patient was a 40 year old male, who complain of numbness in genital area and toes for 1 week. The patient was complaining of straining difficulty and impotence, and he had no sign of other systems. In the brain MRI, there were demyelinating plaques in cerebral periventricular white matter and focal hyperintense lesion in distal conus medullaris without clear limits. Oligoclonal banding was positive in CSF. In somatosensory evoked potential, there was a conduction defect in the right leg. His symptoms partially reduced in a few months. The other case was a 26 year old man, complaining numbness in his genital area for 1 month and in his legs for the last 4 months that becomes violent from time to time. In neurological examination, he had a symmetrical sensory deficit in perineal area. Cerebral and spinal MRI was performed. There were hyperintense lesions in the brain and cervicothoracic area. Some of them were gadolinium-enhanced. There was also a hyperintense lesion that has no clear limits in conus medullaris. There were conduction defects in left median and bilateral fibular somatosensory evoked potentials.
Oligoclonal banding was positive in CSF. He had nearly complete improvement after 1 g/day pulse methyl-prednisolone therapy for 5 days. These two cases were presented as having a very rare isolated entity, conus medullaris involvement in MS.

P115
Clinic neurophthalmologic, electrophysiologic and imaging methods in optic neuritis diagnosis
F. Idiman, S. Otrakbas, S. Men, Ö. Özkân, B. Ugurel, E. Idiman; Dokuz Eylül University (İzmir, TR)

Optic neuritis that characterized with demyelination and focal inflammation in optic nerve can be an isolated disease or a finding of MS. It can be a sign at the onset of MS or it can be seen firstly with any attack after years. In 70% of idiopathic acute monocular optic neuritis cases, a lot of silent demyelinating lesions can be established in MRI. In other words, most of the isolated optic neuritis cases are possibly MS patients or can have a risk for MS development. We studied and followed 72 cases with optic neuritis and we had their neuroophthalmologic profiles, VEP examinations, cranial and orbital MRIs. We wanted to evaluate the abnormality rates of clinical and/or electrophysiologic examinations and radiological images, their contributions to diagnosis, their sensitivity and specificity for diagnosis. All patients were complaining of vision loss. The abnormality rate of their neuro-ophthalmologic examination (visual acuity, color vision, papillae and their light reactions, visual field tests, fundoscopic findings) was 95.8%. The abnormality rate of VEP examinations was 91.7%, cranial MRI was 91.6%, and orbital MRI was 44.4%. In 40% of the patients, clinical examinations and all the laboratory studies were abnormal. As seen the highest abnormality was in clinical neuroophthalmologic examination, and among the laboratory studies, the highest abnormality was in electrophysiological studies. We concluded that the most sensitive test was VEP, the most specific test is orbital MRI and cranial MRI contributes to the diagnosis and evaluation of the risk for MS.

P116
Multiple sclerosis and markers of autoimmunity
A. Angelou, T. Afantou, O. Kanta, R. Lagoudaki, E. Stavriliou, E. Sitropoulou, M. Paschalidou, I. Milonas; Aristotle University (Thessaloniki, GR)

Introduction: In Multiple Sclerosis, the autoimmune mechanism is exclusively turning against the central nervous system. On the other hand several MS patients may display immunologial abnormalities in the peripheral blood resembling to those observed in connective tissue diseases. The majority of studies revealed that the prevalence of autoantibodies (Abs) is higher in patients suffering from MS compared to the general population. Objectives: To determine the prevalence of ANA, ACA, AMA, APCA, ASMA, anti-DNA, ANCA, ACL and ENA screen in inpatients with MS and to attempt an evaluation of their significance. Methods: 98 patients with definite MS were admitted to our clinic due to acute deterioration of their neurological state. 39 of them experienced their first relapse of MS while the rest (n = 58) of the group studied had been previously diagnosed and the immunological testing was performed during their new relapse. All patients had a low EDSS score and the blood samples were collected before the administration of corticosteroids. Results: MS patients at onset: ANA ≥1:80 (15.4%), AMA (+) (2.5%), ASMA (+) (18%), APCA (+) (2.5%), ANCA (+) (0%), anti-DNA (+) (0%), ENA screen (+) (0%), IgG ACL ≥15U/ml MPL (0%), IgM ACL ≥7U/ml MPL (25.6%). MS patients at exacerbation: ANA ≥1:80 (27.6%), AMA (+) (1.7%), ASMA (+) (15.5%), APCA (+) (1.7%), ANCA (+) (1.7%), anti-DNA (+) (1.7%), ENA screen (+) (0%), IgG ACL ≥15U/ml MPL (3.5%), IgM ACL ≥7U/ml MPL (24.1%). Conclusions: The data of our study showed a remarkably higher prevalence of Abs in MS patients in comparison to the general population. ANA were detected in a significantly larger proportion of patients with MS that were already subjected to a number of relapses although their clinical state was not markedly impaired. This finding suggests a dynamic immunological dysfunction, which gets intensified in phases of exacerbation. The interpretation of these observations differs between studies. Sometimes high titers of Abs are related to atypical MS and others are considered as an epiphenomenon. Still the possible role of systemic autoimmunity markers in MS remains uncertain, and many think that their presence is pathologically irrelevant or that their pathogenic function is not defined yet. Even though, most clinicians stand sceptically towards the usefulness of their detection and periodically evaluation in patients with definite MS, our study showed that ANA could indicate the underlying autoimmune activity, while the disease progresses.

P117
Consensus definitions of acquired CNS demyelinating disorders of childhood
L. Krupp, W. MacAllister on behalf of the International Pediatric MS Study Group

Background/Goals: The International Pediatric MS Study Group, created by the National Multiple Sclerosis Society, met to address the inconsistency in the terminology of pediatric CNS demyelinating disorders. The goal of this meeting was to establish operational definitions of acquired CNS demyelinating disorders of childhood.

Methods: An agreed upon set of uniform operational definitions was developed, including the following disorders of childhood: acute disseminated encephalomyelitis (ADEM), clinically isolated syndromes (CIS), neuromyelitis optica (NMO), and multiple sclerosis (MS). The main features of the definitions are presented.

Results: Three forms of ADEM are identified: ADEM, Recurrent ADEM, and Multiphasic ADEM. All forms must include encephalopathy, multifocal neurological signs, lack of alternative explanations, and brain MRI showing large predominantly white matter lesions. A single episode of ADEM may last up to three months, even if new symptoms develop during that period. In Recurrent ADEM, the second episode involves the same areas of clinical and radiological localization. In contrast, Multiphasic ADEM involves new areas. The manifestations of CNS dysfunction in either Recurrent or Multiphasic ADEM must develop at least one month after discontinuation of steroids. CIS is distinguished from ADEM by the absence of encephalopathy. Patients with CIS can have either normal or abnormal MRI findings. NMO requires optic neuritis and transverse myelitis, may include brain lesions, and incorporates NMO antibody results as per the modified 2005 criteria. Pediatric MS is defined according to McDonald’s criteria, but there is no lower age limit. Discussion: An international panel of experts has proposed operational definitions that distinguish ADEM, Recurrent ADEM, Multiphasic ADEM, CIS, NMO and MS in childhood. These definitions must be tested with prospective research and will likely need to be modified accordingly. Nonetheless, they represent a starting point for better understanding acquired CNS demyelinating disorders of childhood.

P118
Adult onset of vanishing white matter-like leukoencephalopathy with autosomal dominant inheritance and ovarian failure
C. Lutjohann, A. Fogli, G. Castelnovo, O. Boespflug-Tanguy, D. Rodriguez; CHU de Nimes (Nîmes, F); INSERM UMR 384 (Clermont Ferrand, F); CHU de Clermont Ferrand (Clermont Ferrand, F); U 546 (Paris, F)

Objectives: To describe two patients with clinical, neuroradiological and neuropathological criteria of vanishing white matter (VWM/ CACH) and atypical phenotypes.

Background: Leukoencephalopathy with vanishing white matter syndrome (VWM/CACH) is an autosomal recessive disorder, characterized by the occurrence of acute
episodes of deterioration following minor head trauma or infection, and symmetrical demyelination on MR with cavitation aspects. Mutations in each of the 5 subunits of eIF2B have been identified (eIF2B1 to 5). **Results:** Case 1. A 48-year-old woman presented a cerebellar ataxia and loss of cognitive functions. Mini-Mental State Exam Score was 21 of 30. Clinical examination found a tetraparamidal syndrome. Age of first menarche was in the normal range. Anomorpha occurred at age 52. MR showed diffuse and severe abnormalities of the hemispheric cerebral, cerebellar and brain stem white matter with a low signal intensity on T1W images and a high signal intensity on T2W images, mild dilated ventricles, without any mass effect or abnormal contrast enhancement. On proton density images, deep hemispheric white matter had a low signal intensity, close to the CSF signal, suggesting cavitating white matter degeneration. Mutations were found in eIF2B genes (eIF2BS). Case 2. A 37-year-old man presented three episodes of coma following upper respiratory tract infection episodes. He presented chronic cerebellar ataxia, spinchter troubles and loss of cognitive function (MMS: 21/30). MR found symmetrical demyelination on MR with cavitation aspects Histological findings of his mother were typical of VWM/CACH syndrome. No mutation in the five genes of eIF2B (EIF2B1 to EIF2BS) was found in the index case. **Conclusions:** CACH syndrome can appear at adult onset. They can be associated with adult onset, ovarian failure and dominant inheritance.

**P119**  
**Autoimmune myelopathy associated with CRMP-5-IgG**  
M. Keegan, S. Pittack, V. Lennon; Mayo Clinic (Rochester, USA)

Myelopathies are heterogeneous in aetiology and are increasingly recognized to have an antigen-specific autoimmune basis (e.g., attributable to autoimmunity directed at aquaporin-4, GAD65, or amphiphysin). CRMP-5-IgG is the most commonly recognized neuronal (nuclear or cytoplasmic) autoantibodies associated with cancer, typically small-cell lung carcinoma (SCLC) or thymoma Neurological accompaniments of CRMP-5-IgG are usually multifocal (Yu et al. Ann Neurol 2001), but optic neuropathy (Cross et al. Ann Neurrol 2003) and basal ganglionitis (Vernino et al. Ann Neurorol 2002) are considered syndromic manifestations. Here we describe myelopathy as a syn-dromic manifestation of CRMP-5 autoimmunity. Data were reviewed for 21 patients seen in the Mayo MS Clinic in whom CRMP-5-IgG was detected in the course of serological evaluation for myelopathy. All 21 patients (11 female) presented with myelopathy as the principal or sole neurological impairment. The median presentation age was 54.6 years (32–70 years). Sixteen (76.2%) were current or former smokers. Twelve (57.1%) had progressive myelopathy, 5 (23.8%) had a single acute myelopathy, and 4 (19%) had a relapsing myelopathy. Nine patients (42.9%) had multifocal symptoms and signs: motor neuroopa-thy (3), sensorimotor neuropathy (3), encephalopathy (2), optic neuropathy (2), and chorea (1). Cerebrospinal fluid abnormalities, identified in 17 patients (80.9%), included pleocytosis, supernumerary oligoclonal bands, elevated protein or elevated IgG index. In a median follow-up of 0.7 years, SCLC was detected in 9 patients (42.9%). Eight patients had one or more coexisting autoantibodies: voltage-gated calcium channel (3), gangliosic acetylcho-line receptor (3), GAD65 (2), NMO-IgG (1), ANNA-1 (1), or ANNA-3 (1). Immunosuppressive treatment was initiated in 8 and was deemed beneficial in 3 (37.5%). Autoimmune myelopathy is often progressive and warrants consideration of immunosuppressant therapy. The patient’s autoantibody profile is an important guide to the diagnosis of malignancy.

**P120**  
**Clinically isolated syndrome: subtle changes at neurological examination at follow-up predict conversion to multiple sclerosis**  
P. Vermersch, L. Bellenger, H. Zéphir, J. de Seze; University of Lille II (Lille, F); G-SEP (Lille, F); CHU de Strasbourg (Strasbourg, F)

Background: In patients with a clinically isolated syndrome (CIS), high numbers of magnetic resonance imaging (MRI) lesions and the presence of oligoclonal bands in the CSF are predictive factors of conversion to multiple sclerosis (MS). Clinical characteristics are poorly associated with conversion to MS. **Objective:** To determine if subtle changes at neurological examination predict early conversion to clinically definite MS (CDMS). Methods: Patients presenting with CIS suggestive of MS were included in a prospective study. All patients were assessed clinically at baseline, 3 months later and every 6 months. Brain and spinal MRI in case of spinal cord presentation were performed at inclusion. CSF analysis was performed in most of the patients. The presence of some subtle changes during the follow-up was systematically noticed in the medical record: decrease of vibration at least in one limb, loss of the gag reflex, asymmetry of tendon reflexes or a Babinski sign, nystagmus, disappearance of cutaneous reflexes, Lhermitte’s symptom. Number of patients with conversion to CDMS was considered. **Results:** Seventy-one patients, 45 females and 26 males, were included with a mean age of 31 years. Presenting symptoms were optic neuritis (n = 16), spinal cord (n = 24), brainstem syndromes (16) or multifocal (n = 15). The mean follow-up was 39 months. CDMS was diagnosed in 41 patients (57.7%). Median conversion time was 13.8 months. The clinical examination showed the presence of at least one subtle clinical change at the follow-up visits in 28 and in 8 cases in the group of patients who will convert to CDMS and in the group of patients who will not convert to CDMS respectively (p < 0.01). The risk of conversion to CDMS was higher in patients with oligoclonal bands (p < 0.05) but not in patients with baseline MRI fulfilling the Barkhof criteria. **Conclusion:** Subtle clinical changes at neurological examination are predictive factors of conversion to MS. Prospective clinical follow-up of the patients after a CIS is important.

**P121**  
**Anti-thyroid antibodies in multiple sclerosis**  
O. Eknekci, N. Tauyar, A. Kocaman, M. Karadeniz; Ege University (Izmir, TR)

**Background:** Thyroid dysfunction and anti-thyroid antibodies have been reported in patients with MS and during interferon beta therapy. **Objective:** The aim of this study was to determine frequency of anti-thyroid antibodies and autoimmun thyroid disorders in patients with multiple sclerosis. **Methods:** Seventy-nine patients with clinically definite MS were included (61 females and 18 males). The mean age of the MS group was 40.5. The control group had been selected from patients with the diagnosis of headache, back pain and without inflammatory neurologic disease. Twenty patients were included in control group with a mean age 37.5 (12 females and 8 males). In all patients, serum samples were analysed for TSH and free thyroid hormone levels (FT3 and FT4). Anti-thyroid antibodies such as antithyroglobulin antibodies (TG Ab), anti-thyroid peroxidase antibody (TPO Ab) were also measured. All patients with high anti-thyroid antibodies were evaluated by ultrasound and thyroid scintigraphy. **Results:** High levels of anti-thyroid antibodies were found in 18 patients with MS (%22). Eight of them had Hashimoto’s disease and 10 patients had only high level antibodies. Three patients with Hashimoto’s disease and 7 patients with positive levels of anti-thyroid antibodies had undertaken treatments with interferon beta before diagnosis of thyroiditis. **Conclusion:** The results suggested a correlation between autoimmune thyroid disease and MS as interferon treatment.
whether they are predictors of future progression. This work was based on 547 clinically and laboratory supported definite MS patients diagnosed and followed regularly by the same person in County Fejér from 1980–2004. A cohort of 280 patients diagnosed from 1990–2001- corresponding to 2166 patient year- was separately analysed. 

**Results:** The average exacerbations rate in yearly diagnosed cohorts was 1.21 in the year of the diagnosis followed by 0.47 in the second and 0.31 in the 3rd year and 0.24 after 12 years. The yearly average exacerbation’s rate in yearly cohorts from 1990–2001 varied from 0.32 – 0.5. The average exacerbation’s rate in yearly diagnosed cohorts decreased in the second year and a further decrease was seen in the third year except the years 1991, 1996 and 1999, where the rate in the third year was a little higher compared to the second year. Out of 547 cases 321 had 2, 3 or 4 exacerbations from the diagnosis until the end of 2003. The average interval between exacerbations in the group with 2 exacerbations was 38.7 month, in the group with 3 it was 56.5 and in the group with 4 was 38.9 month. In those having 3 or 4 exacerbations the interval between the 3rd and 4th exacerbation was less than between the first and second. Those who have fewer months between the first and second exacerbation had a faster progression (10.6 month for those having 3 exacerbations in 1 – 3 years versus 51.71 month for those of 6 – 9 years intervals). It seems that the interval between the first and second exacerbation determines the interval between later exacerbations and therefore the progression of the disease. In the group of patients having 2 exacerbations the average time interval was 5.43/18.53/40.42 month for those having the second exacerbation within 1/2 /3–4 years. The longest interval between the 1st and 2nd exacerbation was in the age group at onset 10–20 years (51.7) and was 28 month at 20 –30 of age, 46.3 month at age 30–40 and 32.3 month at age 40–50. 

**Conclusions:** The average exacerbation’s rate in yearly diagnosed cohorts dropped rapidly in the years following the diagnosis. The time interval between the exacerbations in patients having 2 – 4 exacerbations was determined by the interval between the first and second exacerbation. Older age at first exacerbation shortened the time to get the second one.

**P123**

**Accidental diagnosis of multiple sclerosis – clinical and ethical implications**

H. Roshansaf, S. Fredrikson; Karolinska University Hospital Huddinge (Stockholm, S)

The more frequent use of magnetic resonance imaging (MRI) of the brain of various reasons (trauma, headache etc) may show unexpected abnormalities, including findings compatible with multiple sclerosis (MS). The radiological “en passant”, findings may cause clinical and ethical dilemmas in a person without symptoms indicative of MS. We describe four such patients encountered at our centre over the last year. 

**Case 1:** A 31 year old female was applying to a police academy and needed a complete health examination. The patient had no subjective symptoms and reported good previous health. Examination revealed unilateral hearing loss. MRI of brain showed a pontine lesion. CSF analysis showed oligoclonal bands (OB). Six months later the patient developed sensory disturbances in both legs. 

**Case 2:** A 23 year old female, previously healthy, was found confused with seizures. A neuroinflammatory lesion on MRI was suspected and CSF showed only one band. She was discharged from hospital with a diagnosis of encephalopathy. She had no other neurological focal symptoms or signs. Two years later she had a new attack of confusion and sensory symptoms. A new MRI scan of the brain showed lesions compatible with MS. CSF showed OB. Later, the patient has developed incoordination and fatigue. 

**Case 3:** A 33 year old female was biking through central Stockholm and got hit by a car at a traffic light. She was transferred to Emergency Room and complained of having headache but no other neurological symptoms. An acute CT of the brain showed signs of white matter lesions. MRI of the brain showed two large lesions, of which one was contrast-enhancing. CSF analysis showed OB. Later, sensory symptoms and Lhermitte parestesiae have developed. 

**Case 4:** A 37 year old female was applying to get a driving license. She reported no neurological symptoms. A compulsory health examination including testing of visual acuity and visual fields revealed a right sided defect of her visual fields. MRI of the brain showed more than 20 white matter lesions. CSF analysis showed OB. 

In summary, “accidental” diagnosis of subclinical MS is not uncommon and may have psychological consequences for the patient. Practical and ethical implications of such an unexpected (subclinical) diagnosis will be presented and discussed. A systematic study of the occurrence of subclinical MS is warranted since the number of “accidentally” diagnosed cases are expected to increase with further spread of MRI scanning.

**Subclinical multiple sclerosis-how long does it remain asymptomatic?**

A. Siva, A. Altintas, S. Saip, H. Uygugüll, E. Karasulun, S. Albayram, N. Kocer, I. Çivam; Istanbul University (Istanbul, TR); VKV American Hospital (Istanbul, TR)

**Objective:** To study prospectively Subclinical Multiple Sclerosis (MS) cases diagnosed by magnetic resonance imaging (MRI).

**Background:** Subclinical-MS is defined when clinically silent disease is diagnosed by chance, either at autopsy or through neuroimaging (MRI). The increasing use of MRI in various neurological problems and other causes may reveal cases of subclinical MS, whose long term clinical behaviour is unknown. 

**Design/Methods:** Seven (3 women) cases, who were admitted to our MS Clinic because of an incidental MRI diagnosis of MS were included in this study. All were asymptomatic for MS and had a cranial MRI done because of causes unrelated to MS (such as primary headaches). Initial MRI studies were evaluated independently by two neuroradiologists blinded to the presentation of the cases, who confirmed that MRIs were highly suggestive of MS in all and fulfilling Fazekas MRI Criteria. The mean age at the time of the initial MRI was: 38.28± 17.34. The total follow-up time after the initial MRI was: 57.28 ±42.4 months. All study cases were followed regularly, both clinically and by MRI. 

**Results:** Three of the cases (two men) developed neurological symptoms and signs suggestive of MS during their follow-up period along with new MRI lesions, and therefore converted to clinical MS. The mean time for conversion to clinical MS after the initial MRI study was: 40.33 ± 21.12 months. The total follow-up time after the initial MRI in cases who converted and who didn’t convert to clinical MS were: 58.25 ± 54.62 and 56 ± 30.26 months respectively. All, but one of the cases continued to develop new MRI lesions (some enhancing), consistent with MS, independently from conversion to MS. 

**Conclusions:** Subclinical-MS diagnosed by MRI, may remain asymptomatic for many years or a lifetime, despite continuing MRI activity. This observation is of importance in understanding the disease behavior and in making long term treatment decisions.

**P125**

**Primary progressive multiple sclerosis: a comparative study of the diagnostic criteria**

J. de Seze, M. Debouverie, N. Wauquier, G. Steinmetz, S. Pittion, H. Zephir, M. Fleury, F. Blanc, P. Vemmersch; CHU de Strasbourg (Strasbourg, F); CHU de Nancy (Nancy, F); CHU de Lille (Lille, F)

In approximately 85% of cases, the onset of multiple sclerosis (MS) is marked by relapses and a relapsing –remitting course. In the remaining cases, the course of the disease is progressive (PPMS) from the onset and disability accumulates over time. Successively 3 set of criteria for PPMS have been proposed by several panel of expert (Thompson et al., 2000; McDonald et al., 2001; Polmann et al., 2005), lesions, or four to eight brain MRI lesions with positive visual evoked potential (VEP). To date, no study has focused specifically on the application of the different criteria for PPMS including the new
proposed criteria. In the present study we retrospectively assessed the different sets of criteria for PPMS in order to determine their sensitivity in a non research, clinical setting. We retrospectively reviewed the records of 5419 MS patients followed-up in three MS centers, 695 (12.8%) were classified as PPMS. Of these PPMS patients, 261 had had at least a one-year disease progression and all the necessary tests to apply the different diagnosis criteria, including brain and spinal cord MRI, VEP and CSF analysis, and were included in the study. Applying the Thompson criteria, 168 patients (64.4%) had definite PPMS, 84 patients (32.2%) had probable PPMS and 9 patients had possible PPMS (3.4%). Applying the McDonald criteria, 180 patients (69%) had PPMS. Applying the revised McDonald criteria, 194 patients (74.3%) had PPMS: 71 patients (27.2%) had three criteria, a subgroup of patients where CSF analysis could be considered unnecessary, 103 patients (47.1%) had two criteria, 64 patients (24.5%) had one criterion and three patients had no criteria. The last two groups were classified as not PPMS. Our study demonstrates that the revised McDonald criteria are more sensitive than the previous McDonald criteria as it is now possible to include patients with normal CSF in the diagnosis of PPMS if they have an imaging work-up highly suggestive of MS. However, McDonald criteria are less sensitive than the Thompson criteria. There is now a need for a prospective study of a cohort in order to confirm these data and to assess the specificity of each set of criteria.

P126

Acute demyelinating encephalomyelitis: a cohort study of 60 patients

J. de Seze, M. Debouverie, H. Zephir, C. Lebrun, F. Blanc, V. Bourg, S. Wiertlewski, D. Laplaud, E. Le Page, R. Deschamps, P. Cabre, J. Pelletier, I. Malikova, P. Clavelou, V. Jaillon, G. Defer, P. Labauge, O. Gout, C. Boulay, G. Edan, P. Vennersich; CHU de Strasbourg (Strasbourg, F); CHU de Nancy (Nancy, F); CHU de Lille (Lille, F); CHU de Picardie (Amiens, F); CHU de Languedoc (Montpellier, F); CHU de Toulouse (Toulouse, F); CHU de Poitiers (Poitiers, F); CHU de Nantes (Nantes, F); CHU de Rennes (Rennes, F); Fondation Rothschild (Paris, F); CHU de Poitou Charente (Poitou Charente, F); CHU de Marseille (Marseille, F); CHU de Clermont-Ferrand (Clermont-Ferrand, F); CHU de Caen (Caen, F); CHU de Nimes (Nimes, F); CHU de Mulhouse (Mulhouse, F).

Acute demyelinating encephalomyelitis (ADEM) is characterized by a severe inflammatory attack frequently secondary to infectious events or vaccinations. To date there is no clear criteria for ADEM rather for the first clinical event than for the risk of an evolution through multiple sclerosis. The aim of this study was first to define the spectrum of ADEM in a large multicentric cohort then to found predictive criteria for an evolution through clinically definite multiple sclerosis. We retrospectively studied 60 patients without any previous history suggesting inflammatory event. We excluded patients under 15 years. Clinical presentation were considered atypical when we observed aphasia, hemiplegia, seizure or confusion. All patients had a CSF analysis and a brain MRI. Spinal cord MRI was performed in 43 patients. After a mean time follow-up of 3.4 years, patients were classified into 3 subgroups: monophasic ADEM (MDEM) (n = 6), monophasic ADEM (n = 35) and multiple sclerosis (n = 19). We only performed statistical analysis for the 2 last subgroups. We did not found any differences concerning demographical data. ADEM had more frequently atypical symptoms (74.3%) than multiple sclerosis (42.1%) (p = 0.018). Oligoclonal bands (OCB) were more frequently observed in multiple sclerosis (84.2%) compared with ADEM (20%) (p < 0.001). ADEM had more frequently grey matter involvement (basal ganglia or cortical lesion) (60%) than multiple sclerosis (10.5%) (p < 0.001) and less frequent corpus callosum involvement (22.3% versus 78.9%, p < 0.001). If we considered at least 2 of the 3 following criteria for the distinction between ADEM and multiple sclerosis: atypical clinical symptoms, OCB or grey matter involvement, 29 of the 35 patients (82.9%) had monophasic ADEM, and 18 of 19 patients (94.7%) with multiple sclerosis were classified in the appropriate category. This study underlined several differences concerning the risk of an evolution through clinically definite MS after a ADEM.

We propose differential criteria whom appear discriminant. These criteria should be tested in a larger prospective cohort.

P127

Diagnosis and management of patients experiencing a clinically isolated syndrome suggestive of CNS demyelinating disease

K. Selmaj, S. Bohlega, M. Chofflon, S. Ghebely, R. Gouader, E. Havrada, D. Karassiss, A. Miller, H. Pakdaman, B. Singhul, L. Vécsey; Medical University of Lodz (Lodz, PL); King Faisal Specialist Hospital and Research Centre (Riyadh, SA); Hôpital Cantonal de Genève (Geneva, CH); Lebanese Hospital Geitawi (Beirut, LBN); Razi Hospital (Tunis, TN); University Hospital (Praga, CZ); Hadassah Hebrew University Hospital (Jerusalem, IL); Carmel Medical Center (Haifa, IL); Beheshte University (Tehran, IR); Medical Research Centre (Mumbai, IND); University of Szeged (Szeged, HUN)

Following the introduction of new criteria for multiple sclerosis (MS) diagnosis (McDonald et al., 2001; revised in 2005), earlier diagnosis of the disease is feasible, even after a single demyelinating event. However, management of these patients with early symptoms suggestive of MS is still controversial. Patients experiencing a first clinically isolated syndrome (CIS) suggestive of central nervous system demyelinating disease are at risk of developing MS (up to 85% may eventually convert to clinically definite MS). Guidance for managing these patients is not yet available, although there is evidence that early therapeutic intervention is beneficial in MS. With this aim, a group of MS experts have developed an algorithm for the management of patients presenting with CIS. Once a patient is diagnosed with CIS, magnetic resonance imaging (MRI) of the brain and spinal cord should be performed to look for evidence of lesions. About one third of patients with CIS have a normal MRI at baseline, and these patients should undergo clinical and MRI follow up, ideally every 3 months. There is evidence to suggest that some patients in this group may be at higher risk for conversion to MS than others (e.g. those with motor, cerebellar, or brainstem symptoms/signs; positivity for, or presence of, oligoclonal antibodies in the cerebrospinal fluid [CSF]; or pathological evoked potentials [EPs]). Of the patients with CIS and abnormal MRI, a number of different factors may indicate increased risk of conversion to MS including fulfillment of the revised Barkhof criteria (1997; revised in 2000) and multifocal presentation or signs of brain atrophy. Patients who meet the revised Barkhof criteria or show positive CSF or visual EP results should be eligible for disease-modifying drug therapy if they fulfill any of the following conditions: motor, cerebellar or brainstem involvement, multifocal presentation, poor recovery after the first demyelinating event, active MRI (> 9 T2 lesions, ≥ 1 Gd-enhancing lesion or signs of early axonal damage), or confluent lesions. Patients who do not meet these criteria should undergo clinical and MRI follow up, ideally every 3 months. These guidelines will help physicians to manage patients presenting with CIS, with the aim of delaying conversion to MS and maximizing the benefit of therapeutic intervention.

P128

Clinical and magnetic resonance imaging findings of siblings from a Turkish family with mother diagnosed as multiple sclerosis: case report

O. Aylan, R. Soyin, M. Kayan, T. Tombul, O. Ural; Ataturk's Hospital (Ankara, TR); Veznecilik Ul University (Van, TR).

Background: Subclinical demyelinating lesions may occur in brains of the first-degree relatives of multiple sclerosis (MS) patients. The high rate of consanguineous marriages in Turkey makes it an interesting population in the context of genetic susceptibility to MS. We report the clinical and cranial magnetic resonance imaging (MRI) findings of six siblings from a Turkish family with mother diagnosed as MS. Methods: Neurological examination and cranial MRI exami-
inations were performed in four daughters and two sons from a family with mother clinically diagnosed with definite MS. The mother and father are second-degree relatives. Results: Neurological examination was normal in all siblings except one daughter who had left hemihypesthesia and loss of joint position sense. MRI findings were abnormal in all individuals. There were multiple foci of demyelinating sub cortical and periventricular lesions in all of them. Conclusions: Some reports provide detailed neurologic as well as neuroradiological findings on members of MS families. However diffuse white matter lesions in individuals of MS families may occur, it can not find a history of underlying medical illness of demyelination. Our results suggest that foci of demyelination might be expected in clinically normal offspring of parents with MS, possibly reflecting a genetic predisposition to subsequent development of MS. Clinical follow up and MRI studies of the first-degree relatives when classifying them as healthy are sometimes important.

P129
Clinical, laboratory, MRI, and electrophysiologic findings in acute transverse myelitis: comparison between patients with and without multiple sclerosis
S.H. Hwang, S.B. Kwon, S. Jung, K.H. Kwon, B.C. Lee; Kangnam Sacred Heart Hospital (Seoul, KOR)

Background: Acute transverse myelitis (ATM) is a pathogenetically heterogeneous inflammatory disorder affecting the spinal cord at one or more segments. Therefore, the identification of discriminatory findings providing clues of the underlying etiologies is needed. Moreover, there is paucity of comparison studies correlating the MRI and electrophysiologic changes in ATM with and without multiple sclerosis (MS). In Korea, the spinal cord is the most commonly affected site of MS and its clinical features often resemble those of ATM. As the diagnoses of these two conditions are different, it is important to distinguish them from each other. The purpose of this study was to evaluate discriminatory findings that could help differentiate ATM from MS. Methods: We analysed the clinical, imaging, electrophysiologic, laboratory findings and outcome profiles in myelitis with and without MS. Results: We identified 70 patients, and compared non-MS-related ATM (ATM, n = 52) to myelitis associated with MS (ATM-MS, n = 18). The ATM patients were significantly older than ATM-MS patients at the time of the diagnosis (p < 0.05). A motor weakness was more frequent in ATM than in ATM-MS where symptoms were predominantly sensory (p < 0.05). Spinal cord MRI revealed monosegmental involvement of spinal cord was more frequent in ATM-MS, in contrast to ATM, where lesions involved two or more vertebral levels (p < 0.05). CSF oligoclonal bands were frequent in ATM-MS but not statistically significant. Abnormalities (assessed by summation of number of abnormal studies) of neurophysiological tests including visual evoked potential (VEP), brain stem auditory evoked potential (BAEP) and median and tibial somatosensory evoked potentials (SEP) are more frequent in ATM-MS (p < 0.05). Clinical outcomes defined on the basis of three months modified Rankin scale was better in ATM-MS than in ATM (p < 0.05). Conclusion: ATM and ATM-MS may be differentiated on the basis of clinical, length of vertebral involvement on MRI, electrophysiological and outcome data, and these findings may provide therapeutic implications for patients with myelitis.

P130
The natural history of primary progressive multiple sclerosis in Lorraine, eastern France
M. Debouverie, S. Pittion-Voovitch on behalf of the Lorsep Group

Background: Primary progressive multiple sclerosis (PPMS) has a distinct progression phenotype. Only few longitudinal natural history studies on PPMS have been carried out. In the current studies, we examined the patient characteristics, disease progression, and associated risk factors in the PPMS population of Lorraine, France. Method: The natural history of the PPMS population in Lorraine, France was assessed using the LORSEP (Lorraine Multiple Sclerosis) cohort of definite MS. The main parameters examined were the time to progression from onset to Expanded Disability Status Scale (EDSS) scores of 4, 6, and 7 and the time between assignment of EDSS scores of 6 and 7. Risk factors for progression including sex, age of onset and symptoms at onset were also examined, and progression time to EDSS 7 based on progression time to EDSS 6 studied. Results: Of the 2871 patients with definite multiple sclerosis in the original study, 359 (12.5%) had PPMS. The mean duration of disease was 13.6 years (range, 1.7 to 53.6 years), the median age at onset was 41.7 years (25%[Q1] = 34.7, 75%[Q3] = 50.0), and the male/female ratio was 1/1.36. The median time to EDSS scores of 4, 6 and 7 were (in years) 5.0 (95% CI = 2.8 to 3.7), 9.9 (95% CI = 9.0 to 10.6), and 17.0 (95% CI = 14.9 to 19.0). Five years after onset, 25% required a cane, and after 15 years, this increased to 75%. It was not possible to predict the time to EDSS scores of 6 or 7 or the time between assignment of scores of 6 and 7 based on the sex, age at onset, or symptoms at onset.

P131
To assess the prevalence of Sjogren’s syndrome in patients with multiple sclerosis in Korea
H.J. Kim, J.H. Min, M.J. Kim, J.J. Sung, K.W. Lee; Seoul National University Hospital (Seoul, KOR)

Background & objective: Sjogren’s syndrome (SS) may be considered in the differential diagnosis of multiple sclerosis (MS). SS is a chronic inflammatory disease characterized by keratoconjunctivitis sicca, xerostomia, and other clinical manifestations. Conclusions: We performed a cross-sectional study assessing the prevalence of SS in patients with MS in Korea. Method: We retrospectively studied 60 patients with multiple sclerosis as defined by recent criteria. Clinical laboratory, and MRI findings in the these patients. For the SS screening, we used the revised European criteria. The criteria was used to study 11 patients the complained of xerophthalmia and xerostomia. Xerostomia was evaluated clinically and by Scirmer test without local anesthesia. Xerostomia was evaluated clinically and by salivary scintigraphy. We also performed minor salivary gland biopsy in 5 patients the complained of sicca complex (xerophthalmia and xerostomia). The presence of Ro(SS-A) and La(SS-B) antibodies was also evaluated. We considered patients meeting four or more criteria as having definite SS. Results: Five patients met criteria to be diagnosed with SS (at least four criteria). All patients were women, aged with 28 to 55 years symptom onset. This prevalence is higher than in the general population and implies that SS can mimic MS. Conclusion: Our study shows that out of 60 consecutive patients with MS, 5 patients had a diagnosis of SS. This rate is slightly higher than the usually accepted prevalence of SS in the general population. So we propose that screening for SS should be considered in all patients with MS.
with clinical and electromyographical findings typical of chronic inflammatory demyelinating polyradiculopathy (CIDP) have been described. **Methods:** We reported the clinical, radiological, biological, neuropathological and outcome data of 1 woman and 4 men having both relapsing CNS and peripheral nervous system (PNS) demyelination disease, different from typical MS and CIDP. **Results:** They all have a relapsing disease course of MS over time where PNS involvement seem to be secondary to the CNS involvement. They all fulfilled Barkhöf’s criteria for spatial dissemination on magnetic resonance imagery (MRI), and McDonald’s criteria for MS. No one had oligoclonal bands in CSF, and only one had transient pleocytosis. Two of them had typical white matter changes and also grey matter lesions. No multisystemic inflammatory disease and no metabolic or immunological factor for peripheral neuropathy was found. On electromyography, they all fulfilled Nicolas et al’s criteria, and 4 of them fulfilled the more stringent Ad Hoc’s criteria of 1991 for CIDP. Electromyography showed no conduction block but a homogeneous demyelinating peripheral neuropathy. Nerve biopsy was performed in 2 patients; histological data were in favor of CIDP. Improvement of the clinical status and the neuropathological parameters were observed in 3 patients after IgG IV (n = 1) or cyclophosphamide (n = 2). **Discussion:** In all patients the occurrence of PNS and CNS demyelination, the lack of oligoclonal bands, the diffuse homogeneous peripheral demyelination without conduction block highlight pathogenic mechanisms different from MS and CIDP. The chronology of the clinical events suggest a peculiar entity different from what occur in acute demyelinating encephalomyelitis (ADEM). These cases suggest that immunological reactivity against antigens common to peripheral and central myelin may explain the demyelinating disease of both CNS and PNS. However, these antigens had to be determined.

**P133 Neurosarcoidosis in a well-established definite multiple sclerosis: a case report**


We report an exceptional case of neurosarcoidosis developing more than 15 years after onset of MS. An Algerian woman was first seen in 1990 for a history of progressive walking disturbances, upper limb weakness, acroparesthesia and bladder dysfunction for several years. There was no superimposed relapse. Biological work-up was negative for general inflammation, viral infections and auto-antibodies. CSF showed pleocytosis (12 plasma cells/mm³), elevated IgG Index (1.60) and oligoclonal bands. VEP were altered in both eyes. Brain MRI was suggestive of MS, with typical ovoid lesions, predominantly in the periventricular regions but also in the brainstem. She was diagnosed as primary progressive MS and received no specific immunomodulating treatment. She returned in 2005 for therapeutic advice, because of a subacute deterioration since April 2004. MS relapse was then diagnosed and treated with IV methylprednisolone. MRI showed one gadolinium enhancing lesion diagnosed by the radiologist as a new MS lesion. Her state deteriorated further in 2005 and her neurologist questioned the need for chemotherapy with cyclophosphamide or mitoxantrone. Because of the unusual clinical presentation and aspect of the scan of 2004, a new brain MRI was performed. It revealed a voluminous pseudotumoral lesion, with mass effect on the lateral ventricle, a large zone of oedema, with intense and heterogeneous enhancement. There was no biological sign of inflammation, but chronic lymphopenia. CSF analysis gave similar results. A stereotactic biopsy showed granulomatous lesions, with several lymphohistiocytoid granuloma, without caseiform necrosis. Pathology was suggestive of neurosarcoidosis. She had bilateral hilar adenopathies on thoracic CT scan, also compatible with sarcoidosis, but fibroscopy failed to confirm the diagnosis. Ocular fundoscopy showed sequela of possible iritis. Complementary investigations showed no hypercalcemia and normal angiotensin conversion enzyme level. The patient received IV methylprednisolone, followed by oral prednisone 1 mg/kg/day with a progressive tapering over more than 1 year. Improvement was rapid and continuous, both clinically and on successive MRI scans. This case report stands out in many ways. It leads to question the previous diagnosis of MS in the setting of this new diagnosis of neurosarcoidosis. It emphasizes the worth of searching for an alternative cause in case of an unusual clinical evolution or MRI aspect.

**P134 Spinal multiple sclerosis in Taiwan: a cross-sectional retrospective study**

C.P. Tsai, C.Y. Liu, K.H. Chang, L.S. Ro; Taipei Veterans General Hospital (Taipei, TW); Chang Gung Memorial Hospital (Taipei, TW)

Spinal multiple sclerosis (MS) has been reported in western populations. Like opticospinal MS, the concept of spinal MS as a distinct entity or as a subtype of MS has not been well defined. Addressing the clinical courses of spinal MS would help to delineate the whole feature of idiopathic inflammatory demyelinating diseases. This study aims to assess the clinical characteristics and response to therapy of spinal MS. A cross-sectional retrospective review of patients with MS or with myelitis presenting with restrictive and recurrent symptoms on spinal cords, recruited during Jan 92 and Dec 01 in 2 medical centers in Taiwan, was performed. Patients with optic neuritis were excluded from the study. Retrospective review was performed on 18 patients (4 men/14 women) with spinal MS. The ages at onset were 45 ± 6.3 years for men and 34.1 ± 11.4 years for women. 2 to 8 episodes of paresis attack were encountered within 5 to 14 years of follow-up. At the first attack, 5 patients experienced quadriparesis, another 5 encountered paraparesis, and the remaining 4 had monoparesis. All the motor and sensory symptoms that occurred in the beginning were asymmetrical, and involved more than 2 segments of spinal cord (21/27, 77.8%) (5.1 ± 3.8 segments). During the follow-up, affected spinal levels can accumulate to as many as 16 segments (5.9 ± 4.5 segments). Those having higher CSF white blood cells (WBC) count showed lower immunoglobulin gamma (IgG) index (p = 0.167). On the other hand, those who presented with higher IgG index (> 0.69) have less accumulative cord lesions (p = 0.044). Visual evoked potentials were abnormal in 2 patients; both of them encountered 7 attacks, with mean annual relapsing rate of 1.17 and 0.66 respectively, higher than the other 16 patients (p = 0.031). For their treatment, 13 patients received corticosteroids in the acute stage and 3 patients underwent subsequent interferon-beta therapy. Spinal MS is similar to that of optociphal spinal MS in Taiwan in various aspects such as age at onset, percentage of female patients, and annual relapse rate. Patients with primary progressive courses were not seen in this study. Higher CSF cell count and lower IgG index in 2 patients may imply 2 disease entities. For patients who presented with detrimental courses initially, early diagnosis and treatment with interferon-beta should be emphasized. Further investigation need to be done to assess the association of CSF WBC count, IgG index and interferon-beta with spinal MS.

**P135 PEDIAS: pilot multicentre observational study aiming to standardise diagnosis of an inflammatory neurological event leading patient to consult for the first time**

O. Gout, T. Moreau, S. Allouche, M. Debouverie on behalf of the Club Francophone de la Sclerose en Plaques

**Background:** Multiple sclerosis (MS) is the most common cause of neurological disability in young adults with a wide distribution within European countries, ranging from 1/1000 to 1/800 inhabitants in 2004 with 50,000 to 70,000 patients in France. Great variety of MS clinical expression delays diagnosis. Previous studies showed that first consultation is mostly preceded by undetected inflammatory events, such as optic neuritis, isolated brainstorm or spinal cord syndromes. More-
over 60% to 80% of patients with clinical isolated syndrome (CIS) suggestive of MS and brain lesions at magnetic resonance imaging (MRI) will develop clinically definite MS (CDMS). These observations emphasize importance of early recognition of inflammatory process, and early therapeutic management. Objectives: PEDIAS was designed to standardize diagnosis of a first event of inflammatory demyelination (FEID) and suggestive antecedents of MS. Percentage of real CIS or CDMS will be evaluated. Methods: PEDIAS was designed to enrol in 14 neurological reference French hospitals, 170 patients (about 8.5% of 2,000 new cases of MS diagnosed in France yearly), over 18, hospitalized or consulting for a FEID, and to document environmental and familial factors likely to impact disease management. Two visits were scheduled. At initial visit (IV), patient was provided with a self-reported questionnaire. At the next planned final visit (FV), patient returned it, neurologist reviewed data and completed neurologist questionnaire. Patient questionnaire included demographic, sociographic features, neurological relevant history with regard to prior symptoms lasting more than 24 hours and familial neurological history. Neurologist questionnaire specified onset of disease flare-up, supposed neurological lesion localisation, Expanded Disability Status Scale (EDSS) score, available complementary investigations (MRI results, Barkhof Criteria evaluation, and Cerebrospinal Liquid analysis). Neurologist confirmation of previous relevant inflammatory events permits to conclude to CDMS diagnosis in some patients. Results and Conclusions: A total of 191 patients were recruited in 12 months by 32 active investigators in 21 wards. So far, data from 114 patients are available for both IV and FV to be analyzed. Study is ongoing and results will be provided when available.

P136

Allergic reactions and the risk of multiple sclerosis
L. Bolokadze; Kharkov Medical University (Kharkov, UKR)

Background: It is unclear whether allergic diseases are associated with multiple sclerosis (MS), but histamine 1 receptor blockers, used in the treatment of allergic conditions, decreased the severity of experimental autoimmune encephalomyelitis (an animal model of MS). Objective: To assess the association of allergy history and use of histamine 1 receptor blockers with the risk of MS. Methods: Using a case-control study nested in the Kharkov Medical Center of Multiple Sclerosis, we identified 99 incident cases of MS with at least 5 years of follow-up before their first symptoms (index date). Up to 10 controls matched to the cases by age, sex, general practice, and time in the cohort were selected. Previous history of allergic disease and use of histamine 1 receptor blockers in the 5 years before the index date were assessed through computerized medical records. Results: History of any allergic condition in the 5 years before the index date was not associated with MS risk (adjusted odds ratio [OR] 1.3, 94% CI 0.9 to 1.9). However, use of sedating histamine 1 receptor blockers was associated with decreased MS risk (adjusted OR 0.3, 94% CI 0.2 to 0.8). Conclusion: These results do not support a strong link between allergic conditions and multiple sclerosis (MS) risk but suggest a possible beneficial effect of antihistamines on the onset of MS.

P137

Chronic inflammatory demyelinating polyneuropathy developed in a patient with multiple sclerosis treated with interferon-beta-1b
B.J. Kim, S.B. Koh, K.W. Park, D.H. Lee, C.N. Lee; Korea University Medical Center (Seoul, KOR)

Some of the cell and humorally mediated immune responses that contribute to the development of chronic inflammatory demyelinating polyneuropathy (CIDP) resemble those implicated in multiple sclerosis (MS). Because of these pathogenic similarities between the two diseases, preliminary studies have evaluated the effects of interferon beta formulations in the treatment of CIDP. However, the efficacy of Interferon, which has been widely used for relapsing-remitting MS (RMS), is controversial in the cases of the patients with CIDP. We report here a 31 year old woman with relapsing-remitting type MS treated with IFN beta-1b over 2 years who developed overt CIDP. On neurologic examination, all tendon reflexes were decreased. Electrophysiologic study revealed demyelinating type motor-sensory polyneuropathy. She responded favorably to steroid. This case suggests that IFN-beta-1b treatment may not prevent development of CIDP.

P138

The association of celiac disease with multiple sclerosis
R. Abolfazli, A. Mirbaghi, M. Rabhani, H. Pakdaman; Tehran University of Medical Science (Tehran, IR); Shahid Beheshti University of Medical Science (Tehran, IR)

Multiple sclerosis (MS) & celiac disease (CD) are both considered immune-mediated diseases. Improved screening for antibodies associated with CD (i.e., anti-gliadin[AGA], anti-endomysial, and anti-tissue transglutaminase) has improved the detection of CD in recent years. Numerous neurologic conditions have a reported association with established CD. Previous researchers have investigated the role of a gluten-free diet in the treatment of MS & found no benefits. We have investigated the association of CD with MS. Methods: Using ELISA, we estimated serum IgG & IgA anti-gliadin & IgA anti-endomysial antibodies in 34 MS patients (29 females), which were new cases or previous cases without immunosuppression treatment at least for the last 6 months. (mean age 29.6 years, range 15–46 years, 30 RR MS & 4 SP MS). None of the patients had specific symptoms suggestive of CD, any suggestive family history, or features suggestive of malabsorption. 34 random anonymous blood donors (17 female) were used as serologic controls (mean age 31.4 years, range 19–50 years). The individuals in MS group & in control group with anti-gliadin antibody (IgG or IgA) above the normal range underwent duodenal biopsy. Results: In MS group, high levels of IgG Agra were found in 2 of 34(5.9%), just like IgA Agra (5.9%) & in controls IgG Agra was detected in 5.9%and IgA Agra in 2.9%, (p-value = 1.00 and 0.6). So there were no significant differences between IgG and IgA Agra levels in patient and control groups. IgA anti-endomysial was not found in any of the patients, similar to the controls. After biopsy, only in 2 patients of MS group there were mild villi changes with chronic inflammatory cells but the specific pathological features of celiac were absent. The other biopsies were normal. Normal values of IgA anti-endomysial in MS group were higher than controls (1.66 versus 1.04, p-value = 0.041). Conclusions: 11.8% (5.9% IgG, 5.9% IgA) of our MS patients and 8.8% (5.9% IgG, 2.9% IgA) of our blood donor controls had high levels of Agra, with normal IgA anti-endomysial which is more specific for CD, while the GI biopsies in both groups were not specific for celiac. Therefore AGA in any neurologic case should be interpreted with caution status. Present study showed there is no association between MS & CD.

P139

A comparison of interferon beta treatment outcomes in patients with CIS: BENEFIT, CHAMPS and ETOMS
W. Cendrowski; Neurological Out-Patients Clinic (Warsaw, PL)

Background: Strong ration argues for using interferon beta (IFN beta) in patients with clinically isolated syndromes (CIS) who are at risk of conversion into clinically definite multiple sclerosis (CD MS). Objective: To compare preventive effects of Betaferon® (BENEFIT study), Avonex® (CHAMPS study) and Rebif® (ETOMS study) in CIS patients showing the risk of CDMS development. Patients and Method: Three cohorts of 468, 383 and 262 patients with CIS were compared in this meta-analysis. Results: Smaller proportions (28%, 21%, 34%) of CIS patients progressed into CDMS after 2 years in the Betaferon® group (875 μg weekly), in the Avonex® group (30 μg per week).
week) and in the Rebif® group (22 µg once a week) as compared to placebo groups (45%, 35%, 45%); p between 0.047 and 0.00007. The mean number of days to CDMS development was prolonged by 3 IFNs placebo groups (45%, 35%, 45%); p between 0.047 and 0.00007. Mean or median number of new enhancing (Gd+) lesions was significantly reduced in the Betaseron® group and the Avonex® group (0 and 0.4) as compared to the placebo groups (2.0 and 1.4). Reduction was not the case in the Rebif® cohort and in control cohort in the ETOMS study (0.5 vs. 0); p = 0.809.

Conclusion: Despite clinical differences at onset and at initial MRI indices this comparison proved that 3 products of IFN beta partially prevented or delayed conversion of CIS into CDMS and hampered evolution of new T2-related and enhancing brain lesions.

P140

Clinically isolated syndromes: practical evaluation of multiple sclerosis risk and therapeutic options
W. Cendrowski; Neurological Out-Patients Clinic (Warsaw, PL)

Background: Clinically isolated syndromes (CIS) suggestive of multiple sclerosis (MS) may be classified to a number of subtypes. Therapeutic options in these subtypes are unlike and disputable.

Objective: To present classification of CIS subtypes and therapeutic choices depending on clinical features, MRI indices and oligoclonal bands (OB) in the CSF. Patients and Method: Conversion risk of CIS into clinically definite MS (CDMS) was analyzed in 6 series of 276 patients within 4.62 years (range 0.91 –14.08 years). Classification of CIS included 6 subtypes: 1) mild attack with recovery, normal brain MRI, absent OB, 2) light attack with improvement, 1 T2-related lesion, negative OB, 3) moderate attack with incomplete recovery, 2 T2-lesions or 1 contrast enhancing lesion (CELI), positive OB, 4) severe attack, 3–8 T2 lesions or 1 CEL, brain atrophy, present OB, 5) mild attack and some scores of T2 brain lesions (≥9), 6) severe attack with single subventral lesion. Results: Risk of CDMS in the 1st subtype is minimal (9%) and rational choice is to wait until the second relapse. Probability of CDMS development in the 2nd and the 6th subtype is small (17%). Control MRI and complementary diagnostics are strongly indicated. Methylprednisolone (MP) in the 1st and the 2nd subtype is not recommended. Risk of CDMS in the 3rd, 4th and 5th subtype is moderate (41%) or high (62%). MP and subsequently interferon beta have to be considered in these subtypes. Early planned therapy is therapeutic option in the 4th and 6th subtype resistant to MP treatment. Conclusion: Various options for delaying or initiating MP and immunomodulating therapy may be used in CIS subtypes depending on disability, clinical course, brain MRI pathology and presence of OB in the CSF.

P141

8-year follow-up of rehabilitation outcomes in secondary progressive multiple sclerosis
W. Cendrowski, A. Kwolek, E. Wieliczko; Neurological Out-Patients Clinic (Warsaw, PL); Institute of Physiotherapy-University of Rzeszów (Warsaw, PL)

Background: Long-term follow-up study is required to establish whether the benefit of rehabilitation in secondary progressive multiple sclerosis (SP MS) is maintained over a significant period of time.

Objective: To assess 8-year impact of multidisciplinary inpatient rehabilitation on disability (DS) level in SP MS patients. Patients and method: Forty patients (22 females, 18 males) with SP MS have been included into this study. Mean age of patients was 53.3 ± 10.2 years and mean duration of the disease 22.8 ± 8.90 years. Patients have undergone on average 3 monthly cycles of inpatient and multidisciplinary rehabilitation. Disability level was evaluated at Kurtzke’s scale and disability progression ratio (DPR) was calculated as a quotient of DS points divided by months of MS duration. Results: Prior to the first rehabilitation cycle DS score was 5.47 ± 1.34 pts. and after last course it slightly improved to 5.28 ± 1.27 pts. (Wilcoxon test, p = 0.001). At 8-year follow-up there was evident worsening of DS level in all patients and 3 of them died (7%). DS score significantly increased to 7.09 ± 0.96 pts. (p = 0.0001). Mean DPR per month during cumulative rehabilitation period was lower than in the course of post-rehabilitation period: –0.062 ± 0.63 vs. +0.19 ± 0.14; p = 0.0001. Conclusion: Multidisciplinary inpatient rehabilitation temporarily slowed DS progression in SP MS. This benefit declined over time, reinforcing the need for continuity of symptomatic and neuroprotective therapy.

P142

Communicating the diagnosis of multiple sclerosis – A qualitative study
A. Solari, N. Acquarone, E. Pucci, V. Martinelli, M.G. Marrosu, M. Trojano, C. Borreani, M. Messmer Uccelli; C. Besta National Neurological Institute (Milan, I); Organisation & Development Department (Genova, I); Hospital of Macerata (Macerata, I); Scientific Institute and University Ospedale San Raffaele (Milan, I); University of Cagliari (Cagliari, I); University of Bari (Bari, I); Istituto Nazionale per la Cura dei Tumori (Milan, I); Italian Multiple Sclerosis Society (Genova, I)

Background: Studies on communicating a diagnosis of multiple sclerosis (MS) are few, and all reveal communication and information deficits. Objective: To explore personal experience of the communication of the MS diagnosis from the perspective of people with MS and health professionals. Design: Qualitative study of data obtained in two sets of focus group (FG) meetings with people with MS, and one FG meeting with health professionals. Setting: One patient FG involved people from northern Italy; the other involved people from central and southern Italy. The health professional FG recruited participants from all three areas of Italy. Participants: Twenty-three people with MS (16 women; age range 23 –70) and nine health professionals (four neurologists, three psychologists, and two nurses) took part. Analysis: Methods of framework analysis were applied to meetings transcripts to identify key topics and categories. Results: The experience of communicating/receiving a diagnosis of MS was varied. Very poor levels of support and information provision were reported, particularly for diagnoses given less recently. It was generally agreed, however, that the process of communicating the diagnosis had improved in recent years. It was felt that there was a need to improve diagnosis delivery (in terms of personalization, setting and continuity). Conclusions: Effective communication of the diagnosis of MS is of paramount importance to people with MS, and also health professionals. Improving the quality of the diagnostic encounter requires more than a meaningful patient-neurologist relationship, but also structural and organizational changes. This study was supported by the US National MS Society (Grant No. PP 1201 to AS).

P143

Spinal cord dysfunction suggestive of clinically isolated syndrome: prognostic marker for conversion to multiple sclerosis. A prospective study
M. Martinez Gines, Y. El Bedel, A. Esquivel, J.A. Gazmán de Villoria, J. Romero, C. de Andrés; Hospital Gregorio Marañón (Madrid, E)

Introduction: Approximately 85% of patients with multiple sclerosis (MS) initially present with a clinically isolated syndrome (CIS) and 40% of them develop clinically definite multiple sclerosis (CDMS) after 3 years. CIS was defined as the clinical onset of neurological
MS symptoms

P144

Lack of correlation between vestibular or ocular motor symptoms and signs in patients with multiple sclerosis

C.B. Pereira, A.M.K. Kanashiro, D. Callegaro; Faculdade de Medicina USP (São Paulo, BR)

Introduction: Patients with MS exhibit a variety of vestibular and ocular motor syndromes. This deficits may be an evidence of a relapse, of a greater disability and sometimes of another disease such as benign paroxysmal positional vertigo (BPPV). The aim of this study was to evaluate the clinical correlation of vestibular and ocular motor symptoms and signs in patients with MS.

Patients and Methods: Twenty consecutive MS patients with symptoms or signs of lesion of the vestibular or ocular motor systems were analyzed. Vertigo and diplopia were considered as symptoms of vestibular and ocular motor systems disorders, respectively. Disequilibrium was considered only when associated with vertigo. Abnormal vestibular or ocular motor signs were: ophthalmoplegia, skew deviation, abnormal saccades, smooth pursuit or optokinetic nystagmus and presence of spontaneous, gaze-evoked, head-shaking or positional nystagmus.

Results: Patients age ranged from 19 to 53 years old (media = 36) and there were 12 women. Ten patients had vertigo or diplopia and correspondent abnormal vestibular or ocular motor signs. Four patients had episodic vertigo and a normal examination. One of these had symptoms suggestive of BPPV that was not confirmed. Six patients showed no correlation between signs and symptoms: two complained of constant vertigo with normal examination, one complained of constant vertigo and had a bilateral abducens paresis (from a previous relapse) and three had abnormal signs without symptoms. No vertigo neither diplopia had a bilateral abducens paresis (from a previous relapse) and three with normal examination, one complained of constant vertigo and correspondent abnormal vestibular or ocular motor signs were: ophthalmoplegia, skew deviation, abnormal saccades, smooth pursuit or optokinetic nystagmus and presence of spontaneous, gaze-evoked, head-shaking or positional nystagmus.

Conclusion: Vestibular and ocular motor abnormal signs and symptoms are common in MS patients, however as many as 50% of MS patients in this study had no correlation between symptoms and abnormal signs. Among 20 patients, 30% had either constant symptoms with normal neurological examination, or abnormal signs without correlating symptoms. Further, 20% of patients had episodic vertigo without abnormal signs and one of these had symptoms suggestive of BPPV. Abnormal ocular motor or vestibular signs without symptoms may help identifying subclinical lesions, they may also be present as a sequela of a previous relapse, and may be associated with a greater general disability. On the other hand, some symptoms without abnormal signs may occur in other situations like BPPV. Therefore, this kind of symptoms and deficits may be considered with caution in patients with MS.

P145

Plantar pressure distribution in multiple sclerosis patients depend on pyramidal and cerebellar systems impairment

I. Nikiforova, O. Iva, A. Petrov, A. Ivce, L. Petrov, T. Yivetkova, I. Stolyarov; Institute of Human Brain RAS (St.Petersburg, RUS)

Introduction: Gait problems in patients with multiple sclerosis (MS) are caused by muscle weakness, spasticity, loss of balance, sensory deficit and other factors. The aim of this study is to determine correlation between pressure distribution parameters and pyramidal system (PS) and cerebellar system (CS) impairments.

Material and Methods: 81 patients: 26 men and 55 women (age 37 ± 10 years, body mass index (BMI) 23 ± 4 kg/m²), with relapsing-remitting MS according to McDonald’s criteria, were examined several times. Neurological status of examined patients is characterized with Expanded Disability Status Scale (EDSS) 2.2 ± 0.2, Pyramidal Function Score (PSF) 2.0 ± 0.3, Cerebellar Function Score (CFS) 1.8 ± 0.7. PSF was estimated as 0-no pathology, 1-signs without disability, 2-minimal disability, 3-moderate paraparesis or hemiparesis, 4-marked paraparesis or hemiparesis. CFS was estimated as 0-no pathology, 1-signs, 2-mild ataxia, 3-severe ataxia, 4-severe ataxia. Plantar pressure distributions were performed with emed-AT 25 system (novel, Munich, Germany). Five dynamic records of each foot were made with first step protocol. novel database medical was used to collect clinical and pressure measurement data. Peak pressure (PP), mean pressure (MP), maximum force (MF), pressure-time integrals (FTI), force-time integrals (FTI), contact time (CT) were calculated in novel-projects with novel automask for hindfoot (HF), midfoot (MF), five metatarsal heads (MH1-MH5), big toe (T1), second toe (T2), and lateral toes (T3-H). Parameters were calculated for each subject. The relationship between PSF, CFS and pressure distribution parameters under foot areas was checked with Pearson’s correlation coefficient (CC). Significance level: p < 0.01. Results and Conclusion: CC between PSF and CFS is equal to 0.639. Increase of contact time for total object and under all foot areas (except lateral toes) is correlated more with impairments in PSF. Decrease of peak and mean pressure and maximum force under MH2, MH3 and hindfoot is correlated with impairments in both PS and CFS; under MH4-in CS only. Increase of peak and mean pressure and maximum force under second and lateral toes correlates also with impairments in CFS only. Increase of force- and pressure-time integrals under the toes correlates with impairments in both PSF and CFS. Study supported by RFH 06-06-00497a.

P146

Pain and pain-related quality of life at the early stages of multiple sclerosis

B. Brochet, S. Bokouri, M. Deboire, E. Salort-Campana, M. Bonnet, K.G. Petyt; University V. Segalen (Bordeaux, F)

Background: Most epidemiological studies performed in MS patients with various disease duration reported pain frequency between
40 and 50%. Pain may exist since the beginning of the disease but its frequency and impact at these early stages remain unknown. **Objectives:** To measure frequency of pain at the early stages of MS and its impact on quality of life (QOL). To study correlation between pain and clinical sensory abnormalities in early MS patients.

**Methods:** A population-based sample of 69 consecutive patients have been recruited by the AQUSEP network less than six months after a diagnosis of MS. 68 participated to this study; 57 with relapsing-remitting MS (RRMS) and 11 with progressive MS. Pain was measured using questions of the SEP-59 QOL questionnaire (French adaptation of the MS-QOL-54). A standardised neurological examination was performed to establish sensory function, sensory Kützke functional system score (sensory FSS), EDSS and MSFC. Patients were reassessed after one, two and three years. Results: 76.4% of MS patients reported pain at baseline and 64.7% scored pain between 3 and 6 using a 6-graded verbal scale. 44.1% of patients reported that pain alter QOL at least moderately. 29% of patients without pain had reduced nociceptive sensitivity against 95.5% of patients with pain (p < 0.01). Sensory FSS was significantly higher (p = 0.045) in patients with pain than in patients without pain. Overall quality of life score decrease (SEP-59) was significantly associated with pain. During follow-up a significant but mild improvement of pain and pain-related disability were observed. Pain was significantly associated with MSFC at baseline and during follow-up but was only moderately associated with EDSS at the first yearly follow-up. MADRS scores (depression) did not differ significantly according to the presence of pain. **Conclusion:** Pain is frequent at early stages of MS and affects specifically daily QOL. Pain is associated with clinically evidenced spino-thalamic tract dysfunction. Pain is associated with neurological disability but not depression in this population. This work is supported by grants of ARSEP and Schering France SA.

**P148**

**Mood in relapsing-remitting multiple sclerosis is influenced by different factors depending on disease duration**

E. Reynolds, D. Langdon, D. Kidd; Royal Holloway, University of London (Egham, UK); Royal Free Hospital (London, UK)

**Research question:** What factors influence depressed mood at different stages of relapsing-remitting multiple sclerosis (RRMS).

**Method:** Two groups of RRMS patients were recruited. The early group had a mean symptom duration of 2.5 years (min 1 years, max 4 years), included 12 individuals (10 females), had a mean age of 34 years (SD 4.2) and a median Hauser Ambulatory Index (HAI) of 2. The late group had a mean symptom duration of 15 years (min 10 years, max 28 years), included 20 individuals (15 females), had a mean age of 44 years (SD 5.8) and a median HAI of 2. The groups were matched for disability (HAI), fatigue (Fatigue Impact Scale, FIS), depression (Beck Depression Inventory, BDI) and cognition scores (National Adult Reading Test, NART; Graded Naming Test, GNT; Paced Auditory Serial Addition Task, PASAT). **Results:** In the early group, but not in the late group, depression was significantly associated with years since symptom onset, with the most recently diagnosed being the most depressed (r = -0.71, p < 0.05, controlling for disability, age and gender). In contrast fatigue was significantly associated with depression in the late group, but not in the early group (r = -0.87, p < 0.001, controlling for disability, age and gender). Cognitive deficits were not associated with depression or fatigue in either group. **Conclusions:** Depression appears to be linked to different factors in the early and late stages of RRMS. In the early stages, temporal proximity to diagnosis seems to be a major influence. By the later stages, fatigue is linked to depression. The early stage group are living with a new threatening challenge, hence their mood is linked to duration. As a group they may also be experiencing more aggressive disease. The late stage group are necessarily selected for not having converted to the progressive stage, and may have learnt to live with a relatively stable, less threatening disease. Their mood is linked to fatigue, which may indicate how much their disease interferes with their life. Implications for clinical practise. Depression in early and late stage RRMS may have different triggers and maintaining factors and this should be considered when selecting treatment options.

**P147**

**Clinical course of Asian multiple sclerosis from the onset of optic neuritis or myelitis**

B.J. Kim, M.S. Park, M. Kim, J.S. Bae, J.Y An, S.Y. Oh; Samsung Medical Center (Seoul, KOR); Seoul Medical Center (Seoul, KOR); Catholic Medical Center (Seoul, KOR)

The optic-spinal form of multiple sclerosis (OSMS) may be differentiated from classic multiple sclerosis because of its restricted distribution in the central nervous system. The nature of the disease is still obscure in the aspect of the clinical course or laboratory findings. This study is to investigate the prognosis and clinical progression of OSMS from the initial episode of optic neuritis or myelitis. Retrospectively enrolled 75 Koran patients with clinically definite MS by Poser criteria were grouped according to the initial presenting symptoms; optic(ON), spinal(SMS), and combined(CMS). In conclusion, older male patients in Korea more frequently presented optic neuritis or myelitis as an initial manifestation. Relapses were limited in the optic nerves or spinal cord in most cases of SMS(86%), which possibly suggested different pathogenesis.

**P149**

**Objective and subjective evaluation of voice quality in multiple sclerosis**

M. Dogan, I. Midi, M. Yazici, I. Kocak, D. Ince Gunal; Marmara University Faculty of Medicine (Istanbul, TR)

**Objective:** To evaluate the voice quality in patients with multiple sclerosis (MS) by subjective and objective methods. **Study design:** Comparative, controlled, cross-sectional study. **Materials and Methods:** Female patients with multiple sclerosis (n = 27) and age- and sex-matched healthy controls (n = 27) were included in this study. Vocal functions were evaluated by a multidimensional set composed of videolaryngostroboscopy examination, acoustic analysis and subjective measurements (GRBAS and “Voice Handicap Index”). **Results:** Jitter percent, shimmer percent and soft phonation index (SPI) values were higher in MS patients compared to controls (Jitt, p < 0.001; Shim, p < 0.033; SPI p < 0.0001). Maximum phonation time was significantly shorter for MS patients compared to controls (p < 0.0001). Stroboscopic examination revealed that 16 out of 27 MS patients have a “posterior chink” as glottic closure pattern with higher SPI values (40%). Noise to harmonic ratio (NHR) and mean fundamental frequency (Fo) values were similar for MS and control groups (NHR, p = 0.73; Fo, p = 0.976). **Conclusion:** In this study, most of MS patients had dysphonia due to the weakness of voice. MS tends to worsen acoustic parameters including fundamental frequency, SPI and jitter values. These results are consistent with the more asthenic voice quality observed in MS group.
Clinical and demographic features of primary progressive multiple sclerosis in patients followed up in a medical faculty neurology department, Istanbul

B. Topcular, N. Sozer Topcular, G. Akman-Denir, M. Eraksoy; Istanbul University Medical Faculty Department of Neurology (Istanbul, TR)

Multiple Sclerosis (MS) is the most common cause of neurological disability in young adults. According to accumulating data there are 4 types of disease course: Relapsing Remitting, Relapsing Progressive, Primary Progressive (PP) and Secondary Progressive MS. PPMS is characterized by progressive course from onset and until now there are no medications capable of modifying disease course. Therefore, natural history, demographic and clinical aspects of PPMS patients are of value, for the future clinical trials. In this retrospective study, we investigated demographic, clinical, radiological and laboratory aspects of PPMS patients followed in our MS unit. There were 2800 MS patients (January 2006) and of these 536 (12%) suffered from PPMS. Sixty-four patients that filled study criterias also filled diagnostic criteria suggested for PPMS (Thompson A and revised McDonald). Patients who were lost to follow-up or with insufficient data were excluded. Female to male ratio (F/M) was 0.64. Spinal cord symptoms were the most common initial manifestations (96.6%) and rest of patients presented with brainstem-cerebellar symptoms. The mean age of patients was 48 years (22–72) and mean age at onset was 34 years (13–57). Mean disease duration was 14 years (1–32). Mean EDSS was 4.5 (3–8). Oligoclonal bands were positive in 98.6% of patients that had cerebrospinal fluid analysis. Fifty percent of patients had both cortical and spinal lesions as 37.5% had only cortical lesions on MRs proceeded for the initial symptoms. There were no significant differences in EDSS scores between the patients who received immunosuppresant treatments (i.e. Azathioprin) or not. In study group there was only one case of familial MS and 2 patients initial symptoms were preceded by pregnancy. In 3 patients, there were also extrapyramidal signs. When compared with RRMS patients in our department F/M ratio, initial manifestations, age at disease onset, progression index were significantly different. PPMS patients had later disease onset (p < 0.001), male dominance (p < 0.01) and higher progression indexes (p < 0.001). Except male dominance our findings are similar with previous European studies and showed that PPMS has its own demographic, clinic and radiologic aspects which are different from those of RRMS. Further prospective studies with serial advanced MR imaging technics and genotype/phenotype correlations would be informative and might clarify aetiopathogenesis of this group and provide treatment approaches.

Multiple sclerosis diagnosis assessed after a psychiatric event

F. Blanc, M. Fleury, L. Lita, E. Ruppert, D. Fernoby, P. Vermersch, J. de Seze; University Hospital of Strasbourg (Strasbourg, F); University Hospital of Lille (Lille, F)

Background: Studies of psychiatric morbidity in multiple sclerosis (MS) reported that onset of neurological symptoms came first in most cases. However, rare cases of psychiatric manifestations as a first manifestation of MS have been described. Aim: We report 6 cases of MS patients with psychiatric symptoms at the beginning of the disease, as a first or second event. Methods: We retrospectively studied 6 patients with definite MS who had had psychotic symptoms, manic behaviour or melancholia, as a first or second event. None of the patients were diagnosed as MS before the psychiatric episode. We describe psychiatric and neurological features, MRI findings, clinical outcome, treatment of psychiatric symptom and treatment for MS.

Results: Among these six patients, three (one woman and two men) developed persecutory delusions, two women a melancholia and one man a manic behaviour. Their mean age was 38.5 years (range 20–51 years). None of them had family history of psychiatric morbidity. For half of them, psychiatric symptoms were the first event of MS and for the others the second event of MS. None of them had immunomodulator or immunosuppressor treatment before or during the psychiatric event. The duration of psychiatric event was less than 6 months for 5 patients. Mean EDSS score during psychiatric event was 1 (range 0 to 4). Four patients had EDSS score of 0. All patients but one were treated by antipsychotic. One patient was treated by infusion of corticoid. Examination of the cerebrospinal fluid revealed an oligoclonal pattern and increased protein for all patients. Four patients without visual impairment had prolonged latencies in the visual evoked responses. All patients with persecutory delusions had anti-nuclear antibodies. Brain MRI showed numerous hypertensive lesions especially in the periventricular white matter of the temporal horns, the brainstem, and the subcortical white matter of the frontal lobes. Three patients had cervical medullar hypertensive lesions on MRI. At the end of the follow-up (mean = 8 years; range 1–24 years), 4 patients were again in a relapsing remitting form of MS. The mean EDSS score was 3.4 (0–9). Conclusion: Our study shows that acute psychiatric symptom may reveal MS. Our MRI findings suggest that temporal horns and subcortical frontal lobes are the main structures involved in these cases.
but its severity is not consistently associated with the standard measure of physical disability (EDSS/Expanded Disability Status Score). Methods: To determine the association between cerebellar and cognitive function we utilized an MS clinical database of 1035 patients with definite MS in Victoria. All patients were phenotyped by an MS specialist neurologist. All patients had EDSS, Kurtzke Functional Scores (KFS) recorded and performed the Symbol Digit Test (SDT), a surrogate marker of attention and executive function. Patients with cerebellar or pyramidal dysfunction had a modified verbal SDT to eliminate biases due to disability. Cognitive dysfunction was assessed in a subset of 136 patients by a previously validated formal neuropsychological test comprising 5 domains: attention, visuoconstructional, memory, executive and language (ordinal scale of 100 points). To assess the relationship between components of physical disability and cognitive impairment we used a multi-category ordinal logistic regression model. Patients were categorized in 4 groups by total neuropsychological score. In addition we measured the intercaudate ratio (ICR-distance between medial borders of caudate nuclei/skull diameter), a Magnetic Resonance Imaging (MRI) measure of cerebral atrophy. Results: Analysis demonstrated association between cerebellar dysfunction and impaired cognition. Patients with worse cerebellar symptoms had greater probability of cognitive dysfunction for each possible cut-point value of cognitive symptom score. Specifically, each 1-unit increase in cerebellar symptom score was associated with around 41% increase in the odds of having poor cognitive function (OR = 1.41 [1.09, 1.82], p = 0.009). This effect appeared to be independent of disease duration, ICR and KFS (excluding cerebellar and mental components). Conclusion: We showed that, in patients with MS, worsening cerebellar dysfunction is associated with cognitive decline independent of disease duration and cerebral atrophy as measured by ICR. Whilst it could be interpreted that cerebellar dysfunction is a surrogate measure of central pathology these findings could also imply that some aspects of cognitive decline in MS are driven by abnormalities in cerebellar pathways.

P154

Analysis of clinical manifestations in the initial phase of multiple sclerosis from database of Latvia
M. Metra, L. Elise, A. Millers, M. Murzina, A. Paegle, J. Kalnina; MS Centre of Latvia (Riga, LV); Riga Stradins University (Riga, LV)

Background: Multiple sclerosis (MS) is a chronic disease of the central nervous system (CNS) that affects approximately 2.5 million people worldwide. People with MS can suffer from visual, sensory, motor and other disturbances. So as the symptoms of MS are similar to many different diseases sometimes the time to make a diagnosis of MS can take a couple of years. Goals: The aim of the study was to analyse the information from the data base of MS centre of Latvia, to describe the clinical characteristics of our population and to help to the general doctors, ophthalmologists and neurologists to find these symptoms and to help to the patients as early as possible.

Materials and Methods: Retrospective data from summarized information in the hospital surveys of MS patients and entered in database in the period since 1997 – 2006 was analysed. The analysis is made from the history cases of 653 patients with MS. The diagnosis of MS patients entered in our MS data base is made according to McDonald diagnostic criteria. Patients were divided into 8 groups of symptoms: ocular signs, brainstem signs, sensory disturbances, motor disturbances, cerebellar signs, bladder and bowel dysfunction, cognitive dysfunction, sexual dysfunction. All these symptoms were checked during the initial phase of the disease. Results: The prevalence rate of MS in Latvia is about 49 per 100,000. The average onset of the disease was 24 years of age. The female-to-male ratio was 2.3:1. In a population of 653 MS patients we have found 541 patients with motor disturbances, 479-visual disturbances, 544-sensory disturbances, 421- cerebellar dysfunction. There were found different kind of sensory disturbances: numbness in lower extremities- 406, upper extremities-344, pain-168, hfeel from cold in lower extremities-141 patients. The most common disorder of bladder and bowel disturbances was imperative urinary incontinence. From cognitive dysfunction symptoms’ group the most common there were fatigue-273, the worsening of memory-109. Conclusion: Latvia is one of the countries with rather high prevalence of MS. In the initial stage of the disease patients are suffered from very different kind of symptoms. There is not a large difference among ratio of the main groups of MS symptoms.

P155

The role of noninvasive assessment of bladder dysfunction in multiple sclerosis patients
A. Horvat Ledinek, U. Rot, S. Šega Jazbec; University Clinical Center (Ljubljana, SI)

Objective: Bladder dysfunction affects approximately 75% of patients with multiple sclerosis (MS). The primary problems are failure to store urine and inability to empty the bladder normally. Symptoms of failure to store urine in the bladder include urinary urgency, frequency, incontinence, and nocturia. Symptoms associated with failure to empty the bladder include difficulty with micturition, hesitancy, and a sensation of incomplete bladder emptying. Evaluation of bladder dysfunction should begin with a history and measuring of post voiding residual volumes. The aim of the study was to evaluate the frequency and type of bladder dysfunction and relations between urinary symptoms and objective measurement post voiding volume. Methods: We included 144 patients (112 females, 32 males) with MS according to McDonald criteria. Voiding function have been assessed using multivariate questionnaire, modified international prostate symptom score. Post voiding residual volume was measured ultrasonographically. Urinary symptoms were assessed by bowel/bladder Functional System score of the EDSS (Expanded Disability Status Scale (EDSS)). Results: Bladder dysfunction was found in 79.1% patients. Mean ages of patients were 38.9 ± 2.6 years and the median EDSS was 3.0 ± 1.9. Urgency was the most frequent symptom (51.4%), followed by frequency (46.1%), hesitancy (35.1%), incomplete emptying (25.3%) and mixed incontinence (13.5%). We found that 64.7% of patients had post void residual volume over 100 ml. There was no correlation between urinary symptoms assessed by EDSS and objective post voiding residual volume over 100 ml. Conclusion: According to our findings, symptoms of failure to store urine; urgency and frequency, were the most common type of bladder dysfunction. We didn’t find correlation between urinary symptoms assessed by EDSS and post voiding volume, therefore, ultrasound scanning of residual volume is recommended in every MS patients.

P156

Clinical characteristic of optic neuritis in multiple sclerosis: a retrospective study of 98 patients in Venezuela
A. Soto, G. Orozco, M. Camacaro, M. Gallardo, L. Vink; Hospital Domingo Luciani (Caracas, VE); Hospital Juan Daza Pereira (Barquisimeto, VE) and The MS Study Group of Venezuela

Optic Neuritis is commonly observed in MS. There are few reports regarding this topic in Venezuela. Methods and Results: We investigated 98 patients with Definitive Relapsing Remitting Multiple Sclerosis (MS) according to McDonald’s criteria. 85 females (86.7%) and 13 males (13.26%), mean age 36.8 (range 12 – 55), EDSS of 1 to 3 in 74 patients (75.5%) and 3.5 to 6 in 19 (19.3%). Eighty seven patients (88.7%) had unilateral Optic Neuritis and 11 (11.2%) bilateral optic neuritis. Papillitis was present in 46 patients (46.9%) and retrobulbar optic neuritis was present in 52 patients (53%). Oligoclonal bands (OCB) were present in the CSF in 70 patients (71.42%), 15 patients (15.3%) had normal (CSF), 5 patients (5.1%) had reinforcer...
premature death of the cellist Jacqueline du Pre´ from Multiple Sclerosis (MS). The subsequent award winning film ‘Jackie and Hillary’ prompted me to review literature on this unusual woman bilaterally abnormal in 22.4% of subjects.

**Methods:** This report is based on information obtained from the book; personal observations during repeated encounters with Jacqueline du Pre´ in various social settings; interviews with her husband’s relatives, with friends and her carer. **Results:** She inexplicably suddenly and temporarily left her husband one year before her mouth with food after grabbing it with both hands with voracious appetite i.e. unconventional eating pattern; placidity, explosive laughter and possibly hypersexuality characterised her behaviour late in her illness. No data on imaging or underlying pathology is at my disposal. **Discussion:** Based on clinical data I propose that the unstable mental state with out-of-character behaviour lasting one year before onset of neurological symptoms and signs were integral part of the disease and its first sign. Pure psychiatric presentation of Multiple Sclerosis are described in the literature and are uncommon. Observing her antisocial unconventional eating habits one could not help but be reminded as being identical with the text book pictures which illustrate descriptions of Klüver-Bucy syndrome. To my knowledge Klüver-Bucy syndrome was not yet described in MS. This absence is rather surprising as bilateral temporal lobe lesions in MS are theoretically not inconceivable. **Conclusion:** This unusual woman had an unusual MS: pure psychiatric presentation; Klüver Bucy Syndrome; fulminating course and premature death. Integrating the psychiatric symptoms into her illness — both at the onset and late in the course of the disease — explains and justifies her out-of-character behaviour. I also try to give homage to the artist, her distinguished husband and to the memory of her loving and caring mother-in-law Aida Barenboim.

**P158**

**Multiple sclerosis and distress**

A.G. Betske, I. Sandanger, B. Czujko, E.D. Pedersen, K.M. Myhr; University Hospital Akerhus (Lørenskog, N); Molde Hospital (Molde, N); University Hospital Haukelands (Bergen, N)

**Objective:** To explore the occurrence of distress among MS patients and its relation to demographic and clinical characteristics. **Method:** All out-clinic MS patients from four municipalities in the county of Akershus, Eastern Norway was defined as the target population. Distress was evaluated by the Hopkins Symptom Checklist 25 items (HSCL-25) and compared to a Norwegian control population. HSCL-25 evaluates the presence and intensity of anxiety and depressive symptoms over the previous week, ranging from 1 (not bothered) to 4 (extremely bothered). Scores above 1.67 for men and 1.75 for women are defined as distress [1]. Expanded Disability Status Scale (EDSS) for physical function, and Mini Mental State Examination (MMSE) and Paced auditory serial-addition task (PASAT) for cognitive function were also included. **Results:** A total of 166 definite (Poser) MS patients agreed to participate and 140 of these completed all questionnaires and scales and thus available for evaluation. The initial course was primary progressive MS (PPMS) in 37 (26.1%) and relapsing remitting MS (RRMS) in 105 (73.9%). Forty-six (32.9%) were men and 94 (67.1%) were women, the mean age at onset of MS was 31.7 (SEM = 0.9) years, and the age at examination was 51.6 (SEM + 0.9) years. Disease duration was 19.9 (SEM +1.0) years and the mean EDSS was 4.09 (SEM +0.18), (range 1.0–9.0) with a bi-modal distribution, showing peaks at 1.5–3.0 and 6.0–7.0. Forty-two (30.0%) patients were distressed according to the HSCL-25 compared to 13.8% in a Norwegian control population (p<0.001). Distress was unrelated to gender (p = 0.47), age (p = 0.08), disease duration (p = 0.27), initial disease course (p = 0.22), EDSS (p = 0.74), PASAT (0.53) and MMSE score (p = 0.70), (patients with MMSE below 24 were excluded from this study). **Conclusion:** Distress occurred twice as frequently in this population of MS patients compared to the normal population, and it was independent of clinical and demographic variables. Awareness for symptoms related to anxiety and depression is important to establish appropriate treatment.

**Reference**


**P159**

**Multiple sclerosis in continuing care residents: impact of multiple sclerosis symptoms on quality of life**

S. Warren, K. Turpin, D. Mikel, K. Warren, J. Christopherson, T. Rust; University of Alberta (Edmonton, Alberta, CAN); MS Society of Canada (Edmonton, Alberta, CAN)

**Background:** Researchers have found that five to ten percent of the Canadian multiple sclerosis (MS) population reside in continuing care. While literature exists regarding the quality of life (QOL) of persons with MS in general, literature regarding the QOL of those in continuing care is virtually non-existent. Thus, the purpose of this study was to explore factors which may be associated with the QOL of persons with MS living in continuing care. **Methods:** Thirty-nine MS continuing care residents completed an interviewer administered questionnaire containing valid and reliable inventories to measure disability (EDSS), symptoms (MS-Related Symptoms Scale-MRSS), anxiety and depression (Hospital Anxiety and Depression Scale-HADS), loneliness (Emotional/Social Loneliness Scale-ESL), and QOL (The World Health Organization Quality of Life – Brief Questionnaire (WHOQOL-BREF). In addition, two questions from the MS Quality of Life-54 Questionnaire were administered: 1) rating of overall QOL; and 2) rating of how one feels about their life as a whole. Multiple linear regression models were used to identify the factors possibly associated with the four summary QOL scores of the WHOQOL-BREF: physical, psychological, social, and environmental. **Results:** Poorer physical QOL was found in those persons with increased frequency and multiplicity of symptoms (β = −0.605, p = 0.002) and fewer hours of visitation by family members (β = −0.411, p = 0.025), together explaining 44.1% of the variance. Poorer psychological QOL was found in those persons with a higher HADS depression score (β = −0.532, p = 0.000), and poorer ratings of how one feels about their life as a whole (β = 0.376, p = 0.003), together explaining 51.9% of the variance. Poorer ratings of how one feels about their life as a whole (β = −0.531, p = 0.025) was also associated with poorer social QOL, but only explained 9.6% of the variance. Poorer environmental QOL was found in those persons with greater disability, but only explained 13.5% of the variance. **Conclusions:**
contrast to the general MS population in which depression, disability, fatigue and relapses are the most commonly supported findings associated with poorer QOL, psychosocial factors appear to dominate the influence on this group’s QOL. While it may be the falling body of the MS patient that leads to the need for continuing care, it may be the subsequent assualment on the mind and spirit that cause the greatest deterioration in QOL.

P160

Paroxysmal unilateral dystonia and pathological laughter as first manifestation of multiple sclerosis
M. Aguirregamuzcorta, L. Ramis-Formentà, J. Gich, J. Serena; Hospital Dr. Josep Trueta (Girona, E)

We report a case of 37-year-old woman who consulted in the emergency department and complained of episodes of paroxysm involuntary movements in left extremities with stiffness and pain that lasted a few seconds each one. These movements had been present for one week and its frequency had been increasing up to 7–8 episodes each day. She had not any disease, any toxic contact and she did not take any drug. General and neurological examination were normal except for episodes of sudden paroxysmal dystonia in left extremities with flexion of elbow, wrist and knee and extension of ankle. During these episodes the patient had easy laughter and lack of inhibition. When motor symptoms disappeared, this abnormal behaviour also stopped. Electroencephalogram and cranial computed tomography were normal. Cerebral magnetic resonance imaging showed white matter lesions, some of them with pathological enhance, in periventricular region, left semioval center, right hemi cerebel and right pontum. Autonomic dysfunction showed positive oligosymSUBH.

P161

Paroxysmal unilateral dystonia and pathological laughter as first manifestation of multiple sclerosis
M. Aguirregamuzcorta, L. Ramis-Formentà, J. Gich, J. Serena; Hospital Dr. Josep Trueta (Girona, E)

We report a case of 37-year-old woman who consulted in the emergency department and complained of episodes of paroxysmal involuntary movements in left extremities with stiffness and pain that lasted a few seconds each one. These movements had been present for one week and its frequency had been increasing up to 7–8 episodes each day. She had not any disease, any toxic contact and she did not take any drug. General and neurological examination were normal except for episodes of sudden paroxysmal dystonia in left extremities with flexion of elbow, wrist and knee and extension of ankle. During these episodes the patient had easy laughter and lack of inhibition. When motor symptoms disappeared, this abnormal behaviour also stopped. Electroencephalogram and cranial computed tomography were normal. Cerebral magnetic resonance imaging showed white matter lesions, some of them with pathological enhance, in periventricular region, left semioval center, right hemi cerebel and right pontum. Autonomic dysfunction showed positive oligosymSUBH.

P162

Is there any relation between functional system impairment with depression in patients with multiple sclerosis?
F. Kaplan, H. Boztas, C. Yucelen, N. Yucemen, N. Mutlu; Ankara University, School of Medicine (Ankara, TR)

Studies have repeatedly reported that the prevalence of depression in multiple sclerosis (MS) is high even when compared with other groups with a chronic illness. The issue about whether psychiatric changes are a reaction to the disorder or part of the disease process itself remains controversial. The aim of this study is to determine whether there is any relationship between functional system scores and the presence of depression in patients with multiple sclerosis.

Method: 37 (25 female, 12 male) patients with less than 5.5 EDSS score, who were in their remission phase (36 patients with relapsing-remitting MS and one patient with secondary progressive MS), and who accepted to participate to the study between July 2004 and December 2005 were included in this study. The patients underwent a complete neurological examination, functional systems were assessed according to the functional system scores of Kurtzke, and the patients’ disabilities was assessed by using Kurtzke Expanded Disability Status Scale (EDSS). Then the patients examined by a psychiatrist using some structured tests including Structured Clinical Interview of Diagnose (SCID), Hamilton Depression Scale. Results: Mean EDSS value was 3.3 (1.5–5.5). Seventeen patients (45.9%) had depression according to SCID. There was not any relationship between EDSS and depression. Depression was more frequent in patients with cerebellar involvement than in patients without it using functional system scores (p = 0.037). There was not any relationship with other functional systems involvement and depression.

Conclusion: We found a relationship between cerebellar signs and depression even the absence of serious disability in patients with MS. To our knowledge, the relationship between cerebellar signs and depression has not been reported in MS patients in English based literature previously. MS patients with any cerebellar signs should be evaluated by a psychiatrist even the absence of serious disability or of any complaint in daily life.

P163

Paroxysmal brainstem symptoms in multiple sclerosis
Y. Stern, I. Kishner, J. Chapman, A. Achiron; Sheba Medical Center (Ramat-Gan, IL)

A variety of paroxysmal symptoms has been described in multiple sclerosis (MS) including trigeminal neuralgia, dysarthria, ataxia, tingling, and diplopia, though their incidence is relatively low. We report on a 40-year-old female patient known to suffer from relapsing-remitting MS of three years duration. The patient presented with an acute onset of paroxysmal brief attacks that included left gaze deviation and dysarthria. The attacks lasted up to 2–3 minutes, were frequent occurring every few minutes throughout the day, for a period of several days. The patient could perceive the incoming attack but no external trigger could be determined. During the attack the patient was alert; if she was talking the speech became slurred and incomprehensible and was accompanied by left conjugated gaze deviation. Between the attacks the patient was neurologically intact with no dysarthria and with full eye movements. Treatment with trileptin resulted in remission of the attacks within a few days. The patient’s symptomatology was suggestive of brainstem involvement and indeed brain MRI examination disclosed a new active demyelinating lesion in
Multiple sclerosis patients and sexual dysfunction
I. Fedotova; Kharkov Medical University (Kharkov, UK)

Objective: Sexual dysfunction (SD) severely affects the quality of life in patients with multiple sclerosis (MS). The aim of this study is to investigate the type and frequency of sexual complaints in MS patients, to analyze their relationship to various clinical and psychosocial variables and to clarify the differences between MS patients with and without SD.

Methods: 64 relapsing-remitting (RR), 10 secondary progressive and 5 primary progressive MS patients were included in this study. A structured face-to-face interview regarding sexual function and other physical problems which may interfere with sexual functioning was administered to each patient. They also filled out Multiple Sclerosis Intimacy and Sexuality Questionnaire-19 (MSISQ-19), which includes items for primary (direct physical), secondary (indirect physical) and tertiary (psychosocial) causes of SD. Disability, cognitive functions and psychological functioning were also evaluated.

Results: 62 patients (78.5%) reported primary SD; decreased libido was the most frequent complaint (79.5%). These patients were older and more disabled, however 39% had low disability scores. SD was a common problem for both men (78.5%) and women (79.5%). MS patients with SD were more disabled at the time of study (p < 0.001) and abnormal bleeding days (p < 0.01). 27.6% of the patients complained menstrual disturbances in comparison to 20% of controls.

Conclusion: SD is an underestimated, common problem and may affect the normal sexual life and even fertility of the MS patients (p < 0.04). No difference had been founded in thyroid hormone levels in the two groups. There were some relationships between usage of different interferon products and some specific menstrual irregularities. Conclusion: Menstrual irregularities are really more common in MS. We should study to find the possible causes and mechanisms of them and correct the problem inorder to provide a normal physical and psychological life for female MS patients.

Neurogenic bladder disturbances in multiple sclerosis: social relevance and consequences for job-related rehabilitation
G. Lehrieder; Kiliani-Klinik (Bad Windsheim, D)

Symptomatic management of neurogenic bladder disturbances in patients with multiple sclerosis is important not only due to the high frequency up to 90% among ms-patients. Untreated bladder disorders increase the risk of infections and complications in the upper and lower urinary tract. Urgency and incontinence have a major social impact on the patients. Despite of these relevances neuro-urologic symptoms are often made taboo. We examined the relevance of bladder disorders in young ms-patients with respect to their quality of life (QoL) and especially under aspects of employment. The cohort consisted of 170 consecutive patients of the neurologic department of the kiliani-klinik. The group was representative for patients requiring inpatient rehabilitation. The male-female ratio was 36:64%. According to Kuntzke's Expanded Disability Status Scale (EDSS) 57% could walk without aids, 30% needed one- or both-sided aids to walk (i.e. EDSS 6.0 to 6.5). 13% were wheelchair bound or without independent transfer. We found a frequency of 62% of patients suffering from bladder disturbances. Most important symptoms among the employed patients were high frequency and urgency of urination. Incontinence and pain in the escalation of bladder disturbance don’t play a relevant role in the patients who are not on pension. Only one third of the patients suffering from urinary symptoms were adequately treated. Symptom-related decrease in quality of life and occupational participation only due to the urinary symptoms was estimated by the patients themselves in a range of 30 to 40% of the total ms-related QoL-decrease. In more than 10 percent bladder dysfunction is a relevant, non seldom the only, reason for starting one’s disability pension. Job-related rehabilitation of MS-patients has to focus not only on motor or visual disturbances but also on the bladder problems. Diagnostic evaluation, enhancing knowledge and selfcompetence on the side of the patient is needed. But employees with ms and bladder disturbances need more than starting the appropriate therapeutic strategies: They need understanding for their special problems by their superiors. In their working places frequent breaks are necessary, short distances to the next toilet are desirable and fewer external work recommended. Pension insurance institutes and employers must be informed about the needs of the patients with neurogenic bladder dysfunction to preserve the ability to participate in working life.

Menstrual disturbances and related plasma hormone levels in female multiple sclerosis patients
M. Nabvi, E. Garshasbi, M. Jalali, M.R. Nejati; Shahed University (Tehran, IR)

Background: Menstrual irregularities are common complaints of female MS patients in clinic. It may be due to dysfunction of H.P. axis, injury to H.P. due to demyelination or axonal loss, defects of target organs due to a chronic immune mediated disorder and the effect of some medications such as interferones or symptomatic drugs. The problem can be a matter of disturbances and lead to psychological problem and may affect the normal sexual life and even fertility of the patients.

Methods: To evaluate the relative prevalence of menstrual irregularities and related hormones changes in plasma, we have assessed 58 definite iranian MS patients and compare the results with 58 matched healthy female. Results: 55.2% of the patients complained menstrual disturbances in comparison to 20% of controls. More patients had oligomenorrhea (p < 0.03), hypomenorrhea (p < 0.001) and abnormal bleeding days (p < 0.01). A high frequency up to 90% among ms-patients. Untreated bladder disorders increase the risk of infections and complications in the upper and lower urinary tract. Urgency and incontinence have a major social impact on the patients. Despite of these relevances neuro-urologic symptoms are often made taboo. We examined the relevance of bladder disorders in young ms-patients with respect to their quality of life (QoL) and especially under aspects of employment. The cohort consisted of 170 consecutive patients of the neurologic department of the kiliani-klinik. The group was representative for patients requiring inpatient rehabilitation. The male-female ratio was 36:64%. According to Kuntzke's Expanded Disability Status Scale (EDSS) 57% could walk without aids, 30% needed one- or both-sided aids to walk (i.e. EDSS 6.0 to 6.5). 13% were wheelchair bound or without independent transfer. We found a frequency of 62% of patients suffering from bladder disturbances. Most important symptoms among the employed patients were high frequency and urgency of urination. Incontinence and pain in the escalation of bladder disturbance don’t play a relevant role in the patients who are not on pension. Only one third of the patients suffering from urinary symptoms were adequately treated. Symptom-related decrease in quality of life and occupational participation only due to the urinary symptoms was estimated by the patients themselves in a range of 30 to 40% of the total ms-related QoL-decrease. In more than 10 percent bladder dysfunction is a relevant, non seldom the only, reason for starting one’s disability pension. Job-related rehabilitation of MS-patients has to focus not only on motor or visual disturbances but also on the bladder problems. Diagnostic evaluation, enhancing knowledge and selfcompetence on the side of the patient is needed. But employees with ms and bladder disturbances need more than starting the appropriate therapeutic strategies: They need understanding for their special problems by their superiors. In their working places frequent breaks are necessary, short distances to the next toilet are desirable and fewer external work recommended. Pension insurance institutes and employers must be informed about the needs of the patients with neurogenic bladder dysfunction to preserve the ability to participate in working life.

Imaging - Part I

Retinal nerve fibre layer atrophy in primary progressive multiple sclerosis

Axonal loss is thought to be the predominant cause of disability in progressive multiple sclerosis. Whilst magnetic resonance imaging is...
able to detect brain atrophy, and thus indirectly infer neuronal and axonal loss, total brain volume loss could also be due to loss of myelin or of glia. The retinal nerve fibre layer (RNFL) and macula are accessible to study with non-invasive imaging techniques, such as optical coherence tomography (OCT). The RNFL is composed nearly entirely of unmyelinated axons of retinal ganglion cells, and the macula is densely populated with retinal ganglion cells, giving measures of axonal and nerve cell loss respectively. We studied nine patients (6 male; 3 female; mean age 49.3 years, age range 32 to 64 years; EDSS 5.5 to 7, median EDSS 6.0; disease duration 9.3 years, range 5 to 26 years) with primary progressive multiple sclerosis. All had progressive myelopathy at onset. None had a history of optic neuritis or progressive optic neuritis at onset, although one patient had noted progressive visual deterioration in the eighteen months prior to study. OCT measures of the RNFL thickness (RNFLT) and macular volume were acquired using a Stratus OCT3 device. Twelve controls (8 male; 4 female; mean age 44.3 years, age range 29 to 63 years) had RNFLT and macular volume studies as described above. Mean patient RFNLT and macular volume were significantly reduced when compared with mean control values. Mean patient RNFLT was 84.35 micrometres SD 10.5 micrometres, compared with mean control eye RNFLT 96.0 micrometres SD 11.2 micrometres (p = 0.03). Mean patient macular volume was significantly smaller, 6.46 mm$^3$ SD 0.28 mm$^3$ as compared with control eye macular volume 6.88 mm$^3$ SD 0.30 mm$^3$ (p = 0.001, adjusted for age and sex). RNFLT and macular volume measures using OCT provide evidence of retinal axonal and ganglion cell loss in primary progressive MS.

### P168

**Brain MRI findings in relapsing neuromyelitis optica**

J.A. Cabrera-Gomez, L. Quevedo-Sotolongo, A. Gonzalez-Quevedo, S. Lima, Yanely Real-Gonzalez, M. Cristoforo-Conrinnas, K. Romero-Garcia, C. Ugarte-Suarez, J. Jordan-Gonzalez, I. Gonzalez de la Nuez, I. Garcia Labrea, R. Tellez, I. Pedroso-Ibáñez, R. Rodriguez-Garcel, A.Y. Cabrera-Nuñez, Yisel Real-Gonzalez; CIREN (Havana, CUB); INN (Havana, CUB); ELAM (Havana, CUB); CIMEQ (Havana, CUB); School of Medicine (Cienfuegos, CUB); School of Medicine (Santa Clara, CUB)

**Background:** Some studies showed abnormalities in brain magnetic resonance imaging (MRI) as to relapsing Neuromyelitis Optica (R-NMO) from 12–46%. These abnormalities are described as compatible/non-compatible with Multiple Sclerosis (MS). **Objective:** To describe the abnormal brain MRI lesions in R-NMO with imaging studies conducted with more sensitive white matter changes techniques. **Methods:** 30 patients with R-NMO (Wingerchuck et al.) were selected. All brain MRI studies were performed with a 1.5 Tesla Siemens MRI system according to the Standardized MR Imaging Protocol for Multiple Sclerosis from the Consortium of MS Centers Consensus Guidelines. **Results:** Brain MRI images were evaluated in 29 R-NMO cases because in one case the MRI images were not appropriate for the study. Of these 29 brain MRI studies, 19 cases (65.5%) had at least one or more lesions (1–57) and 10 were negative (34.4%). Brain MRI findings in 19 cases, were characterized in T2/ Fluid-attenuated inversion-recovery (FLAIR) by the presence of subcortical/deep white matter lesions in 16 (84.2%) cases (1–50), most of them <3 mm and without juxtacortical localization. Periventricular lesions observed in 13 (68.4%) cases, but morphologically they were not oval, ovoid nor perpendicularly orientated. Infiltratorial lesions, all >3 mm, were observed in 4 (21.05%) cases without cerebellar involvement. T1 studies demonstrated absence of hypointense regions. Optic nerve enhancement was observed in 6/19 patients (31.5%). All brain MRI abnormalities observed were not compatible with Barkhof et al. criteria of MS. **Conclusions:** This study based on a Cuban patient population, with long duration of disease, good sample size and detailed characterization by MRI, demonstrated the brain MRI pattern of R-NMO patients which is different from MS.

### P169

**Brain magnetisation transfer imaging in neuromyelitis optica, transverse myelitis, and multiple sclerosis**

A. Boster, J. Perunal, M. Mackenzie, C. Caon, F. Bao, Z. Latif, O. Khan; Wayne State University School of Medicine (Detroit, USA)

**Objective:** To assess magnetization transfer (MT) imaging changes in the brain of patients with neuromyelitis optica (NMO), transverse myelitis (TM), and MS. **Background:** Most patients with NMO have unremarkable brain MRI scans or develop changes later during the course of the disease. In contrast, patients with TM restricted to one or two segments of the spinal cord and without fulfilling criteria for MS or NMO have normal brain MRI scans. Furthermore, it has been shown that MTR of normal appearing brain tissue in NMO is normal in contrast to the well described abnormalities in MS. We investigated corpus callosum MTR changes in NMO, TM, and MS compared to healthy controls (HC). **Methods:** Seven NMO IgG antibody positive patients, 8 TM patients, 10 RRMS patients and 6 healthy controls (HC) underwent MRI with MT images. Conventional Brain MRI scans and MT scans were obtained on a 1.5T scanner. For MTR calculation, a region of interest (ROI) from the genu and splenium of corpus callosum were marked out using a semi-automated technique and then mapped out on to the co-registered MT images. **Results:** MTR values from the NMO were significantly lower than TM patients and HC but similar to patients with RRMS. **Conclusions:** NMO-IgG antibody positive NMO patients demonstrate MTR changes in the corpus callosum indicating that more diffuse tissue damage may occur in these patients distant from the classic spinal cord and optic nerve injury. Patients with TM appear to have disease restricted to the cord without evidence of myelin damage in the brain.

### P170

**Multiple sclerosis in African-Americans with multiple sclerosis: insights into disease severity and comparison with Caucasian multiple sclerosis**

O. Khan, M. Mackenzie, C. Caon, L. Dantzer, F. Bao, Z. Latif, A. Tsels, C. Ozust, A. Hudson; Wayne State University School of Medicine (Detroit, USA)

**Objective:** To determine the clinical characteristics of African-Americans (AA) with MS and identify potential features into disease severity and distinction from Caucasians (CAC) with MS. **Background:** Previous multi-center collaborative studies have shown that AA with MS experience more severe disease course and greater disability in shorter period of time than CAC with MS. In this work, we extend our previous observations by including MR imaging features of AA with MS as well as response to DMT in a large data set from a single MS Clinic. **Methods:** 118 well-characterized AA MS patients and 118 well-characterized AA MS patients. **Results:** Mean age, EDSS and disease duration (DD) were 43.2 years, 4.8 and 9.3 years, respectively, for the entire cohort (n = 96). 40 of 96 (41.7%) patients were heterozygous carriers for APOE E4. Mean age, EDSS, and DD were 41.1 years, 5.0, and 8.7 years, respectively. In the APOE E4 positive group, time to EDSS of 6.0 (n = 21) and 6.5 (n = 12) were 4.8 and 6.6 years, respectively. In the APOE E4 negative group, time to EDSS 6.0 and 6.5 were available when applicable. MRI and CSF data were also available. Presence of APOE E4 was determined after establishing demographic features, minimizing bias from the retrospective collection of clinical data and its potential association with APOE E4 allele. We were able to analyze brain and cervical spinal cord MRI characteristics in AA and compare them to CAC MS patients. Furthermore, we were also able to perform MT, MRS, and DTI in a smaller number of AA MS patients. **Results:** Mean age, EDSS and disease duration (DD) were 43.2 years, 4.8 and 9.3 years, respectively; for the entire cohort (n = 96). 40 of 96 (41.7%) patients were heterozygous carriers for APOE E4. Mean age, EDSS, and DD were 41.1 years, 5.0, and 8.7 years, respectively. In the APOE E4 positive group, time to EDSS of 6.0 (n = 21) and 6.5 (n = 12) were 4.8 and 6.6 years, respectively. In the APOE E4 negative group, time to EDSS 6.0 and 6.5 were 6.3 and 10.4 years, respectively. Preliminary analysis showed that APOE E4 positive AA MS had greater involvement of spinal cord MRI abnormalities than APOE E4 negative patients. Furthermore, AA MS patients had a significantly smaller brain and spinal cord volume compared to CAC. AA with MS responded less favorably to DMT than CAC with MS. **Conclusions:** Our study
The present study aimed to reveal the characteristics of brain and the presence of brain lesions do not exclude the diagnosis of NMO. Neuromyelitis optica (NMO) is characterized by M. Nakamura, I. Nakashima, K. Fujihara, I. Miyazawa, T. Misu, with NMO-IgG Brain MRI findings in Japanese multiple sclerosis patients P172

Objective: To investigate the usefulness of long-term brain proton MRS in RRMS and to examine effect of disease modifying therapy on cerebral axialon injury in RRMS. Background: In-vivo techniques of quantifying cerebral axonal injury and recovery brain such as proton brain MRS are gaining popularity given the improved acquisition techniques and reproducibility and the growing emphasis on axialon injury in MS. Measuring N-acetylaspartate (NAA) referenced against creatine (Cr) is a reproducible method of assessing axonal injury. We are investigating the long term effect of glatiramer acetate (GA) on axonal injury in RRMS and whether this may be useful in studying cerebral axialon injury over a long term. Methods: In an ongoing long-term prospective study, we performed combined MRI/MRS on 22 treatment naïve RRMS patients, 18 of whom commenced treatment with GA and 4 remained untreated by choice. Imaging was repeated annually for 4 years. Blinded MRS analysis of NAA/Cr were carried out in a volume-of-interest (VOI) centered on the corpus callosum that also allowed for the examination of the normal appearing white matter (NAWM). Results: Baseline (n = 18) mean age, disease duration, and EDSS were 43.5 years, 8.2 years, and 2.77, respectively. Baseline (n = 18) mean NAA/Cr in the entire VOI was 1.97 (+0.24) and 2.075 (+0.30) in the NAWM. After 4 years of follow up, 15 of the 18 patients in the treated group were still participating in the study with a mean NAA/Cr of 2.21 +0.16 in the VOI and 2.27 + 0.20. Three of four untreated patients began treatment with GA during the study. Individual results with be presented. Six healthy controls were also followed for 4 years. Detailed image analysis including correlation with conventional lesion load will and the risk of developing chronic destructive lesions in areas of decreased NAA/Cr also be presented. Conclusions: This is the longest prospective study employing annual brain proton MRS imaging in RRMS and provides insight into mechanisms involving chronic axonal injury. It also demonstrates effect of glatiramer acetate on axonal injury and recovery in a long-term study. We believe that NAA may be a reasonable marker for assessing long-term disease progression and therapeutic response.

P172

Brain MRI findings in Japanese multiple sclerosis patients with NMO-IgG

M. Nakamura, I. Nakashima, K. Fujihara, I. Miyazawa, T. Misu, Y. Itayama; Tohoku University School of Medicine (Sendai, JP)

Introduction: Neuromyelitis optica (NMO) is characterized by severe optic neuritis and transverse myelitis. Whether NMO is a variant of multiple sclerosis (MS) has been controversial, but a study on NMO-IgG, an NMO-specific serum autoantibody, strongly suggested that NMO is distinct from MS. Since recent studies showed that some patients with NMO-IgG had brain lesions, in the newly proposed diagnostic criteria for NMO by Wingerchuk et al. (2006), the presence of brain lesions do not exclude the diagnosis of NMO. The present study aimed to reveal the characteristics of brain and spinal cord MRI findings in NMO-IgG-positive patients in Japan. Patients and methods: We investigated the findings of the brain and spinal cord MRI in 15 Japanese patients who were seropositive for NMO-IgG. Results: Among them, 7 patients (47%) had brain lesions, and 14 patients (93%) had longitudinally extensive lesions (> three vertebral segments). The only patient without a longitudinally extensive spinal cord lesion, however, had extensive spinal cord atrophy indicating a history of an extensive cord lesion. Among the 7 patients with brain lesions, 5 had only optic neuritis and transverse myelitis as clinical symptoms. The other 2 patients had no episode of optic neuritis but had histories of cognitive dysfunction, hemiparesis, or cerebellar ataxia. On brain MRI, diffuse white matter lesions, juxtacortical lesions, callosal lesions, periventricular lesions, and longitudinally extensive lesions along pyramidal tracts were commonly seen in the patients with NMO-IgG. Cerebellar hemispheric lesions were seen in one patient who developed cerebellar ataxia, and a hypothalamic lesion was seen in another patient who showed hyperprolactinemia at the relapse of visual disturbance. None of the patients had a cavitary brain lesion. Although some brain lesions in the patients with NMO-IgG resembled those of MS, ovoid lesions with open-ring enhancement typically seen in classical MS were not observed in any patient with NMO-IgG. Conclusions: Our study showed that brain lesions were not rare in the Japanese patients with NMO-IgG, and the MRI features of the brain lesions in the patients appeared to be different from those in MS. In particular, like longitudinally extensive spinal cord lesions, longitudinally extensive lesions along the pyramidal tract in the brain may be unique to the patients with NMO-IgG.

P173

Lack of relationships between MRI periventricular lesions and oligoclonal IgG bands detection in Caucasian multiple sclerosis

A. Pichiecchio, R. Bergamaschi, D. Franciotta, E. Tavazzi, E. Caverzasi, G. Poloni, S. Bastianello; Mondino Neurological Institute (Pavia, I)

Introduction: Neuropathologic studies suggest that MS plaques are possibly the main source of oligoclonal immunoglobulins. The lack of oligoclonal IgG bands (OCBs) in a small percentage of MS patients could thus be related to a predominant subcortical plaque distribution, with drainage of interstitial fluid through periarteriolar pathways. This hypothesis was supported by the strong association between periventricular lesions and OCB recently reported in Japanese MS patients(Nakashima I. et al. Acta Neurol Scand 2006; 113: 125 – 131). Objectives: To correlate the presence of cerebrospinal fluid (CSF) OCBs and periventricular lesions in Caucasian patients with multiple sclerosis(MS). Materials and Methods: We retrospectively analyzed brain magnetic resonance imaging (MRI) of 46 Caucasian MS patients (14 males, 32 females, 38.8 years mean age, 2.3 mean disease duration, 44 relapsing-remitting, 2 clinically isolated syndromes, 1.4 mean EDSS), who underwent spinal tap close to the radiological examination. All patients fulfilled 2001 McDonald’s criteria for MS diagnosis. Patients were divided in two subgroups with respect to the presence/absence of OCBs in CSF: 23 patients had 2 or more bands (OCB+ group), 23 patients one or no band (OCB– group). No significant difference in disease duration at the time of lumbar puncture and MRI examination was observed among the two groups. All imaging studies were performed on a 1.5 T Philips Gyroscan system. Two independent neuroradiologists identified and counted the lesions in periventricular and subcortical regions. The maximum section of each lesion was also measured using the MRIcro software. Results: In the OCB+ group, 16/23 patients (70%) showed predominant periventricular than subcortical lesions, with a total mean surface equal to 217 in voxel units. In the OCB– group, 19/23 patients (83%) had predominant periventricular lesions with a total mean surface equal to 225.7 in voxel units. No significant difference in the distribution of subcortical vs. periventricular plaques in OCB+ vs. OCB– MS patients was found. No significant difference was also found in the mean periventricular lesions’ section among the two
groups, for both number of lesions and total section surface.

Conclusions: Different genetic backgrounds could partially explain the discrepancies between our study and the Japanese one. Alternative hypotheses (subpial B-cell niches?) could be taken into account to tackle the issue of site of production of OCBs.

P174

Proton magnetic resonance spectroscopy and magnetisation transfer imaging of normal appearing brain tissue in multiple sclerosis patients with minimal lesion load

M. Sager, A. Maj, M. Bieniek, K. Schmaj; Medical University of Lodz (Lodz, PL)

Heterogeneity of multiple sclerosis (MS) is observed in different aspects of disease including clinical manifestation and magnetic resonance imaging (MRI). One of the atypical groups are patients who fulfilled clinical diagnosis of MS but with no or minimal lesion load on conventional magnetic resonance imaging (MRI). Aim: The main goal of this study was to investigate, using spectroscopy (H-MRS) and magnetization transfer imaging (MTI), normal appearing brain tissue (NABT) in patient with minimal lesion load (atypical MS, aMS) and MS patients with typical MRI meeting the MRI criteria (tMS).

Method: Thirteen aMS patients (8 women, 5 men) and ten tMS patients (5 women, 5 men) were included in the study. Additionally, 8 healthy volunteers (HC) (7 women, 1 men) participated in the study as a references groups. All groups were matched according to age and both MS groups according to EDSS and disease duration. In all participants, MRI protocol included: conventional MRI (PD/T2, T1-weighted images), flash 2D with and without magnetization transfer and single voxel spectroscopy (steam TE 20 ms, TR 6000 ms, 64 averages) from right and left centrum semiovale (CS) were performed. Based on two flash 2D images, magnetization transfer ratio histogram were calculated and mean magnetization transfer ratio (mean MTR), pick high and pick position of MTR histogram were assessed. From H-MRS data, NAA/Cr, Cho/Cr, m ln/Cr and NAA/m ln ratios were calculated. Data analysis included multimodal comparison between aMS and tMS to HC.

Results: NAA/Cr ratio from left CS was significantly lower in aMS than in HC (1.64 ± 0.17 vs. 2.07 ± 0.4 p = 0.01) whereas in tMS there was no obvious difference in NAA/Cr ratio from left CS in comparison to HC (1.96 ± 0.62 vs. 2.07 ± 0.4 p > 0.05). In tMS, m ln/Cr ratio from right CS and mean m ln/Cr (from both CS) was significantly higher compare to HC (0.75 ± 0.2 vs. 0.46 ± 0.1 p = 0.008; 0.71 ± 0.2 vs. 0.54 ± 0.15 p = 0.064) whereas in aMS there was no difference in m ln/Cr ratio compare to HC. Additionally, lower NAA/m ln ratio from right CS in tMS but not in aMS than in HC was detected (2.5 ± 0.9 vs. 4.5 ± 2.28 p = 0.02). Mean MTR value in aMS was significantly lower than in HC (36.4 ± 3.91 vs. 40.29 ± 3.6 p = 0.05) whereas in tMS there was no obvious difference. Conclusion: Different pattern of H-MRS metabolites and MTR changes in aMS and tMS when compared to HC patients indicate that character of damage in NABT might be different in these two forms of disease.

P175

Conventional MRI and diffusion tensor imaging in Brazilian patients with optic spinal multiple sclerosis Asian type

M. Papais Alvarenga, R. Papais Alvarenga, C. Cristina Ferreira Vasconcelos, S. Vieira Alves Leon, A. Marcos da Silva Catharino, F. Erthal, L. Brandão; UNIBIO (Rio de Janeiro, BR)

The optic spinal form of MS Asian type is defined by the clinical involvement of the optic nerve and spinal cord. Brainstem signs do not exclude this diagnosis (1999). Patients and Method: We analyzed here 30 consecutive OS-MS patients attended in the Hospital da Lagoa (Rio de Janeiro, Brazil) from January/2005 to April/2006. An interview was held in order to identify clinical data. The patients were submitted to neurological evaluation and were examined with routine MR imaging, and diffusion tensor MR sequence on a GE Excite 1.5T imager. Diffusion tensor sequence was performed with 23 directions and pox processed using the function software to obtain the fractional anisotropy map. Results: OS-MS patients were mainly women (83%) and Afro Brazilians (58.3%). At the time of MRI, the mean time of disease was 7.9 years (1–21 years) and the median of the EDSS was 7.5 (2–8). The MRI showed cerebral lesions 83.3% and reduction of anisotropy 50%; brainstem T2 lesions with reduction of anisotropy 33.4%; optic nerve abnormalities at STIR technique 70%; large lesions at spinal cord 83.3%. 16.7% of the patients fulfilled the MRI MS criteria (2001). Discussion: The absolute criteria for NMO proposed by Wingerchuk et al. (1999) must be reviewed. NMO syndrome in Brazilian is characterized mainly by optic and spinal cord bouts, but brainstem symptoms could occurred as postulated by Jun-Iori-Kina (1999). Conventional MRI and DTI imaging were useful to identify cerebral and brainstem lesions.

P176

Combination of single-voxel proton MR spectroscopy and diffusion MR imaging in the evaluation of tumefactive demyelinating lesions

S. Lavrinic, T. Stosic-Optincal, S. Gavrilovic, M. Gavrilovic, V. Peric; Clinical Center of Serbia (Belgrade, CS)

Background: Tumefactive demyelinating lesions comprise circumscribed solitary lesions within cerebral hemispheres, greater than two centimetres in diameter, with conventional MR imaging appearance similar to neoplasm. Clinical presentation is usually atypical for multiple sclerosis, and correlates to the presence of focal brain lesion with mass effect. Methods: We report 7 cases of a previously healthy individuals, average age of 32 years, who presented with rapid onset of focal neurological deficits. In all cases, after the initial brain CT, a tumor was suspected. Results: Common MRI finding included a formidable solitary brain lesion, with perifocal edema and mass effect. In 3 cases there was inhomogeneous postcontrast enhancement and 4 lesions demonstrated ring enhancement. In general, diffusion weighted MR imaging revealed mildly increased apparent diffusion coefficients within the lesions, while single voxel proton spectroscopy showed reduction of NAA, increase in Cho, mi and lipids. Marked reduction of Cr was also observed. These finding were highly indicative for the demyelinating plaque in acute stage. The patients responded favourably to corticosteroid therapy, with significant reduction of neurological deficits. Follow-up MRI examinations revealed no progression to multiple sclerosis in 5 patients. Conclusion: In cases of tumor-like demyelinating lesions, especially in patients without a history of multiple sclerosis, neuroimaging necessitates implementation of the advanced MR techniques, such as diffusion MR imaging and MR spectroscopy, which can give important insight in physiological and metabolic information, complementing morphologic findings from conventional MRI.

P177

Progressive axonal loss in early relapsing-remitting multiple sclerosis. A 1H-MRS and MRI longitudinal two-year follow-up study

A. Fascul-Lozano, M.C. Martinez-Bisbal, I. Bosca, C. Valero, F. Coret, B. Martinez-Granados, L. Martí-Bonmatí, B. Celda, B. Casanova; Hospital la Fe (Valencia, E); Universidad de Valencia (Valencia, E); Hospital Clínico (Valencia, E); Hospital Quiron (Valencia, E)

Objective: To investigate the progression of spectroscopically measured axonal damage in the normal-appearing white matter of brainstem and its relationship with changes in brain T2-hyperintense lesion volume (T2LV), clinical inflammatory activity and disability in early relapsing-remitting multiple sclerosis (MS) patients. Methods: Forty-three relapsing-remitting MS patients and ten age-matched
healthy subjects were prospectively studied for two years. EDSS was calculated each three months and the MS Functional Composite scale once a year. T2-weighted MR and 1H-MRS imaging were acquired at time of recruitment and annually. The T2LV was calculated using a semiautomatic program; NAA, Cr and Cho resonances areas were integrated with jMRUI program and the ratios were calculated for the sum of the volume elements that represented the brainstem. We considered brainstem, where large tracts join together from both hemispheres, as a suitable region to detect early axonal damage in agreement with preliminary pathologic studies. Results: The basal NAA/Cho ratio at brainstem was significantly decreased in MS patients compared with controls. After two years, there was a decrease in the NAA/Cho (−9%; p = 0.002) and the NAA/Cr ratio (−13%; p = 0.001) while the T2LV increased (19%; p = 0.043) in MS patients. Control subjects had no significant metabolic changes at the pons. A subgroup analysis showed a higher NAA/Cho decrement in patients with more than one relapse. At the final of the study, a significant decrease in the NAA/Cho ratio (−18%; p = 0.001; C10) was observed in patients with one relapse. At the final of the study, a significant decrease in the NAA/Cho ratio (−18%; p = 0.001; C10) was observed in patients with one relapse. At the final of the study, a significant decrease in the NAA/Cho ratio (−18%; p = 0.001; C10) was observed in patients with one relapse. At the final of the study, a significant decrease in the NAA/Cho ratio (−18%; p = 0.001; C10) was observed in patients with one relapse.

P178
Metabolite changes in the white matter 3 years after a clinically isolated syndrome suggestive of multiple sclerosis

In a previous study, using single voxel proton magnetic resonance spectroscopy (1H-MRS) we demonstrated that myo-inositol (Ins) was significantly elevated in the normal appearing white matter (NAWM) at 19 weeks after the onset of a clinically isolated syndrome (CIS) and that this was associated with the subsequent development of clinically definite multiple sclerosis (CDMS; Fernando et al., 2004). The aim of this study was to investigate the NAWM of patients three years after a CIS using 1H-MRS and correlate it with clinical status at that time. Methods: Single voxel 1H-MRS (PRESS with TR 3000ms, TE 30ms) was performed on the NAWM of 67 patients (58 optic neuritis, 5 brainstem, 4 spinal cord syndromes, 41 female, 26 male, median age 33 years, median EDSS 1) a median of 38 months after a CIS and in 46 healthy controls (24 female, 22 male, median age 36 years). Absolute concentrations of N-acetyl-aspartate and N-acetyl-aspartyl-glutamate (tNAA), total creatine and phosphocreatine (Cr), choline containing compounds (Cho), glutamate plus glutamine (Glx) and myo-inositol (Ins) were estimated using the LCModel. Results: Compared with control subjects, the concentration of Ins (mean 3.31 mM, SD 0.84 versus mean 3.86 mM, SD 0.93; p = 0.0005) was elevated in CIS NAWM, especially in those with abnormal brain MRI (mean 3.95 mM, SD 0.96; p = 0.0003) and in those with CDMS (mean 4.29 mM, SD 0.95; p = 0.000002) and McDonald MS (mean 4.03 mM, SD 0.94; p = 0.0001) at 3 years. An increase in Cr was also observed in patients with abnormal brain MRI (mean 4.09 mM, SD 0.62 versus mean 3.72 mM, SD 0.43; p = 0.011) and in those who developed McDonald MS at 1 year (mean 4.25 mM, SD 0.67; p = 0.001) and 3 years (mean 4.11 mM, SD 0.60; p = 0.025). There was a trend for a reduced tNAA in CIS patients (control mean 8.46 mM, SD 0.83 versus CIS mean 8.14 mM, SD 0.79; p = 0.087), which was significant in those who developed CDMS at 1 year (7.81 mM, SD 0.79; p = 0.009). NAWM Ins correlated with EDSS at 3 years (r = 0.318, p = 0.009). Conclusions: The results suggest that glial proliferation (as demonstrated by the increase in Ins and Cr) is a prominent feature during the early clinical stages of MS and that it may have functional significance in relation to disability as well as subsequent conversion to MS. The lack of a significant decrease in tNAA suggests that axonal damage is a relatively less prominent feature in the NAWM in the first 3 years after the onset of a CIS, except in those who develop CDMS within 1 year.

P179
A functional connectivity study in multiple sclerosis patients comparing 2 principles of data sampling
M. Amann, J. Hirsch, L. Achtinichits, L. Kappos, E.W. Radue, A. Gass; University Hospital Basel (Basel, CH)

Background: Functional connectivity MRI (fcMRI) is based on synchronous low-frequency MR signal fluctuations in resting-state brain. These fluctuations are synchronised between functionally related cortical areas. To date two main data sampling strategies are used: a) high-sampling rate to avoid cardiac and respiratory aliasing; b) moderate sampling rate for higher spatial coverage, (undersampling cardiac and respiratory signal). We compared the 2 strategies in an fcMRI experiment. Methods: Ten normal controls and 10 relapsing-remitting MS patients (6 w, 4 m, age 20–56 years). Primary motor (M1) areas were detected in a fMRI block design study: right hand finger flexion-extension paced by 1Hz flashligh. Then, 2 fcMRI experiments in inactive subjects were performed. For the moderate sampling scheme, 300 samples (5 slices positioned on the motor cortex) were acquired with TR = 1s, whereas high-sampling rate fcMRI was performed with TR = 0.25s (1100 samples, 5 slices positioned on the motor cortex). The results suggest that this was associated with the subsequent development of clinically definite multiple sclerosis (CDMS; Fernando et al., 2004).

P180
Altered functional and structural connectivities in patients with multiple sclerosis: an fMRI and MR tractography study at 3 T
M. Filippi, M.A. Rocca, P. Valsasina, E. Pagani, A. Falini, G. Scotti, G. Comi; Scientific Institute San Raffaele (Milan, I)

Using functional magnetic resonance imaging (fMRI), an abnormal pattern of movement-associated cortical activations has been demonstrated in multiple sclerosis (MS) patients with different disease phenotypes. Damage or dysfunction of neuronal connections among cortical areas, related to structural damage of specific brain pathways, might contribute to explain these functional abnormalities. To determine the functional and structural substrates of motor network dysfunction in patients with relapsing-remitting (RR) MS without overt motor impairment, using analysis of functional connectivity and MR tractography. Using a 3 Tesla scanner, in 12 right-handed patients with RRMS and 14 sex- and age-matched
healthy volunteers, we acquired, dual-echo (DE), diffusion tensor (DT) MRI with diffusion gradients applied in 32 non-collinear directions and fMRI during the performance of a simple motor task with the dominant hand. A tractography algorithm was run on DT MRI data from patients and controls. On the fractional anisotropy (FA) maps, one region-of-interest on the midsagittal slice was drawn to select the entire corpus callosum (CC). The left (L) primary sensorimotor cortex (SMC) was selected as the seed region to compute correlation maps with other brain regions, using statistical parametric mapping (SPM2) and dynamic causality modelling. Between-group comparison in fMRI and DT MRI metrics and correlations between fMRI and DT MRI metrics were assessed using SPSS. In MS patients median DE lesion-load (LL) was 10.6 ml. Compared to control, MS patients had significantly higher mean diffusivity (p = 0.03) and lower FA (p = 0.003) in the CC. Compared to controls, MS patients had increased functional connectivity between: a) the supplementary motor area and the right (R) cerebellum (p = 0.05), b) the R primary SMC and the R cerebellum (p = 0.01), and c) the L inferior frontal gyrus (IFG) and the L primary SMC (p = 0.004). They also showed reduced connectivity between: a) the R cerebellum and the L IFG (p = 0.009), and b) the L secondary sensorimotor cortex and the L IFG (p = 0.01). No correlation was found between coefficients of altered connectivity and DE LL and DT MRI metrics of CC damage. In clinically stable RRMS patients, altered functional connectivity in the motor network is observed. The lack of correlation between measures of abnormal connectivity and DE LL and DT MRI metrics of CC damage prompts the investigation of structural damage of intra-hemispheric pathways.

P181

Functional MRI and neuronal plasticity depending on the motor training in multiple sclerosis

C. Ballario, M. Ferri, J. Nagel; Fundacion R de Neurorehabilitacion (Rosario, RA); Instituto Gamma (Rosario, RA)

Introduction: The existence of neuronal plasticity phenomena in the cerebral cortex of people with multiple sclerosis, which lead to changes in the Functional Magnetic Resonance (fMRi) has already been shown. However, the way the brain of people affected with MS responds, after motor training development, is an enigma still unsolved. Our intention is to know if a training program with a temporal extension (10 days in a row) can optimize the recruiting additional brain areas to perform cognitive tasks.

Aims: To compare changes in the activation patterns in FMRi of patients with MS before and after an extensive motor training compared to healthy subjects of the same age and sex. Materials and Methods: 10 patients with RRMS were recruited, right handed, between 22 and 56 years old, with a EDSS score < 3.5 and a Nine Hole Peg Test < 24 seconds. Free of relapses during the 3 months before the inclusion of the patient into the study. Apart from them, 10 healthy subjects were recruited, with the same age and sex. A FMRi was took, performing a motor paradigm which consisted in doing a flexion-rest extension-rest, all this within six minutes. Then patients and controls were asked to do the motor paradigm on a daily basis, 10 minutes during 10 days. On the eleventh day the FMRi study was repeated. Activation areas and their activation percentages were compared between cases and controls pre and post training. Results: Coinciding with previous studies, there was an increase in the activation area in patients compared to the controls performed on the pre-training FMRi. The areas that showed statistical significance were right Prefrontal and Basal Ganglia; and bilateral Motor Cinguli. Regarding the post-training study, a significant reduction in the activation was observed, both in the controls and in the patients, in the following areas: bilateral Primary Motor and Premotor; and left Prefrontal and Basal Ganglia. The difference in the activation percentages between cases and controls was not significant ("p" ns). Conclusions: The findings in the present study indicate that our patients’ brains were capable of reshaping the motor activity after a long period of training, obtaining a significant reorganization in the explored areas. A broader knowledge of these reached plastic phenomena could contribute in the selection of some therapies and influence the results of a “specifically designed”

P182

fMRI during PASAT and PVSAT in mild multiple sclerosis, moderate MS and normal volunteers

G. Nagels, E. Vandervliet, W. Van Hecke, S. Engelborghs, M.B. D’hooghe, P. Craes, F. Parizeel, P.P. De Deyn; Nationaal Multiple Sclerose Centrum (Melsbroek, By; Universitair Ziekenhuis (Antwerpen, By); Universiteit Antwerpen (Antwerpen, By)

The aim of this study was to examine whether there is evidence for a cerebral plasticity which follows a similar pattern as in motor and sensory paradigms, during cognitive paradigms in MS. Twenty patients with definite multiple sclerosis according to the Poser criteria were included, who had not had a relapse for at least 30 days before entry into the study, who did not use sedatives, and who had a visual function above 20/40 as measured on a Snellen chart. Ten patients were selected to have an EDSS score between 0 and 3.5, inclusive (MS 0 – 3.5 group), and ten patients were selected to have an EDSS score 4 and 7, inclusive (MS 4 – 7 group). A control group of ten healthy volunteers was matched to the patient group for age, gender and schooling level. All subjects underwent Paced Auditory Serial Addition Test (PASAT) and Paced Visual Serial Addition Test (PVSAT) in a block design during fMRI (1.5 T Siemens Sonata, 40 mT/m gradients, standard CP head coil, TE 50 ms, TR 3000 ms). Cognitive performance, assessed with the pre-fMRI PASAT, did not differ significantly between the experimental groups. PVSAT performance during fMRI was also not significantly different between groups (ANOVA, df = 2; F = 2.241; p = 0.126). PASAT performance during the fMRI however, was significantly worse in the MS 4 – 7 group than in the control group (ANOVA, df = 2; F = 5.139; overall p = 0.013; post hoc p = 0.009). There was no significant difference in fMRI-PASAT performance between controls and MS 0 – 3.5 patients, or between both patient groups. Total activation volume during PASAT was higher in the MS 0 – 3.5 group, compared to the control group (t-test, p = 0.023). There were no significant differences in activation volumes between controls and MS 4 – 7 patients. Activation volumes for PASAT were not significantly different between groups. In a GLM with activation volume as dependent variable, treatment group was a significant factor (df = 2, F = 3.737, p = 0.030), while modality (auditory or visual) was not. These fMRI findings support a cognitive cerebral plasticity in MS. The lack of a difference in activation volumes during PVSAT may be a result of the relative difficulty of the PASAT versus the PVSAT at an equal interstimulus interval of three seconds.

P183

Differential fMRI activation patterns on the Computerised Tests of Information Processing in patients with multiple sclerosis with low Expanded Disability Status Scale scores

L. Walker, A. Smith, M. Freedman, C. DeHeuledeemeester; The Ottawa Hospital (Ottawa, CAN); University of Ottawa (Ottawa, CAN)

Background: Cognitive deficits have long been identified in individuals with multiple sclerosis (MS) but insights into their neural substrates have only recently been suggested. Methods such as functional magnetic resonance imaging (fMRI) have demonstrated that patients with MS demonstrate differential activation patterns on cognitive tasks compared to healthy controls. It appears that their brains attempt to compensate for dysfunction, often by recruiting additional brain areas to perform cognitive tasks. Goals: In the current study we investigated the performance and fMRI activation patterns of patients with MS on the Computerized Tests of
Information Processing (CTIP). Methods: Twelve cognitively impaired patients with MS (with little or no physical disability) and 12 sex, age and education matched healthy controls were administered the CTIP while in the MR scanner. The CTIP measures reaction time (RT) and errors on three tasks with increasing cognitive demands (i.e. simple RT, RT when a choice is required, and RT when semantic search is required). Results: Both patients and controls demonstrated the expected increases in RT and errors with increasing task complexity, with patients showing longer RTs and more errors than controls across all three tasks. The discrepancy between number of errors made by patients and controls grew with increasing task complexity. Second level whole brain random effects analyses for the Semantic minus Choice tasks revealed significant differences in blood flow between the two groups. Patients showed attenuated neural activity compared with controls in several left hemisphere regions including the precuneus, visual cortex and middle temporal gyrus. Patients showed more activity in motor areas in the right hemisphere, including the precentral gyrus and premotor cortex. The Choice minus Simple contrast revealed significantly more activity in patients than controls in left superior occipital gyrus. Conclusions: In summary, patients were not able to substantially activate areas in the left hemisphere to perform the Semantic task as accurately as controls but were able to do so more efficiently for the more simple Choice task. This suggests that as information processing requirements increase, the MS patients are not able to recruit appropriate brain regions to perform the tasks accurately. These results provide insights into the impaired information processing speed that is prevalent in MS patients.

P184

Differential fMRI activation patterns on a response inhibition task in patients with multiple sclerosis with low Expanded Disability Status Scale scores
A. Smith, L. Walker, M. Freedman, C. DeMeulemeester; University of Ottawa (Ottawa, CAN); The Ottawa Hospital (Ottawa, CAN)

Background: Although deficits in executive functioning are well known cognitive sequelae of multiple sclerosis (MS), less is known about patients' performance on response inhibition tasks in particular. Behavioural observation of cognitively impaired MS patients often reveals impulsivity. However, knowledge about associated activation patterns on functional magnetic resonance imaging (fMRI) is lacking. Goals: In the current study we investigated the performance and fMRI activation patterns of patients with MS on a response inhibition task (Go/No-Go). Methods: Ten cognitively impaired patients with MS (with little or no physical disability) and 10 sex-, age- and education-matched healthy controls were administered the Go/No-Go task while in the scanner. The task measures reaction time and errors on two different conditions (i.e. press for X [X] and press for all letters except X [non-X]). Results: Both patients and controls demonstrated longer reaction times on the more challenging response inhibition condition (i.e. non-X). Patients and controls did not differ significantly from each other in their reaction times. Nonetheless, patients made more errors than controls. The discrepancy between the number of errors for patients and controls was greater in the non-X condition. Second level whole brain random effects analyses for the non-X minus X contrast revealed that patients demonstrated more compensatory activation than controls in premotor areas, orbital frontal cortex and inferior frontal gyrus. Patients demonstrated less activity than controls in more posterior brain regions, specifically, in the supramarginal gyrus and the occipital cortex. Conclusions: In summary, despite patients' neural compensation in frontal areas, their lack of ability to compensate in posterior regions may explain their poor performance in response inhibition. Difficulties with response inhibition may manifest itself behaviourally as impulsivity. This study thus provides new insight into the neural processing of the impulsivity often observed in cognitively impaired individuals with MS.

P185

Imaging cognitive fatigue in encephalomyelitis disseminata: a fMRI study
R. Lange, T. Hassa, C. Weiller, C. Dettemers; Universitätsklinikum Freiburg (Freiburg, D); Kliniken Schmieder (Gailingen, D); Kliniken Schneider (Konstanz, D)

Background: Fatigue is a very common symptom of encephalomyelitis disseminata which has a great impact on quality of life and productivity. Diagnosis of fatigue is currently made by structured interviews and self assessment scores. The aim of our study is to investigate the organic basis of fatigue and to develop an objective marker of fatigue in encephalomyelitis disseminata using functional magnetic resonance imaging (fMRI). Methods: We investigated 19 patients with confirmed diagnosis of encephalomyelitis disseminata reporting fatigue in a cross-sectional, prospective study. Patients with severe motor disabilities as well as patients with moderate or severe cognitive impairment were not included in the study. Characterization of the patient group included the modified fatigue impact scale (MFIS), expanded disability status scale (EDSS), Beck depression inventory (BDI) and a neuropsychological test battery. The well known n-back task, which induces a high cognitive load concerning working memory, attention and concentration was used in the blocked fMRI experiment. N-back levels one and two were pseudo-randomized in six blocks per session. Five consecutive sessions resulted in a total investigation time of 20 minutes. A group of eight healthy subjects were investigated with the same paradigm and served as control group. Results: The mean EDSS was 3.1 (± 1.6), MFIS 42.7 (± 14.8) and BDI 9.2 (± 6.5). Patients activated a widespread network of frontal, pre-frontal, parietal, cerebellar and paracentral areas. Differential effects between the n-back difficulty levels showed increased activation for the more difficult 2-back condition in the parietal and frontal regions as known from the n-back task. Comparison with the control group yielded stronger activation in the cingulate gyrus in the patient group for the easier 1-back condition. Conclusion: The n-back task is well suited to investigate ED-patients using a fMRI experiment within less of half an hour with consistent results. ED-patients seem to require more effort to perform even the easier condition of the task as indicated by increased activation in the cingulate gyrus. The absence of differences in the more difficult condition may be due to already maximized effort in both groups. As fatigue was the leading symptom in our patient group, the described effect could be attributed to ED-related fatigue. More sophisticated regression analysis within the patient group are in progress.

P186

Differential brain compensation for cognitive versus motor processes in early relapsing-remitting multiple sclerosis subjects without clinical impairment
M. Burke, R. Mahurin, J.D. Bowen; University of Washington (Seattle, USA)

Objective: To assess regional cortical alterations of functional MRI (fMRI) brain activation in relapsing-remitting multiple sclerosis (RRMS) versus controls by a parallel design utilizing standardized clinical cognitive measures in conjunction with functional brain imaging with identical tasks. Background: Clinical staging and disability status in MS are frequently based on results from clinical cognitive assessment. However, it is unclear to what extent cognitive test performance within normal ranges reflects integrity of associated brain networks. Design/Methods: We studied 15 RRMS (mean age 43.6 SD 8.2 years, mean EDSS = 4.5) and 10 control (mean age 45.2 SD 9.6 years) right-handed subjects. Outcome measures included global CNS function, cognitive, and motor tests. Each fMRI task followed a boxcar paradigm on a GE 1.5-T scanner. The two fMRI tasks consisted of a verbal numeric reasoning task (logical reasoning) and a simple motor task (fingertap). fMRI results were compared to prescan performance of identical computer-based versions of the same tasks.
(NeuroCog Assessment Battery). **Results:** The prescan logical reasoning task showed no significant difference between MS and controls (p > 0.05); however, MS subjects reported significantly greater perceived effort than controls (p < 0.01). Statistical parametric mapping of fMRI data revealed greater activation in MS subjects than controls. Between-group differences were found in the orbitofrontal cortex and cerebellum for the logical reasoning fMRI task. Performance on prescan fingertap showed no significant difference between MS and control subjects (p > 0.05), with no between-group differences noted on level and extent of brain activation within the left motor cortex and supplementary motor area. **Conclusions:** Increased brain activations during a logical reasoning task compensates for minor cognitive disability in MS, suggesting that compensatory mechanisms enabled MS subjects to attain control performance levels for the computerized task, but with more perceived effort. This effect was not found for a simple motor task in subjects without motor impairment. These compensatory cognitive processes appear to arise earlier in the disease course than for simple motor activity. Study supported by: Veterans Health Administration.

**P187**

**Development and disruption of functional cerebral grey matter reorganisation depending on disease progression in MS patients (according PET and MRI data)**

A. Ilves, L. Prakhova, G. Katsaeva, N. Tolotayn, A. Pozdnyakov, I. Stolyarov, A. Scoromets; Institute of Human Brain (St Petersburg, RUS); St.Petersburg State Medical University I.P. Pavlov (St Petersburg, RUS); Central Research Institute of Roentgenology and Radiology Health Ministry of Russia (St Petersburg, RUS)

**Background:** Experimental data concerning the changes in cerebral grey matter in multiple sclerosis (MS) are ambiguous to present day. There is proven global and regional brain atrophy, on the other hand regional increase in neuronal activity is revealed. **Objective:** To investigate the structural-functional changes in the cerebral grey matter depending on disease progression in MS patients. **Patients and Methods:** 107 patients with clinically definite MS and 21 age matched healthy control were examined. EDSS range was: 0 and 6. All subjects underwent 1.5-T MRI and Positron Emission Tomography (PET) studies using 18F-fluorodeoxyglucose. To analyse the pathogenesis of disease progression the patients were divided into 3 groups: relapsing-remitting(RR) MS, progressive (PR)MS with EDSS ≤ 6, PRMS with EDSS > 6. All PET data were analysed by homemade Image Analysing programs. BPV (brain parenchyma volume) and basal ganglia volumes were calculated as percentage of intracranial volume with program Java Image provided by “Xnapse Systems” (UK). **Results and Conclusions:** In the progression of MS (in transformation from RRMS to PRMS and EDSS increase) the regional Cerebral Metabolic Rate of Glucose (rCMRglu) reduction of the cerebral grey matter develops initially in the supplementary motor cortex of dominant hemisphere in RRMS, finishing strongly pronounced reduced global CMRglu in PRMS with EDSS > 6 group. In PRMS patients with EDSS ≤ 6 relative increase rCMRglu were revealed also. rCMRglu (functional activity) reduces in regions are directly charged with realization of impaired functions and increases in regions, which are functionally related with structures, charged with realization of impaired functions. The aforesaid enable to conclude about the stable functional cerebral grey matter reorganization, compensatory upcomings in the PRMS patients with EDSS ≤ 6, which have an adaptive role and limits the clinical manifestations of disease. In PRMS with EDSS > 6 group the regional increase rCMRglu was not revealed. Decompen- sation of the functional cortical reorganization in PRMS with EDSS > 6 group correlated with progression of the global brain atrophy (BPV = 95.6% in control group, 85.8% in MS with EDSS ≤ 6, 83.3% in PRMS with EDSS > 6) and regional basal ganglia grey matter atrophy (n.(caudate volume)/0.48% in control group, 0.44% in PRMS with EDSS ≤ 6, 0.35% in PRMS with EDSS > 6), more prominent in thalamus (volume = 1.20% in control group, 1.19% in PRMS with EDSS ≤ 6, 0.49% in PRMS with EDSS > 6). Pathological substrates of chronic black holes (CBH) cannot be defined on conventional magnetic resonance imaging (MRI) in patients with multiple sclerosis (MS). Yet, their in vivo characterization would be valuable as CBH represent more severe damage. **Tissue Specific Imaging (TSI)** is an innovative Double Inversion Recovery variant that produces three images, each suppressing two major tissue types (e.g. grey and white matter) based on T1. Tissues with long T1, corresponding to freely moving liquid, such as cerebrospinal fluid (CSF), are selectively shown. We measured magnetization transfer ratio (MTR) of CBH visible and not visible on 3D-TSI-CSF to test the hypothesis that the former represents more advanced disease pathology. Eighteen patients with MS (Expanded Disability Status Scale (EDSS) 0–7.5, 9 relapsing-remitting (RR) and 9 secondary progressive (SP)) who had an MRI performed at the NIH at least 24 months earlier were included. Apart from the retrospective evaluation of this MRI, the study consisted of a set of 3T and 1.5T MRI. At 3T, inversion recovery prepared 3D fast spoiled gradient-recalled (IR-FSPGR), 3D-TSI and T1-weighted images (pre- and post-contrast) were acquired. At 1.5T, T1 ON and OFF magnetization transfer (MT) images were obtained. A BH identified on the 3T-IR-FSPGR was defined as CBH if present as a hypointense lesion without a concomitant enhancement and already visible on the 1.5T MRI acquired at least 24 months earlier. One identified, those CBH were sorted in two groups. Group-A (g-A) CBH corresponded to CBH also visible as hypointense on 3D-TSI-CSF. Group-B (g-B) CBH were those not appearing on the 3D-TSI-CSF. MTR of g-A and g-B lesions were calculated and compared. All patients showed at least one CBH. G-A lesions were identified in all patients but 2 RRMS patients (EDSS of 1.5 and 0, disease duration 9 and 3 years, respectively). MTR values of g-A lesions (0.224[0.059]) were lower (p < 0.001) than those of g-B lesions (0.319[0.025]) and MTR values of g-A lesions were the closest to CSF-MTR. No significant differences were found between SPMs and RRMS patients relative to MTR values of g-B lesions but a strong trend (p = 0.059) towards a lower g-A MTR was seen in SPMs subjects. For the first time, 3D-TSI-CSF shows in vivo that not all CBH are at the end stage of pathology. Thus, 3D-TSI-CSF imaging offers a promising tool for in vivo characterization of CBH without further post-processing.

**P189**

**Grey matter disease in patients with multiple sclerosis imaged at high field: correlation between cortical and subcortical pathology**


It is well known that grey matter (GM) pathology may affect patients with multiple sclerosis (MS) leading to substantial disability. Cortical lesions as well as reduction of cortical and subcortical structures volumes have been identified in both post-mortem and in vivo studies. However, it is not clear whether focal/diffuse cortical and subcortical pathology occur independently within the same individual or are linked via a similar pathological substrate in MS patients. In the present study, we aimed at investigating correlations between cortical and subcortical disease in a cohort of MS patients imaged at high magnetic field scan. Thirty-one patients with MS (13 secondary progressive [SP] and 18 relapsing remitting [RR], mean ± standard deviation) age 44 ± 7.9, Expanded Disability Status Scale [EDSS] score 3.7 ± 2.1, MS duration 14 ± 7.3 years, composite score: 0.0123 ± 0.498)
and 24 age-gender matched healthy volunteers were included. Each subject underwent a single magnetic resonance imaging (MRI) at 3 Tesla equipped with an 8-channel phased array coil. Conventional T1 pre/post contrast and T2 images as well as three 3D Inversion Recovery Spoiled Grass sequences (SPGR) were obtained. Global and regional cortical thickness (CTh), as well as thalamus and basal ganglia (BG) volumes were measured on the mean image obtained from the 3 co-registered SPGRs by using free-surf (version 20060418 stable release). At the time of the abstract preparation, all the patients and HVs have been imaged. Results from post-processing analyses are available in approximately 50% of the subjects, the majority of whom in the SP stage of MS. Preliminary results showed a substantial reduction in mean thalamic (p ≤ 0.0001) and BG volume (p = 0.001) in patients compared to HVs. Though a trend in atrophied CTh was seen in patients, differences in mean CTh between patients and HVs were not visible in the small sample size analysed so far. Significant correlations were found between thalamic and BG volumes (p ≤ 0.0001, r = 0.9). Neither thalamic nor BG volumes correlated significantly with the mean CTh. Findings related to the entire population will be obtained and presented, thus allowing for more straightforward conclusions. At the moment, the preliminary observations allow us to conclude that subcortical pathology has a high independent component from cortical disease in MS patients.

P190

Comparison of 1.0T and 3.0T MRI findings in follow-up of patients with multiple sclerosis
T. Stosic-Opincal, M. Gavrilovic, S. Lavrnic, S. Gavrilovic, R. Milenkovic, M. Jovancevic; Clinical Center of Serbia (Belgrade, CS); Railway Health Care Institute (Belgrade, CS)

Background: The purpose of this study was to estimate the advantage of 3.0 Tesla (T) over 1.0T field strength MRI systems in evaluation and follow up of patients with multiple sclerosis (MS), by comparing relative sensitivity using similar acquisition conditions.

Method: We performed scans on twenty-three patients with clinically definite MS at 1.0T and 3.0T MR systems on the same day. Scans were repeated on both systems in a six months interval. Similar patient positioning techniques and pulse sequences were used: T1-weighted spin echo (T1W-SE) with and without gadolinium contrast injections, T2W SE and fluid attenuated inversion recovery (FLAIR) imaging. Three experienced neuroradiologists examined images independently by focal lesion counting.

Results: At 1.0T field strength a total of 41 gadolinium-enhancing lesions were detected in 10 of the 23 patients studied. On the 3.0T MR images, 51 lesions were detected in 13 patients representing a 24.4% increase in the number of detected enhancing lesions. 3.0T FLAIR scans showed a 235 hyperintense white matter lesions compared to 205 lesions on 1.0T scans. That represents a 14.6% increase in the number of detected lesions. Six months later a total of 55 gadolinium-enhancing lesions were detected with 3.0T (7.8% increase) compared with 43 lesions with 1.0T MR system (4.9% increase). Increase in number of hyperintense white matter lesions was 2.9% for 1.0T and 7.7% for 3.0T system. Conclusion: High-field 3.0T MR imaging demonstrates better sensitivity in the detection of focal brain lesions in MS. This improvement is more apparent in contrast enhanced lesion detection and less noticeable in FLAIR detected lesions. 3.0T MRI system provides additional diagnostic information in follow-up of patients with MS.

P191

High field-high yield: detecting multiple sclerosis white matter lesions at 9.4 tesla
K. Schmierer, H.G. Parkes, P.W. So, D.H. Miller, T.A. Yousry; Institute of Neurology, UCL (London, UK); Imperial College (London, UK)

Objective: To explore whether compared to magnetic resonance imaging (MRI) at 1.5 Tesla (T) MRI at 9.4T allows more accurate assessment of multiple sclerosis (MS) pathology in MS brain.

Background: Several MR modalities are being applied to probe MS in vivo [1]. The investigation of post mortem MS brain using MRI and histological methods allows the pathological correlates of MR changes to be assessed directly. Limits in image resolution and signal-to-noise at 1.5T impede co-registration between MR and histology. However, accurate detection and co-registration between MR and histology is important in such studies to (i) enable the most reliable MR-pathological correlations (ii) overcome the failure to detect up to 40% of pathologically confirmed white matter lesion (WMLs) on standard 1.5T MRI [2].

Methods: Fixed brain slices (1 cm thick) of four subjects with MS (mean age 68 years [SD 5]) were studied. On a 1.5T scanner dual spin echo (SE) proton density and T2-weighted (repetition time [TR] 2000; echo time [TE] 30–120 ms; flip angle 90°, matrix size 256 × 256, fields of view [FOV] 240 × 180 mm and 300 mm²) images were acquired. Using a stereotactic system ROIs were selected and blocks dissected [3]. Using a special sample holder the blocks were then scanned on a 9.4T Varian Unity (+). Contiguous slices through each block were obtained (FOV 3 cm, matrix size 256 × 192, slice thickness 1 mm). Five sets of SE images (TE 13-60 ms; TR 2 s; 4 averages) were obtained and T2 maps produced. Tissue blocks were then dissected, processed for embedding in paraffin and sections stained for Hematoxylin & Eosin (H&E) and Luxol-Fast blue (LFB). The number of lesions on H&E and LFB stained sections was compared with respective ROIs on the scans acquired at 1.5T and 9.4T.

Results: Five tissue blocks were included, each of which containing – at 1.5T – a single WML only. The first block of which all data became available showed five WMLs on scans acquired at 9.4T. All these WMLs corresponded to WMLs on histological sections. Conclusion: MRI at 9.4T appears to be a powerful tool to detect WMLs due to MS in much more detail than present at 1.5T. Analysis of the remaining four blocks is currently underway. Further studies will include calculated T1, T2 and MTR maps at 9.4T and quantitative measures of axonal damage, inflammation and gliosis.

References
P194

Time between disease onset, MS diagnosis and treatment with disease modifying therapy and their relationship with brain atrophy and burden of disease in low disability relapsing-remitting multiple sclerosis patients
D.L. Cookfair, B. Weinstock-Guttman, F.E. Munschauer, N. Garg, E. Zivadinov; University at Buffalo, State University of New York (Buffalo, USA)

Background: Despite the availability of disease modifying therapies (DMTs) for relapsing remitting (RR) multiple sclerosis (MS), there is sometimes a delay between diagnosis of MS and initiation of DMT, and many patients who experience clinically isolated syndromes do not receive treatment with DMT. Objective: To assess whether longer time between symptom onset and MS DX (TOD), delay in treatment with DMT following DX (DT), and/or total time from disease onset to commencement of treatment with DMT (TT) are associated with increased levels of brain atrophy or burden of disease (BOD) as measured by MRI in patients with low to moderate disability RR MS. Methods: 215 consecutively accrued MS patients who underwent 1.5 MRI scan and clinical evaluation were studied using a cross-sectional design. Study inclusion criteria were: RR disease; EDSS score < 3.5; MS DX ≤ 13 years ago; age 18–65; presence of DMT treatment within the past 2 years; no steroid treatment or relapse in 3 months prior to study entry. Mean (SD) disease duration was 10.1 (1.2) years; EDSS 1.9 (0.7); age 42.7 (8.2); % Female = 82.7%. Brain parenchymal fraction (BPF), gray matter fraction (GMF), and white matter fraction (WMF), T2 and T1 lesion volume (LV) were calculated. Strength and significance of associations were measured using Spearman correlation coefficients (Sp r) followed by stepwise multiple linear regression to adjust for potential effects of age and gender. Results: TOD, DT and TT years showed weak significant correlations with BPF as measured by Sp r (rs: –0.236 to –0.295, p-values < 0.001); GMF (rs: –0.207 to –0.286, p-values < 0.002) and also with BOD measures: T2-LV (rs: 0.292 to 0.248; p-values < 0.004); T1-LV (rs:0.193 to 0.249; p-values < 0.005), but not with WMF. After adjusting for age, gender and steroid use in the past, TOD, DT and TT were still associated with BPF and (cube root) T2-LV. Age+TOD explained 22.7% of variance in BPF (p = 0.017); TT+age explained 5.6% of variance in T2-LV (p = 0.001). DT+age+gender best predicted GMF (R² = 0.347; p = 0.006); and TT+age best predicted (cube root) T1-LV (R² = 0.065; p = 0.04). Conclusions: These data suggest that failure to initiate treatment for RR MS in patients due to lack of a definitive MS DX may increase the likelihood of brain atrophy, while the delay in TT may increase both the likelihood of atrophy and formation of T2 and T1 lesions. Our data provide additional support for early intervention in MS patients.

P193

Functional MRI of the spinal cord in patients with relapsing remitting MS and low disability: a preliminary study
M. Filippi, P. Valsasina, F. Agosta, M.A. Rocca, D. Caputo; MRI Research Group, Fondazione Don Gnocchi (Milan, I); Scientific Institute San Raffaele (Milan, I); Fondazione Don Gnocchi (Milan, I)

Background: Functional magnetic resonance imaging (fMRI) of the spinal cord has proven to be useful in investigating sensory and motor activity in the cervical and lumbar spinal cord in healthy subjects and in patients with spinal cord trauma. To date, spinal fMRI has been applied in a preliminary study of multiple sclerosis (MS) showing, in patients with the progressive phenotypes of the disease, patterns of cord activity resembling those of subjects with cord trauma. Goals: To determine the feasibility of spinal fMRI using a 1.5 Tesla clinical MR system and to evaluate the extent of the activations in the cervical cord of patients with relapsing remitting (RR) MS and low disability. Methods: Cervical cord fMRI (Turbo Spin-Echo sequence: TR = 2850 ms, TE = 11ms, flip angle = 120°, 9 axial slices with thickness = 7 mm, in-plane resolution = 0.39 x 0.39 mm) was acquired from 6 patients with RRMS (3 men and 3 women; mean age = 39.0; mean disease duration = 13.3 years; median Expanded Disability Status Scale score = 1.5) and 5 healthy controls. During the fMRI session, using a block design, the subjects were cued while performing two tasks: a passive motor task consisting of repetitive flexion-extension of the wrist of the right hand and a sensory task, consisting of tactile stimulation of the right hand. FMRI data analysis was performed using a custom-made software, written in MatLab, in the time domain on a pixel-by-pixel basis by computing the cross-correlation R between fMRI temporal series and the exercise paradigm, after images realignment and application of a median filter. Statistical maps of significant activations were generated by segmenting the spinal cord by an image mask. Results: An average intensity increase of the fMRI signal was observed during the performance of both tasks (4.0%±1.1% and 4.3%±1.6% in healthy subjects and in MS patients during the passive motor task; and 2.8%±1.5% and 3.9%±0.8%, respectively, during the sensory task). Although a slightly increase of activation in the cord was visible in the patients, the difference was not significant, perhaps of the small number of patients included. Conclusions: This preliminary study shows that spinal fMRI is feasible in MS patients. The information obtained by the use of spinal fMRI might contribute to improve our understanding of MS pathophysiology and to design effective rehabilitation programs.
valiations were used to assess the predictive value of MRI. **Results:** We found no significant effect of the MRI parameters in a multivariate Poisson regression model. This is similarly true for MRI parameters individually, supporting the results of Held et al. 2005. Despite these negative results, we did check the goodness of predictions for the model including no MRI information (consisting of number of pre-study relapses and disease duration) and models with MRI parameters. The mean squared errors for the predictions were assessed via cross validation. This analysis suggests that an additional inclusion of MRI parameters leads to model over-fits and thus increased errors in estimations. **Conclusion:** We found evidence that if information on previous relapses is available, MRI parameters have no additional value for the prediction of future relapses.

**P196**

A 3 Tesla functional MRI investigation of the role of the “mirror-neuron system” in patients with relapsing-remitting multiple sclerosis

M.A. Rocca, A. Ceccharelli, P. Tortorella, A. Falini, G. Scotti, G. Comi, M. Filippi; Scientific Institute San Raffaele (Milan, I)

**Background:** Several functional magnetic resonance imaging (fMRI) studies of simple movement performance in patients with multiple sclerosis (MS) have suggested that the absence of overt clinical symptoms despite the presence of widespread tissue damage might be related to an increased recruitment of brain networks that are usually active in healthy individuals when performing more complex tasks. **Aims:** To investigate in patients with MS the function of the “mirror-neuron system”, a fronto-parietal network that, in humans is thought to be involved in action observation and imitation learning. **Patients and Methods:** In 16 right-handed patients with relapsing-remitting MS (F/M = 13/3, mean age = 35.0 years, mean disease duration = 9.0 years, median EDSS score = 1.5) and 14 sex- and age-matched healthy volunteers, we acquired fMRI using a 3 Tesla scanner to investigate the performance of two different motor tasks. The first consisted of repetitive flexion-extension of the last four fingers of the right hand [simple task (ST)] alternated to epochs of rest, while the second [mirror task (MT)], consisted of observation of a picture showing another subject while performing the same task alternated to epochs of rest. FMRI analysis was performed using SPM99. **Results:** During the ST, when compared to controls, MS patients had more significant activations of the contralateral primary sensorimotor cortex (SMC) and bilateral supplementary motor area. During the MT, both groups showed the activation of several visual areas, the intraparietal sulcus (IPS), bilaterally, and the left inferior frontal gyrus (IFG). This latter region was significantly more active in patients than controls. The between-group interaction analysis between ST and MT showed that in MS patients, the IPS was more active also during the ST. **Conclusions:** Functional cortical changes can be detected in MS patients not only when investigating the performance of active tasks (ST), but also during passive ones (MT), thus indicating the presence of cortical reorganization. During the performance of a simple motor task, MS patients activate cortical regions that are recruited in healthy subjects when performing a more complex task. This might yet represent an additional mechanism with the potential to limit the severity of the clinical outcome associated with MS-related tissue damage. This study was supported by a grant from FISM (2004/R/7).

**P197**

Multiparametric MRI assessment of brain and cord damage in primary progressive multiple sclerosis: a large-scale, multicentre study

M. Rovaris, E. Judica, E. Perego, B. Benedetti, F. Barkhof, N. De Stefano, Z. Khaleeli, T. Korteweg, D.H. Miller, X. Montalban, C. Polman, A. Rovira, J. Sastre-Garriga, A.J. Thompson, M. Filippi; Scientific Institute San Raffaele (Milan, I; VU University Medical Centre (Amsterdam, NL); University of Siena (Siena, I); Institute of Neurology (London, UK); Hospital Vall d’Hebron (Barcelona, E)

**Background:** Although the mechanisms underlying the accumulation of disability in patients with primary progressive MS (PPMS) are still unclear, a major role seems to be played by “occult” rather than by conventional MRI-detectable tissue damage. The potential of non-conventional MRI in the work-up of PPMS remains, however, a matter of debate. **Goals:** To investigate the usefulness of a multiparametric approach and determine if magnetization transfer (MT) complements conventional MRI for large-scale natural history studies and treatment trials of PPMS. **Methods:** Conventional and MT MRI data from 226 patients with PPMS, which had been locally acquired in participating centers, were sent to Milan for image post-processing and analysis. The imaging protocol consisted of dual-echo, T1-weighted and MT MRI scans of the brain, with axial slice orientation and 5-mm slice thickness. In the vast majority of patients, 3D scans of the cervical cord were also available. Expanded disability status scale (EDSS) score was rated at the time of MRI acquisition and after a median period of 4.5 years. Eighty-four healthy subjects locally underwent the same brain MT MRI protocol to serve as controls. Centralized image analysis comprised T2 lesion volume (LV), normalized brain volume (NVB) and C2 cross-sectional area (CSA) measurement. Following MT ratio (MTR) maps creation, MTR histograms from the whole brain tissue, the normal-appearing white matter (NAWM) and gray matter (GM) were also obtained. **Results:** The mean values of NVB and CSA, as well as those of MTR histogram-derived metrics, showed a significant inter-center heterogeneity. After correcting for acquisition center, pooled average MTR and histogram peak height values were significantly different between PPMS patients and controls for whole brain, NAWM and GM. Significant (weak to moderate) correlations were found between MT MRI changes and T2 LV or NVB. **Conclusions:** MT MRI is sensitive to “occult” tissue damage in PPMS and might provide complementary information to those given by conventional MRI when monitoring the disease evolution. Sequence-related variability of measurements makes a standardization of MT MRI acquisition prior to study initiation essential for the design of multicenter studies.

**P198**

Magnetisation transfer MRI metrics predict the accumulation of disability eight years later in patients with multiple sclerosis

F. Agosta, M. Rovaris, E. Pagani, M.P. Sormani, M. Rodegher, G. Comi, M. Filippi; Scientific Institute San Raffaele (Milan, I)

**Background:** In multiple sclerosis (MS), the relationship between conventional magnetic resonance imaging (MRI) findings and the clinical evolution of the disease is weak. Magnetisation transfer (MT) MRI can provide markers reflecting the more disabling features of MS pathology. **Goals:** Aim of the present study was to assess the value of MT MRI quantities and their short-term changes in predicting the long-term accumulation of disability in patients with MS. **Methods:** Seventy-three patients were included, either suffering from definite MS for at least two years, with a relapsing-remitting (n = 34) or secondary progressive (n = 19) disease course, or from clinically isolated syndrome suggestive of MS (n = 20), with the first clinical attack in the preceding three months and paraclinical evidence of spatial disease dissemination. All patients were followed prospectively with clinical visits for a median period of 8.0 years. Thirteen sex- and age-matched controls with no previous history of neurological diseases and with a normal neurological examination were also studied. Conventional and MT MRI scans of the brain were obtained at baseline and after 12 months in all subjects. At baseline and at 12 months, T2-hyperintense lesion volume (LV), T1-hypointense LV, normalised brain volume (with gray [GM] and white matter...
Determinants of disability in multiple sclerosis: a cross-sectional, multiparametric, quantitative MR-based study of disease phenotypes

A. Pulitzi, M. Boruvka, E. Judica, B. Benedetti, M.P. Sormani, V. Martinelli, A. Fallini, G. Comi, M. Filippi; Scientific Institute San Raffaele (Milan, I)

Background: Diffusion tensor (DT) MRI is able to quantify the severity of tissue damage in multiple sclerosis (MS), both within T2-visible lesions and in the normal-appearing white matter (NAWM) and gray matter (GM). Proton magnetic resonance spectroscopy (1H-MRS) can be used to measure in vivo whole brain N-acetylaspartate (WBNAa), whose decrease is considered a marker of neuronal loss and/or dysfunction.

Goals: To investigate the strength of the correlation between a composite measure of conventional MRI-derived metrics (Z4) and baseline clinical characteristics for subjects entering the CombiRx Trial.

Methods: A microarray study

Experimental models

Neuronal response to experimental demyelination and axonal transaction – A microarray study

G. Lovas, J. Nielsen, L. Hudson; Jahn Ferenc Hospital (Budapest, HUN); National Institutes of Health (Bethesda, USA)

Demyelination and axonal injury constitute the major pathologic features of multiple sclerosis. Both have a profound impact on neurological impairment through blocking physiological neural function. To assess alterations of gene expression patterns in affected neurons, chemically-induced demyelination and axotomy was performed in the pontocerebellar pathway of rats. Four, ten and thirty-seven days following lesion of the cerebellar white matter, contralateral (affected) and ipsilateral (unaffected) neurons of the pontine nuclei were isolated and screened for messenger RNA expression using microarrays (Affymetrix gene chip arrays). Gene expression data from SHAM operated and healthy control animals was also gathered. DNA damage-related transcripts, cyclins, transcription factors, hormone precursors and neuropeulators were among the groups of affected genes. In the case of activating transcription factor 3 (ATF3) and thyrotropin releasing hormone (TRH), the messenger RNA alterations were confirmed at the protein level by immunohistochemistry.
Analysis of the large data sets revealed that demyelination and axotomy primarily induce a 'common neuronal response to injury', besides activating distinct gene clusters specific to the injury type. These results should facilitate the identification of target molecular cascades for potential neuroprotective drug development in the most common human demyelinating disease.

P202

Heat shock protein 70 is essentially involved in the generation of the autoimmune response to myelin antigen in EAE, an animal model of multiple sclerosis

M. Mycko, H. Cwaklinka, C.S. Raine, K. Selman; Medical University of Lodz (Lodz, PL); Albert Einstein College of Medicine (New York, USA)

Protracted inflammation has been associated with the generation of autoimmune responses. One of the major outcomes of inflammatory stress concerns a sharp increase in the chaperonin, heat shock protein 70 (hsp70). Previous experiments in vitro have shown that over-expression of inducible hsp70 enhanced responsiveness to myelin basic protein (MBP). To prove that hsp70 is involved in myelin antigen recognition, we applied a mouse deficient in one of the major genes encoding inducible hsp70, hsp70.1. Hsp70.1⁻/⁻ mice sensitized for experimental autoimmune encephalomyelitis (EAE) with myelin/oligodendrocyte glycoprotein (MOG) peptide 35-55, displayed almost complete resistance to the disease. This resistance correlated with the loss of T cell proliferation to MOG35-55 and interferon gamma production. Interestingly, hsp70 deficiency led primarily to T cell dysfunction demonstrated by antigen presentation assays which showed MOG35-55 reactivity to be abolished when T cells from hsp70.1⁻/⁻ mice were co-cultured with wild type antigen presenting cells. The mechanism of T cell failure was shown to be TCR dependent and to involve activation-induced apoptosis. These results provide compelling evidence for a role for hsp70 in the recognition of autoantigen, MOG, and strengthen the hypothesis that stress-associated induction of hsp70 modulates autoimmune reactions.

P203

Virus-induced chronic neuro-inflammation and failure of neuroprotective processes

A. Bernard, S. Cavagna, C. Rey, M. Fevre-Montagne, N. Davoust, C. Confavreux, P. Giraudon; INSERM (Lyon, F); Hopital Neurologique (Lyon, F)

Viruses are suspected as etiological agents of several human CNS diseases, including Multiple Sclerosis. They may trigger brain disorders leading to neuronal cell death or impairments of synaptic plasticity and neurotransmitter release. Viruses may imbalance CNS homeostasis via viral products or immune responses. However little is known on the in vivo association between chronic inflammation and neurodegenerative processes. We used a virus-induced mouse model of chronic brain inflammation to investigate such an association. Presence of CD4 and CD8 T cells concomitantly with expression of pro- and anti-inflammatory cytokines, as well as MMPs and TIMPs and enhanced extracellular matrix proteolysis characterized this model. In vivo study of brain metabolism using proton spectroscopy clearly indicated a neuronal suffering, demonstrated by the lower expression of the neuronal marker N Acetyl Acetate. To further investigate the relationship between chronic neuroinflammation and neuronal alterations we performed brain gene expression analysis. Genes coding for molecules that ensured homeostasis and neuroprotection: NeuroD1, a member of the bHLH transcription factor family, which plays a pivotal role in neural progenitor differentiation and neurogenesis; -ATPase inhibitory factor known to regulate synaptic transmission and mitochondrial functioning; -Neuronal Pentraxin, a synapse-associated protein cluster factor for the AMPA glutamate receptors that participate to axonal guidance. In addition, the down regulation of ± internexin, neurofilament acting on axonal growth and microtubule stabilization, and SPARC implicated in cell/ECM interaction, pointed out cytoskeleton and ECM integrity impairments, respectively. These data indicate that defect in neuroprotection may result from accumulation of immune cells that sustain inflammation, leading, even in the absence of virus, to neural dysfunction and neurodegenerative process.

P204

A novel TNF family member, TRAIL, induces death of human oligodendrocytes by JNK activation prior mitochondrial pathway

M. Matysiak, A. Jurewicz, S. Andrzeja, K. Selman; Medical University (Lodz, PL)

Background: TRAIL (TNF-related apoptosis inducing ligand) belongs to a broad TNF family of molecules. TRAIL is expressed by autoreactive T cells and was shown to induce death of oligodendrocytes. However intracellular signaling involved in TRAIL-induced oligodendrocytes death is unknown. Methods: We assessed involvement of caspase and c-jun NH2-terminal kinase (JNK) in oligodendrocytes death after TRAIL stimulation. Caspases activation was assessed by Western blotting using antibodies against active form of enzymes, using fluorogenic substrates and pancaspase inhibitor. JNK activation was assessed by using antibodies against phosphorylated JNK isoforms and analyzing c-jun phosphorylation by autoradiography. The role of JNK activation was confirmed by using dominant negative construct of MKK4, a kinase upstream of JNK. Changes in mitochondrial membrane permeability was assessed using ApoAlert Mitochondrial Membrane kit and cell death was analyze by FACS using Annexin-V and PI staining. Results: We defined that intracellular transduction signaling involved in TRAIL-induced death of oligodendrocytes is associated with strong, sustained activation of JNK and dominant negative mutant of MKK4 inhibited TRAIL-induced oligodendrocytes death. The immunoprecipitation showed that JNK3 isoform was predominantly activated upon TRAIL-induced oligodendrocytes death and JNK activation occurred before mitochondrial membrane dysfunction. We did not observed activation of caspase pathway as well as other mitogen activated kinases, p38 and ERK. Conclusions: These results indicate that JNK activation prior to mitochondrial dysfunction is critically involved in oligodendrocytes death induced by TRAIL.

P205

Experimental demonstration of T-cell migration into the CNS across the restrictive epithelium of the choroid plexus

N. Strazzie, R. Creidy, C. Malcus, C. Confavreux, J. Boucaut, J.F. Ghesri-Egée; INSERM (Lyon, F); CNRS (Marseille, F); HCL (Lyon, F)

Growing evidence support the hypothesis that both during “physiological” immune surveillance and in inflammatory diseases, T cell entry from the systemic circulation into the ventricular cerebrospinal fluid (CSF) through the choroid plexuses (CP) or into the subarachnoid spaces surrounding meningeal vessels is an important pathway of brain infiltration. This is supported by studies confronting the surface phenotype of CSF-borne T-cells and the expression of adhesion molecules among the various blood-brain interfaces (Proc Natl Acad Sci, 2003, 100: 8389; J Neuroimmunol, 2002, 129: S1). It is also suggested by the selective accumulation of T cells beyond the CP
fenestrated vessels into the stroma, both in normal rats and after peripheral immune activation (J Neuroimmunol, 2005, 162: 19). Yet, the crucial migration step of T cells across the choroidal epithelium, actually forming the anatomic site of the blood-CSF barrier, remains totally unexplored. Direct trafficking of T cells into the CSF was investigated using a reconstituted rat choroidal epithelium that maintains structural (tight junction proteins) and functional (fence function and transport polarity) specific features of the blood-CSF barrier. Activated T-cells expressing various chemokine receptors, exposed at the basolateral, i.e. blood, side of the choroidal epithelial monolayer, migrated through the cellular interface at a low efficiency. Confocal analysis showed that the migration pathway was paracellular, and did not induce any apparent delocalisation of the pericellular tight junction proteins. Accordingly, permeability studies performed on the epithelial monolayer at the end of the migration experiment revealed that the fence function of the barrier was maintained. Transepithelial T-cell migration was strongly stimulated when the apical, i.e. brain/CSF, side of the epithelium was exposed to chemokines that are involved in the physiopathology of multiple sclerosis, again causing no alteration in the tightness of the junctions. These data provide evidence for controlled T cell migration across the blood-CSF barrier, and, in the context of multiple sclerosis may be relevant to the predominant localization of both focal demyelinated plaques and diffuse white matter injury respectively in periventricular and subpial areas (Brain, 2005, 128: 2705).

Expression of chemokines CCL19, CCL20, CCL21 and CCL22 during ChREAE

B. Bielecki, A. Gorzynska, P. Wołalski, A. Glabinski; Medical University of Lodz (Lodz, PL)

Background: Chemokines are chemotactic cytokines which play an important role in development of experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS). These cytokines initiate migration and accumulation of inflammatory cells in the central nervous system (CNS). We have shown previously that several classical chemokines and their receptors are upregulated during attacks of ChREAE and might be responsible for leukocyte entry into the CNS. Goal: The major goal of this study was to analyzed expression of some relatively recently described inflammatory and homeostatic chemokines in the CNS and peripheral tissues at different stages of ChREAE. Methods: Animals with ChREAE were sacrificed and several organs including spinal cord, brain, spleen and kidneys were obtained. Using quantitative Rnase Protection Assay (RPA) and MicroArrays assays we analyzed expression of several inflammatory and homeostatic chemokines including CCL19, CCL20, CCL21 and CCL22. Localization of chemokine expression was analyzed with immunohistochemistry. Results: In brains we observed increased expression of chemokines CCL19, CCL20, CCL21 and CCL22 during relapses of the disease as compared to disease remission. Expression of these chemokines in control animals was significantly lower. Expression of CCL19 and CCL21 was localized in the vicinity of inflammatory foci within the CNS. Expression of chemokines in kidneys was similar in control animals and in mice with ChREAE. Conclusions: Our results suggest the complex pattern of chemokines expression not only in the CNS but also in peripheral tissues during different stages of ChREAE.

Antigen-specific CD4+CD25+ T cells prevent induction of demyelination in experimental allergic neuritis

S. Hodgkinson, G. Tran, N. Carter, M. Killingsworth; University of NSW (Sydney, AUS); University of Sydney (Sydney, AUS); Liverpool Hospital (Sydney, AUS)

Antibody deposition and complement activation, especially membrane attack complex (MAC) formation is thought to be critical to the mediation of injury in experimental allergic neuritis (EAN). In previous studies, we found active experimental allergic encephalomyelitis (EAE) was less severe in a strain of PVG rats that are totally deficient in the C6 component of complement (PVG/C6−/−), and thus unable to assemble MAC, compared to complement sufficient PVG/c rats. The role of complement in demyelination could not be examined because there is limited central nervous system demyelination in EAE. As PVG rats are not susceptible to either active or passive EAN, a model in which there is demyelination, we bred a Lewis C6− strain by backcrossing C6− from PVG/C6−/− onto an EAN susceptible strain of Lewis rats. After 10 generations we have a congenic Lewis rat that is deficient in C6 (Lewis/C6−/−) that is unable to form MAC. Sera from these rats cannot support lysis of red cells in a haemolytic complement assay unless pure C6 is added. Otherwise, these rats are histologically and immunologically identical to normal Lewis rats, in that they accept skin grafts from Lewis rats. EAN was induced by immunization with bovine peripheral nerve myelin in Freund’s complete adjuvant comparing groups of Lewis and Lewis C6− rats. Disease was monitored by daily clinical scoring and weighting. At day 14 and 21 some animals in each group were sacrificed to examine cauda equina nerves for demyelination, cellular infiltration and ultrastructure. Lewis/C6−/− (n=19) were susceptible to EAN, but with a lower disease severity as compared to the wild type Lewis rats (n=20), from day 13 to 24 day. For example clinical score was 0.5 ±0.5 vs. 1.3 ±0.9 (p<0.001) at day 14 and 1.6 ±0.4 vs. 2.0 ±0.4 at day 21 (p=0.003). There was no difference in weight loss between Lewis C6−/− and Lewis. The degree of demyelination in Lewis/C6−/− was significantly less than Lewis, 8.2 ±8 vs. 28 ±13% at day 14 (p<0.003) but not at day 21, 22.5±14 vs. 30 ±17%. Electron microscopy confirmed there was wide spread nerve demyelination in both groups. These results suggest that although MAC has a role in the severity of the disease and lack of MAC delays the onset of severe disease, that MAC is not required to mediate demyelination. MAC may promote injury by sub-lytic action. Thus MAC is not essential for demyelination.
P209
Therapy with interleukin 5 suppresses autoimmune T cell-mediated peripheral nerve demyelination in EAN and increases CD4+CD25+ cells
S. Hodgkinson, G. Train, N. Carter, N. Verma, K. Plain, M. Killingsworth, B. Hall; University of NSW (Sydney, AUS); University of Sydney (Sydney, AUS); Liverpool Hospital (Sydney, AUS)

Th2 cell cytokines reduce aggressive immune inflammation mediated by Th1 cells. We examined if IL-5, a Th2 cytokine that is considered pro-inflammatory in allergy, asthma and parasitic infestations, had any role in altering the severity of inflammation and demyelination in experimental allergic neuritis (EAN) induced in Lewis rats by immunization with bovine peripheral nerve myelin in Freund’s complete adjuvant. IL-5 treatment had major effects on EAN, a disease of T cell mediated demyelination of peripheral nerve fibres. IL-5 therapy commencing at the onset of disease, usually commencing at day 12, reduced the severity of paralysis and weight loss at days 14 through to 18 (p < 0.01) compared to controls and sham treated controls (n = 14, all groups). The disease did not worsen within a day of commencing IL-5. Nerve demyelination was markedly reduced with differences at both 2 and 9 days after starting therapy, with <4% of nerves demyelinated compared to >25% in controls (p < 0.0001) at 9 days. At the site of immunization, IL-5 increased CD4+CD25+ T cells and reduced Th1 cell infiltration into the nerves. These CD4+CD25+ T cells’ proliferative response to PNM in vitro was significantly enhanced (p < 0.06) by addition of IL-5 to the culture. This study showed IL-5 therapy rapidly inhibited autoimmune T cell mediated demyelination, probably by activation of CD4+CD25+ T cells that are induced to express the IL-5 receptor and respond to IL-5. This study suggests IL-5 may be an anti-inflammatory cytokine of use in therapy of demyelinating diseases and may act by induction of CD4+CD25+ T regulatory cells.

P210
Macrophage phenotype during relapsing experimental autoimmune encephalomyelitis can be predicted by USPIO MRI at disease onset
J. Mikita, M. Deloire, T. Touil, M.H. Cannon, C. Boiziaux, V. Dousseau, K.G. Petty, B. Brochet; University V. Segalen (Bordeaux, F)

Background: Macrophage infiltrates in experimental autoimmune encephalomyelitis (EAE) and multiple sclerosis (MS) can be monitored in vivo by MRI with ultrasmall super paramagnetic iron oxide (USPIO) nanoparticles. In pathological conditions macrophages present at least two main phenotypes: proinflammatory M1 macrophages express inducible Nitric Oxide Synthase (iNOS) while immunomodulatory M2 macrophages express Arginase I. Objective: To study the value of USPIO imaging to predict macrophage phenotype in EAE. Methods: We used a relapsing EAE to study macrophage phenotypes within inflammatory infiltrates at the clinical onset (group I), at the end of first attack (group II) and during the second attack (group III). USPIO MRI was performed at the clinical onset and was repeated for animals of group III. Animals with MRI signal changes were considered as MRI+. On serial thin sections of brainstem activated macrophages were quantified by anti ED1, M1 macrophages by anti iNOS labelling using confocal immunofluorescence and M2 by arginase I labelling; axonal damage and loss in brainstem tissue were quantified by classic immunohistochemistry methods. The obtained results were compared with MRI data. Results: MRI+ rats presented more important macrophage recruitment (ED1+ cell number); cells expressing ED1+ and iNOS+ (M1) were also more abundant in MRI+ animals than in MRI− animals. In regard to clinical disease severity and tissue alterations (axonal loss and tissue damage) significant differences between MRI+ and MRI− animals were observed. M2 phenotype analysis is in progress. Conclusion: MRI performed at the onset of EAE predicts the presence of iNOS-expressing macrophages (M1 phenotype) and extent of nervous tissue lesions in inflamed CNS at different stages of disease course.

P211
A new mechanism of EAE tolerance dependent on NK cell activation by hsp70-peptide complexes
G. Galazka, M. Stasiońek, A. Jarwicz, A. Walczak, A. Zyltch, C. Brozman, C.S. Raine, K. Selmaj; Medical University of Lodz (Lodz, PL); International Institute of Molecular and Cell Biology (Warsaw, PL); Albert Einstein College of Medicine (New York, USA)

In this study, we tested the novel strategy that hsp70 could selectively pick up peptides of immunoregulatory properties from inflamed tissue. We have investigated the effect of a pure hsp70 fraction and an hsp70 fraction associated with peptides (hsp70-pc), isolated from the CNS of control healthy animals or animals with EAE (experimental autoimmune encephalomyelitis), on the subsequent development of immunization for EAE. The hsp70 and hsp70-pc complexes were isolated from brains of healthy mice or mice with EAE and purified using affinity chromatography with ATP- or ADP-agarose column (respectively). We have show that hsp70-peptide complexes (hsp70-pc) isolated from brains of mice with EAE prevented the development of EAE clinically and pathologically when administered before proteolipid protein (PLP-P59-151) immunization. In animals in which EAE had been suppressed by hsp70-pc, lymphocytes showed increased IFN-gamma and NO production. Co-culture of spleen cells from hsp70-pc immunized mice with spleen cells from untreated EAE mice reduced PLP139−151-induced reactivity and induced production of IFN-gamma. In addition depletion experiments, showed that when spleen cells from hsp70-pc immunized mice were deprived of cells expressing NK markers the proliferative response to PLP139−151 was reversed. Finally, we have show that PLP-reactive cells from mice preinjected with hsp70-pc undergo apoptosis in response to restimulation with PLP139−151. To definite prove the role of NK cells in the observed hsp70-pc-induced immune regulation, we performed transfer of NK cells from hsp70-pc immunized mice to recipients sensitized for EAE and abolished disease development. In conclusion, we have demonstrated that peptides derived from inflamed CNS and bound to hsp70 are able to induce a novel regulatory involving NK cells inhibiting autoreactive T cells. These findings might further contribute to our understanding of hsp immunoregulatory mechanisms in autoimmune diseases.

P212
CD 34+ myeloid progenitors and macrophages/microglia: implication in the diffuse axonal loss during the chronic stage of experimental autoimmune encephalomyelitis
G. Andriadis, C. Vuillat, N. Davoust, M.F. Belin, C. Conforeaux, S. Natas; Inserm U433 (Lyon, F); Hôpital Neurologique Pierre Wertheimer (Lyon, F)

Recent studies in multiple sclerosis (MS) suggest that axonal loss may be the pathological correlate for the development of irreversible neurological disability. However the pathogenesis of axonal damage, particularly the exact role of inflammatory cells, remains unclear. In this context, the present study was aimed at characterizing the relation between axonal loss and cellular infiltration at early versus late clinical stages in a model of chronic experimental autoimmune encephalomyelitis (EAE). EAE was induced in C57Bl/6 mice by immunization with myelin oligodendrocyte glycoprotein (MOG) peptide 35−55. Animals were killed at several time points after disease onset until approximately 3 months after immunization. Immunohistochemistry for neurofilament 200 kDa, CD11b, CD4 and CD34 was respectively used to quantify axonal loss, macrophages/microglia, CD4+ lymphocytes and CD34+ myeloid progenitors infiltration in the spinal cord of MOG-EAE mice. In parallel, to identify a putative source of microglial cells, we assessed the blood of EAE mice for the presence of CD34+ myeloid progenitors by flow cytometric analysis.
Significant diffuse axonal loss was present from the first attack and increased dramatically during the chronic phase. Macrophage/microglia infiltration appeared to be restricted to perivascular areas during the acute phase while it became widespread at a late chronic stage. Interestingly, areas of severe axonal depletion were colocated with CD11b+ infiltrates. In contrast, CD4+ lymphocytes density decreased greatly during the chronic phase of this model and did not correlate with increased axonal loss. Moreover we observed from onset of the chronic phase that a subset of CD11b+ cells co-expressed the stem cell marker CD34 especially in perivascular and meningeal areas. This was accompanied by an increase of CD11b+/CD34+ cells from the acute to the chronic phase in the blood of EAE mice. Overall our results suggest a determinant role of macrophages/microglia on chronic progressive axonal damage, sustained by a possible recruitment of CD34+ myeloid progenitors from blood to the central nervous system in EAE. In addition, even though T lymphocytes play a crucial role in the induction of EAE, our data show that axonal loss may be at least partially independent from T lymphocytes activity in this model.

P213

Type 1 interferon receptor (IFNAR)-dependent modulation of myeloid cell activation determines the course of experimental autoimmune encephalomyelitis

M. Prinz, H. Schmidt, C. Detje, A. Mildner, K. Knobeloch, U. Hanisch, R. Gold, B. Becher, W. Brück, U. Kalinits; University Hospital Gottingen (Gottingen, D); Paul-Ehrlich Institute (Langen, D); Institute of Molecular Pharmacology (Berlin, D); Institute for Multiple Sclerosis Research (Gottingen, D); University Hospital (Zurich, CH)

While treatment of MS patients with interferon-beta (IFN-b) leads to a marked decrease in the exacerbation rate as well as to delayed sustained disease progression, the precise mechanisms of the beneficial effects of IFN-b are still enigmatic. In this study we show that type 1 interferon receptor-deficient mice (IFNAR−/−) were highly susceptible to experimental autoimmune disease (EAE) and developed a more severe disease course with increased CNS inflammation, demyelination and lethality. Since IFNAR is expressed in all body tissues, little is known about the actual target tissue during disease. To clarify this, we used Cre/loxP-mediated gene targeting to investigate the cell-specific function of IFNAR in vivo. Mice with a specific IFNAR deletion in the central nervous system (Nestin-CreIFNARfloxflox) revealed no differences in the acute course and showed compatible tissue damage in the CNS compared to WT controls, indicating that type 1 interferons do not have a direct protective impact on the CNS. Interestingly, neither T cell (CD4-CreIFNARfloxflox) nor B cell (CD19-CreIFNARfloxflox)-specific IFNAR deletion influenced the cellular course and composite composition of infiltrating cells. However, IFNAR deletion on macrophages/neutrophiles (Ly6M-CreIFNARfloxflox) led to severe disease with an enhanced effector phase and increased disease lethality as seen in IFNAR−/− mice. Deletion of IFNAR on macrophages induced altered MHC class II expression and change of cytokine and chemokine production. In summary, we show that IFNAR triggering, specifically on myeloid cells, but not on lymphocytes or CNS cells, is crucial for immunomodulatory effects of type 1 IFN during autoimmune CNS disease.

P214

Spinal cord vascular laminin changes in experimental autoimmune encephalomyelitis

S.J. Karlik, W. Roscoe, C. Magalhaes, M. Welsh; University of Western Ontario (London, Ontario, CAN)

A vascular component, characteristic of inflammation, is associated with MS lesions and we have been pursuing angiogenesis as a key participant in CNS lesion growth and development in neuroinflammation. Both guinea pig and mouse EAE models show expression of VEGF in active lesions with an accompanying increase in blood vessel counts. To visualize alterations in vessel morphology, laminin and Factor VIII antigen immuno-staining in spinal cord vasculature were performed in tissue sections from 45 guinea pigs immunized with whole CNS/CFA and 86 C57Bl/6j mice immunized with MOG35-55 peptide during acute and chronic stages of disease. In controls, laminin immunoreactivity revealed multiple small vessels and identified a distinct division between the vascular and parenchymal basement membranes. As the neuroinflammation evolved, a characteristic morphological change was the separation of the two basement membranes with the development of perivascular cuffing in the intervening space. The vessels appeared engorged with inflammatory cells in the intramembranous space and are distorted and enlarged in lesions, taking on an annular appearance in cross section. As the disease progressed into demyelination, the highly defined basement membrane structures became poorly defined. In the chronic animals, structurally undefined deposits of laminin were observed in demyelinated lesions. This could represent vascular remodeling indicative of angiogenesis or tissue remodeling. Vascular morphology in EAE is dramatically altered as a function of disease state and may provide a suitable target for future therapeutic intervention.
P216
A model for specific de- and remyelination in rodent whole brain spheroid cultures
C. Teunissen, E. Vereyken, C. Dijkstra; VUMC Amsterdam (Amsterdam, NL)

Studies on demyelination in Multiple Sclerosis (MS) widely involve the experimental autoimmune encephalitis (EAE) model. Important disadvantages of this model are that it is induced by an autoimmune reaction, while oligodendrocyte damage likely is an earlier pathological event in MS, and that it involves large numbers of animals. The aim of the present study was to develop a specific in vitro demyelination model using whole brain spheroid cultures. The different neuronal and glial cell types present in this model form three-dimensional contacts, leading to myelinated axons.

Methods: Using this system we investigated the characteristics of demyelination based on a new protocol, i.e. exposure to lysolcithin (LPC). Results: Decreased myelin basic protein (MBP) staining and 2', 3'- cyclic nucleotide 3'-phosphodiesterase (CNPase) activity were observed after one week of repeated exposure of the cultures to LPC in a narrow dose-range. Moreover, we were able to show remyelination in this model one week after removal of LPC. Specificity of the treatment for oligodendrocytes was confirmed by immunostaining and concentration measurements of astrocyte, microglia and neuronal markers. We studied the mechanism of oligodendrocyte damage induced by LPC, e.g. the role of oxygen radicals or membrane cholesterol contents.

Conclusions: The specific de- and remyelination observed in this in vitro model supports the use of this model in research into the mechanisms of de- and remyelination in MS. Further, the model can be used in preclinical drug or cell transplant testing, reducing the number and discomfort of animals.

P217
G-CSF stabilises blood-brain barrier characteristics of human brain microvascular endothelial cells in vitro
J. Kraus, M. Hoppen, K.S. Kün, M. Schilling, P. Oeschmann, W.R. Schäbitz; Paracelsus Private Medical University (Salzburg, A); University Hospital (Münster, D); Johns Hopkins University School of Medicine (Baltimore, USA); University Hospital of Giessen and Marburg (Giessen, D)

Introduction: Blood-brain barrier (BBB) breakdown is an early event in the pathogenesis of multiple sclerosis (MS). Granulocyte-colony stimulating (G-CSF) has been approved for the treatment of granulocytopenia. Moreover, G-CSF was shown to be effective in a murine model for cerebral ischemia. The positive outcome was attributed to anti-neurodegenerative and neuroprotective effects. It was also suggested that G-CSF additionally has a stabilizing effect on the BBB. Methods: In order to investigate whether G-CSF exerts a direct effect on the BBB, we applied an in vitro BBB model where immortalized human brain microvascular endothelial cells (HBMEC) in co-culture with rat astrocytes form a tight permeability barrier for 3H-inulin and 14C-sucrose. Results: Addition of G-CSF to the co-culture system led to a further reduction of the permeability for 3H-inulin and 14C-sucrose across the HBMEC monolayers in a dose dependent manner. Moreover, with our in vitro model we mimicked a pathophysiological condition which has been suggested to contribute to the breakdown of the BBB in MS: Withdrawal of the astrocytes from the co-culture (to mimic changes in the microenvironment) led to an increase in the paracellular permeability across HBMEC monolayers. Pretreatment with G-CSF prevented the modulation of the BBB after withdrawal of astrocytes from the co-culture. Discussion: Our data demonstrate that G-CSF exerts a direct effect on human cerebral endothelial cells leading to a direct stabilization of the barrier function in vitro including a prevention of BBB modulation by a possible pathophysiological condition. Moreover, G-CSF revealed to have almost the same effects in our in vitro model as we found for IFN-beta. This direct effect on the BBB in combination with its anti-neurodegenerative and neuroprotective effects could be a hint that G-CSF is a possible candidate for MS treatment.

P218
Motor neuron loss in multiple sclerosis
F. Paul, J. Vogt, O. Aktas, A. Snoradchenko, K. Müller-Wielch, S. Meier, H. Steinhusch, C. Schmitz, R. Nitsch, F. Zipp, Charite-University Hospital (Berlin, D); Maastricht University (Maastricht, NL)

Background: Axonal transection and neurodegeneration secondary to or independent of neuroinflammation have gained increasing interest in multiple sclerosis research as these pathologies seem to correlate better with long term disability than the number of relapses and the visible inflammatory burden on conventional MRI images.

Methods and Results: Using design-based stereology in different EAE (experimental autoimmune encephalomyelitis) models we discovered a significant loss of spinal alpha and gamma motor neurons already early in the course of the disease. At the peak phase of the disease, the neuronal cell loss reached around 70% of the total number of neurons in passive EAE and around 50% in active EAE. In parallel, we were able to demonstrate a significant reduction of the number of motor neurons in the spinal cord of both six MS patients compared to six age and sex matched controls by stereological assessment of spinal cord tissue samples obtained at autopsy. Moreover, nerve conduction studies revealed a significant reduction of amplitudes in comparison to controls. Furthermore, sural muscle action potentials in 60 MS patients compared to 50 age and sex matched controls, while amplitudes of sensory nerves as well as sensory and motor conduction velocities did not differ between both groups. Conclusion: Motor neuron loss in multiple sclerosis as a condition purely affecting the upper motor neurons.

P219
Grey matter pathology induced by immunization with a beta-synuclein peptide
A. Escher, D. Merkler, R. Diem, W. Brack, C. Stadelmann; University of Gottingen (Gottingen, D)

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the CNS presumed to be autoimmune in nature. In a subgroup of patients grey matter regions, such as cortex and spinal cord grey matter, are extensively involved. In the present study, we aim to induce grey matter pathology resembling that found in MS by immunization with a neuronal protein. Beta-Synuclein is a cytoplasmic protein located in the perikarya and presynaptic nerve terminals. Immunization of Lewis rats with the beta-Synuclein peptide93-111 has been reported to induce monophasic autoimmune CNS inflammation with complete recovery (Mor et al., 2003). We found inflammatory infiltrates mainly in the grey matter of the spinal cord. No demyelination was observed. To mimic MS inflammatory-demyelinating pathology, anti-MOG antibodies were transferred into beta-Synuclein peptide-immunized rats. Extensive demyelinated grey matter lesions with iNOS-positive, myelin phagocyting macrophages could be generated. Numbers of APP-positive axons and c-jun-positive neurons were increased in transferred compared with beta-Synuclein peptide-immunized animals. Thus, immunisation with beta-Synuclein peptide93-111 leads to predominant grey matter pathology in Lewis rats and may serve as a tool to study mechanisms of grey matter pathology in MS.
P220
An increase in axonal mitochondrial density may play a role in axonal loss following myelin degeneration in the Plp1 mouse model of demyelination

V.E. Hogan, K.E. White, A. McGill, S. Karim, M. McLaughlin, L. Griffiths, D. Bates, D. Turnbull, P.P. Nichols; University of Newcastle (Newcastle, UK); University of Glasgow (Glasgow, UK)

Background: Axonal pathology in multiple sclerosis (MS) has been described for over a century but new insights into axonal loss and disability have re-focused interest in this area. Recent evidence suggests that mitochondria may play a role. In particular there appears to be a link between the mitochondrial gene defects causing Leber’s Hereditary Optic Neuropathy and MS. Furthermore, impaired axonal mitochondrial function has been demonstrated by MR spectroscopy in MS patients and oxidative damage to mitochondrial DNA has been demonstrated in chronic MS plaques.

Objectives: Our goal was to investigate the hypothesis that the upregulation of mitochondrial function is central to the maintenance of conduction in demyelinating axons but may also contribute to axonal degeneration and loss.

Methods: The Plp1 mouse is a transgenic hemizygous mouse with increased copy number of the wild type proteolipid protein gene. Demyelination of CNS axons develop in later life in these mice. Spinal cord axons were compared between Plp1 and wild type littermates at 2, 4, 12 months post natal with respect to myelination and mitochondrial activity (cytochrome c oxidase (COX) time lapse histochemistry). Tissue was also processed for electron microscopy (EM) for estimation of mitochondrial density per axon and axonal loss.

Results: At 2 and 4 months there was no evidence of axonal loss, demyelination, or changes in mitochondrial activity or density in the Plp1 compared to wild type mice. At 12 months, EM showed disorganisation of the axons with degeneration of the myelin and an increase in axonal mitochondrial density in Plp1 compared to wild type (2.40 ±1.69 μm², p = 0.02). However, COX histochemistry experiments did not show a corresponding increase in mitochondrial activity in the Plp1 mice. At 12 months there was a significant reduction in the area of dorsal column occupied by axons in the Plp1 compared to the wild type (40 ± 46%, p = 0.035), implying axon loss.

Conclusion: Although there was no overall increase in mitochondrial activity in the 12 month Plp1 mice, the loss of axons combined with the increase in mitochondria per axon suggests that within individual axons there is increased mitochondrial activity. This increase in axonal mitochondrial activity, in response to myelin disruption, may lead to increased production of reactive oxygen species and ultimately to axonal damage and subsequent loss.

P221
The production of multiple neurotrophic factors by glatiramer acetate-specific T cells facilitates the generation of oligodendrocytes

V. Skihar, C. Silva, W. Yong; University of Calgary (Calgary, CAN)

Background: The formation of oligodendrocytes from precursor cells is a prerequisite for remyelination in MS. Several neurotrophic factors regulate oligodendrocyte development and their levels may be deficient in MS. Strategies to increase neurotrophic factors may thus promote remyelination in MS. It is now clear that leukocytes are sources of neurotrophic factors. As glatiramer acetate (GA) generates T helper 2 (Th2) cells, we examined whether these produce neurotrophic factors and promote the generation of oligodendrocytes.

Methods: Mice pre-dosed with GA were sacrificed and lymph node cells were restimulated with GA in vitro to obtain GA-specific Th2 cells. Using GeneArray®, the Th2 cells were analyzed for production of neurotrophic factors. Maturation of progenitor cells to oligodendrocytes in the presence of GA-specific Th2 cells was studied in vitro as well as in vivo using hyaluronan-induced demyelination of the adult mouse spinal cord.

Results: GA-specific T cells were found to produce more interleukin-5, a Th2 cytokine, than interferon-gamma, a pro-inflammatory Th1 cytokine. When activated by GA, the GA-specific Th2 cells increased their levels of several growth factors known to regulate oligodendrocyte development, including insulin-like growth factor (IGF)-1 and -2, platelet-derived growth factor A and B, several fibroblast growth factors, leukemia inhibitory factor and vascular endothelial cell growth factor. These growth factors were detectable by 1 day of culture, and were increased at 2 and 3 days of GA-restimulation. When injected in vivo, the GA-specific Th2 cells homed to sites of injury, and immunohistochemistry suggested the presence of growth factors in situ. Ongoing experiments address whether these cells promote the generation of oligodendrocytes in vitro from neurospheres or committed precursors. Qualitative determinations in vivo suggest that there is an accumulation of oligodendrocyte progenitors and oligodendrocytes at the lesion site in lyssolecithin-demyelinated mice. Whether this increase number of oligodendrocytes enhances myelin repair will also be quantitatively addressed.

Conclusion: GA-specific Th2 cells express several neurotrophic factors relevant to the development of oligodendrocytes and these may help account for the therapeutic effect of GA in MS. Supported by an operating grant from Teva Pharmaceutical Industries, Israel.

P222
TLR3 stimulation suppresses relapsing EAE by inducing endogenous interferon-beta

T. Touil, D. Fitzgerald, G. Zhang, A. Rostami, B. Gran; Thomas Jefferson University (Philadelphia, USA); University of Nottingham (Nottingham, UK)

Objective: We tested the hypothesis that polyinosinic-polycytidylic acid (poly I:C), a Toll-like receptor (TLR)3 agonist and potent type I interferon inducer, can modulate the clinical and pathological expression of EAE, an animal model of multiple sclerosis (MS).

Background: Interferon beta (IFNb) is the most commonly used immunomodulatory treatment for MS and is administered to patients as an exogenous recombinant protein. Microbial components can induce the production of endogenous IFNb by antigen-presenting cells (APC) through stimulation of TLRs. Poly I:C is a mimic of double-strand RNA that stimulates APC through TLR3.

Methods: SJL mice were immunized with proteolipid protein peptide PLP139–151 and treated with poly I:C or PBS injected intraperitoneally during the induction or the relapsing phase of disease. Clinical, pathological, and immunological parameters were assessed.

Results: Treatment with poly I:C significantly suppressed clinical and pathological signs of EAE in both treatment protocols. Poly I:C induced significantly increased production of the CC-chemokine CCL2 (MCP-1) by spleen cells cultured ex vivo in the presence of PLP139–151. The expression of IFNb was significantly increased in the spleens of treated mice. Neutralization of either IFNb or CCL2 by spleen cell cultures showed that the production of CCL2 was IFNb-dependent.

Conclusions: Poly I:C treatment suppresses EAE by mechanisms that involve IFNb and CCL2. The induction of endogenous IFNb will be further assessed as a strategy for the treatment of MS.

P223
Do protease inhibitors support regenerative processes in experimental autoimmune encephalomyelitis?

A. Mueller, X. Pedre, A. Steinbrecher; University of Regensburg (Regensburg, D)

Various protease inhibitors (PI) are induced during the disease course of experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis. PI, mostly secreted by leukocytes, astrocytes and neurons after inflammatory stimuli, are
known to attenuate tissue inflammation. However, little is known about the effects of PI on neural and glial regeneration. Methods: EAE was induced in DA rats by immunization with myelin oligodendrocyte glycoprotein peptide 1–125 in complete Freund’s adjuvant. PI expression in the spinal cord from rats with EAE and healthy controls was analyzed by hybridization of Affymetrix microarrays and by RealTime-PCR. Localization of PI was studied by immunohistochemistry. Proliferation of neural stem cells isolated from the ventricular wall of adult rats and incubated with PI was determined by BrdU staining and cell counts. Results: Microarray- and RealTime-PCR analyses identified the selected PI as the overall most strongly induced gene within the spinal cord during MOG-induced EAE in rats. PI was abundant in perivascular inflammatory infiltrates. The cultivation of adult neural stem cells with recombinant PI resulted in a strong and consistent increase of cell proliferation. Discussion: The selected PI is highly expressed in spinal cord during EAE and is associated with inflammatory infiltrates. In addition to its known anti-inflammatory effects our results indicate a possible role in neural repair processes ongoing in the inflamed CNS. At the conference, the effect of the PI on the differentiation of adult neural stem cells will be provided.

P224
Usage of anti-MOG B cell and T cell transgenic mice model to evaluate effects of immunomodulating therapies in EAE – A pilot study with Copaxone® (glatiramer acetate)
T. Ziemsens, S. Dartsch, M. Marggraf, H. Reichmann, H. Schneider; Neurology University Clinic (Dresden, D)

The considerable heterogeneity in the pathogenesis of MS is evidenced by identification of 4 distinct subtypes of MS (Lucchetti et al., 2000). In the “inflammatory” subtypes I and II, the different involvement of autoimmune T and/or B cells seems to have a major impact on the clinical and histopathological characteristics of the disease, thus leading to the different in response of various therapeutic options. Methods: Transgenic mice expressing a targeted heavy chain of the demyelinating anti-MOG 8.18c5 antibody (BCR-tg mice; Litzenburger et al., 1998), and a MOG-peptide specific T cell receptor (TCR-tg mice; Bettelli et al., 2003), were used in this study. In addition to induction of active and passive EAE, spontaneously evolving EAE was also investigated in double-transgenic mice. Glatiramer acetate (GA) was administered in these different models either before induction or the spontaneous evolution of EAE. Clinical disease, histopathology and immunological parameters were investigated. Results: BCR-tg mice develop significantly earlier and more severe clinical disease scores in active and passive EAE compared to WT mice. Histopathologically, large deposits of anti-MOG Ab and complement are found in addition to increased number of cells of the myeloid lineage like macrophages and activated microglia. All correlate quite well with enhanced demyelination, axonal loss and clinical disease scores. Similarly, TCR-tg mice develop significantly earlier clinical disease in active and passive EAE compared to WT mice. Double transgenic, BCR- and TCR tg mice develop a severe spontaneous form of EAE starting 6–8 weeks after birth. GA treatment reduced profoundly severity after active immunization. Treatment with GA in TCR–tg mice prevented clinical and histopathological signs of EAE by reducing the number of activated ependymal T cells. GA treatment also inhibited development of spontaneous EAE. Conclusions: EAE model using anti-MOG BCR- and TCR-tg mice was developed to explore the role of ependymal tig T and B cell responses in demyelinating disease. This approach offers a panel of neurobiological-immunological studies in addition to the clinical and histopathological evaluation. GA is effective in treating EAE in an anti-MOG TCR- and BCR-tg background as well as prevents the development of spontaneous EAE in double tg mice at least up to half a year. Especially this preventive mode of action will be part of additional studies.

P225
Combination treatment with IFNbeta and anti-alpha4 integrin antibody slightly enhances monotherapy effects upon lymphocyte trafficking to the central nervous system
B. Wipke, A. Wadsworth, C. Lorenzana, S. Simmonds, E. Peterson, F. San Pablo, E. Messersmith; Elan (South San Francisco, USA)

Interferon-beta and anti-alpha4 integrin treatments have demonstrated efficacy in reducing human multiple sclerosis relapse rates and gadolinium-enhancing lesions, and direct effects upon trafficking are implied. In this study, a direct comparative measurement of lymphocyte trafficking was performed for IFNbeta and anti-alpha4 integrin monotherapy and combination therapy in rat experimental autoimmun e encephalomyelit s (EAE). 111-Indium-labeled naïve rat spleocytes were transferred into EAE Lewis rats on day 11, and a panel of ten tissues was harvested 16–18 hours later and quantified by gamma counter. Treatment groups were: rat IFNbeta (300,000 IU/day s.c. day 7–12), GGS/3 anti-alpha4 monoclonal antibody (3 mg/kg s.c., day 7 and 10), combination treatment, and vehicle. In-life phase clinical scores demonstrated significant benefit for GGS/3 and combination therapy (p <0.05 vs. vehicle), but IFNbeta alone provided no significant protection (p >0.05 vs. vehicle). Spinal cord and brain in control animals contained modest but consistent amounts of radioactivity. IFNbeta alone had no effect on trafficking to the spinal cord, but decreased accumulation in the brain by ~20% relative to vehicle. In contrast, GGS/3 treatment significantly inhibited accumulation in the spinal cord (85%) and brain (75%) relative to vehicle animals. IFNbeta alone inhibited accumulation in Peyers’ patches (PP) by 44% and in mesenteric lymph nodes (MLN) by only 13%, compared to 96% inhibition (PP) and 60% (MLN) by both GGS/3 and GGS/3+ IFNbeta combination treatment. Combination therapy was equally or slightly more effective than GGS/3 alone in preventing cell accumulation in the brain, spinal cord, inguinal lymph nodes (ILN), MLN, and PP (<10% change in total inhibition compared to GGS/3 alone). From these studies, we conclude that under these conditions, GGS/3 inhibits trafficking more effectively than IFNbeta for all organs where inhibition is observed, and that combination therapy results in only a slight increase over GGS/3 alone in inhibition of cell trafficking to the spinal cord and MLN, but not the brain or ILN.

P226
Symadex, a tyrosine kinase inhibitor, shows effectiveness in experimental autoimmune encephalomyelitis
S.I. Karlik, A. Ayam; University of Western Ontario (London, CAN); Xandius, Inc. (Cambridge, USA)

C-1311 (Symadex) is a novel imidazoacridinone antiproliferative agent that targets receptor tyrosine kinases in the PDGF family, notably FLT3. It disrupts trafficking of autoreactive cells and concomitant angiogenic processes. As we have been investigating the vascular component of neuroinflammation, this mechanism of action provided a motivation to observe its potential effects on EAE. For these studies CNS/CFA-induced EAE in the guinea pig was used during the acute (<d20 post immunization) and chronic (>d20) phases. Initiating continuous treatment before the appearance of clinical signs (d7) fundamentally altered the typical clinical course to yield a monophasic acute disease with prevention of the chronic demyelinating phase. Consistent with the retention of T cell-mediated acute disease, FACS analysis revealed no alteration of immune cell populations, except for a small decrease in B cells. Treatment initiated at various times during the chronic phase showed reversal of clinical and pathological signs. A decrease in the parenchymal immune cell population accompanied a decrease in TUNEL staining of spinal cord sections from treated animals, consistent with prevention of new cellular infiltrates from enlarged perivascular cuffs. Tissue recovery was observed with remyelination.
and vascular alterations revealed through laminin staining were also reversed. Thus, Symadex, in the absence of classical immuno- suppressive properties, shows potent effectiveness in controlling and reversing experimental demyelinating disease, potentially through modulation of vascular changes and inhibition of related trafficking mechanisms.

**P227**

**Ingested (oral) SIRS peptide 1–21 inhibits experimental autoimmune encephalomyelitis**

S.A. Brod, Z.M. Hood; University of Texas (Houston, USA)

**Background:** Ingested type I IFN inhibits clinical attacks, relapses and inflammation in murine experimental autoimmune encephalomyelitis (EAEE). Type I IFN activate human suppressor T cells that produce soluble immune response suppressor (SIRS). **Objectives:** We examined whether a peptide (SIRS) induced by type I IFN would have similar effects to ingested IFN-α in EAE. **Methods:** C57Bl/6 mice were actively immunized with myelin oligodendroglial (MOG) peptide 35–55 (MEGVWYKPSRVRHLYRNGK) in IFA and followed for evidence of disease. Clinical severity was graded daily. For parental treatment, on day –7 preceding active immunization, and continuing through day +14 post immunization, B6 mice were injected with control saline (mock), 0.1, 1, or 10 mcg SIRS peptide 1–21 (NH-MET-Thr-Glu-Glu-Asn-Gln-Gln-Ser-Ser-Pro-Lys-Thr-Thr-Ile-Asn-Ala-Asp-Cys-OH). For oral treatment, B6 mice were fed with 0.1 ml of saline (mock) or 1, 10 or 100 mcg SIRS peptide 1–21 daily. Following sacrifice, spinal cords were removed and evaluated independently for foci of inflammation by a blinded observer. Splenocytes from grouped saline (mock) fed or 100 mcg SIRS peptide fed mice were stimulated with Con A and examined using mouse cytokine inflammatory antibody array. **Results:** SIRS peptide 1–21 showed significant inhibition of EAEE (p < 0.001). Ingested SIRS peptide at 10 and 100 mcg SIRS peptide inhibited EAEE compared to placebo (p < 0.001). There were significantly less inflammatory foci in the SIRS peptide fed group compared to the control mock saline group (p < 0.03). Splenocytes from SIRS peptide 1–21 fed mice showed increased production of CD30L, IL-6, IL-13, I-TAC, TCA-3/CCL1, TNF-α and decreased production of lymphotactin after Con A stimulation. **Conclusion:** Ingested (oral) SIRS peptide significantly inhibits both clinical and inflammatory predominate via counter-regulatory type 2-like cytokines and chemokines IL-13, CD30L and TCA-3 despite increased peripheral expression of TNF-α.

**P228**

**Neuronal damage in MOG-induced optic neuritis correlates with T-cell infiltration**

M.B. Sa¨ttler, D. Merkler, M. Togni, I. Gadjanski, C. Stadelmann, M. Ba ¨hr; University of Magdeburg (Magdeburg, D); University of Gottingen (Gottingen, D); University of Magdeburg (Magdeburg, D)

**Introduction:** Long-term disability in MS patients is primarily caused by axonal and neuronal damage. We demonstrated previously that neuronal apoptosis occurs early during myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAEE). The aim of the present study was to determine the influences of T- and B-cell activation on neuronal damage in MOG-induced optic neuritis. **Methods:** Brown Norway and Dark Agouti rats were immunized using soluble or precipitated MOG. Infiltrating T-cells were identified by CD3-immunohistochemistry whereas the anti-MOG-antibodies within the rats’ serum were evaluated by ELISA. The densities of surviving retinal ganglion cells (RGCs), the neurons that form the axons within the optic nerve (ON), as well as acute and chronic axonal damage within the ONs were evaluated. **Results:** We observed a significant correlation of axonal and neuronal damage and T-cell infiltration within the ONS in MOG-induced optic neuritis. In contrast, no positive correlation was observed for the loss of RGCs or axons and the anti-MOG-antibody titer. In BN rats, an inverse regulation of T- and B-cells was present, but in DA rats both autoimmune responses occurred in parallel. **Conclusions:** Different patterns of autoimmune responses can be induced in MOG-EAEE by modification of the rat strain. A higher rate of T-cell infiltration correlates well with increased axonal and neuronal loss, indicating a pathogenic influence of these cells on the axons in MOG-EAEE. A further characterization of the T-cells infiltrating the ONS might help identify possible underlying mechanisms.

**P229**

**The effect of cytokines in the survival of neural stem cells in vitro**

R. Lagoudaki, N. Taskos, A. Kourakis, A. Kotsis, I. Milonas, N. Grigoraidis; Aristotle University of Thessaloniki (Thessaloniki, GR)

**Introduction:** Recently, research has been focused in the transplantation of neural stem cells (NSC) in Experimental Allergic Encephalomyelitis (EAEE), the animal model of Multiple Sclerosis (MS). Among several issues that remain to be answered is the survival of the transplanted cells in an inflammatory environment, such as the one present during EAEE or MS process. **Materials and Methods:** NSC were isolated from cerebral hemispheres of 1–2 days old C57Bl/6 mice. MTT assay was used for the measurement of NSC proliferation in vitro, under the presence of IFNg, TNFα, IL-4 and TGFβ, in various concentrations (1 to 1000 units/ml). Caspase-3 immunocytochemistry (ICC) was performed in order to detect whether the reduction in the proliferation of the cells was due to apoptosis. ICC was also performed for the detection of Bax, Fas receptor and Fas ligand expression. The apoptotic death of the NSC was verified by the use of RT-PCR for the expression of related genes such as, Araf-1, LICE, Bid, Bax and Bad. **Results:** The presence of IFNg and IL4 reduced the NSC proliferation from 22.38% (33 units/ml) to 31.35% (1000 units/ml) and from 23.4% (33 units/ml) to 47.34% (1000 units/ml), respectively. TNFα induced an even higher reduction at levels ranging from 31.58% (33 units/ml) to 63.16% (1000 units/ml). In the presence of TGFβ, low concentrations (33 units/ml), increased the proliferation capacity of NSC by 5.11%, whereas at high concentrations (1000 units/ml) a decreased by 41.48%, was noticed. Increased expression of caspase-3, in the presence of IFNg (29.87%), IL-4 (23.28%) and TNFα (33.33%), was identified. Contrary, TGFβ induced a reduction of the expression of caspase-3 (8.9%). In the presence of TNFα, a 14.67%, 8.3%, and 68.16% reduction of Fas, Fas-L and Bax, respectively, was observed. However, IFNg resulted in a 43.21%, 25.55% and 49% expression of the correspondent proteins. In the presence of IL4, the correspondent expression of the same proteins was 68.16%, 61.92% and 61.85%. The genes Apaf-1, LICE, Bid, Bax and Bad were co-expressed in the presence of all cytokines tested. **Conclusions:** Our results indicate either increased apoptosis or survival of NSC in the presence of pro- or anti-inflammatory cytokines, respectively. In particular, the major apoptotic pathway followed in the presence of TNFα is the mitochondrial one, whereas in the case of the other two proinflammatory cytokines, via both mitochondrial and death receptor pathways.

**P230**

**CD4+ CD25+ regulatory T cells contribute to the therapeutic effects of glatiramer acetate in experimental autoimmune encephalomyelitis**

Y. Lee, W. Piao, R. Liu, X.F. Bai, M. Price, S. Rhodes, R. Rodebaugh, D. Campagnolo, F.D. Shi, T. Vollmer; Barrow Neurological Institute (Phoenix, USA); Ohio State University Medical Center (Columbus, USA)

CD4+CD25+ regulatory T cells (Tregs) are potent immunosuppressors that are pivotal in the maintenance of self-tolerance. The
involved of Tregs in therapies for immune-mediated diseases has been proposed, but direct supporting evidence is still lacking. While investigating mechanisms underlying the clinical benefits of glatiramer acetate (GA) in an animal model of multiple sclerosis (MS), i.e., experimental autoimmune encephalomyelitis (EAE) in C57Bl/6 mice, we recently demonstrated that GA can protect mice deficient in the Th2 cytokines IL-4, IL-10 and IL-4/IL-10 from acquiring EAE, suggesting that mechanisms other than Th2 cells may be responsible for the therapeutic effects of GA. Here we demonstrate that GA treatment boosts the expression of Foxp3 on Tregs during EAE. Depletion of CD25+ cells completely abrogates the blockage of EAE by GA. Furthermore, adoptive transfer of purified Tregs from GA-treated EAE mice is more effective in suggesting EAE development than Tregs from untreated EAE controls. Thus, our current data provide evidence that Tregs may be the major contributor to GA’s therapeutic action in EAE and, possibly, MS. Further mechanistic studies to reveal the molecular events linking GA with Tregs may optimize GA treatment and lead to the development of new, even more effective therapies that utilize this mechanism of action.

P232
Myelin has limited immunosuppressive activity
J.W. Lindsey; University of Texas-Houston (Houston, USA)

Objective: The objective of this work was to compare the immunosuppressive activity in myelin and whole brain homogenate.

Background: Multiple sclerosis is an immune mediated disease which affects myelin in the central nervous system. Lesions in the white matter typically have inflammatory infiltrates and demyelination. Demyelinating lesions also occur in grey matter, but the grey matter lesions typically have minimal inflammation. In previous work, we demonstrated that homogenates of whole brain tissue are immunosuppressive in culture. Hypothesis: Our hypothesis in this work is that myelin is deficient in this intrinsic immunosuppressive activity, thus making the white matter microenvironment permissive for inflammation and immune-mediated damage.

Methods: Myelin and other subcellular fractions were isolated from whole brain tissue of naïve mice using sucrose gradients. Lymph node cells from mice immunized with ovalbumin were cultured with antigen and various amounts of myelin, whole brain homogenate, or other fractions. The proliferative response was measured after 3 days, and the suppressive activity of each dilution was calculated. The suppressive activity of myelin and brain homogenate was compared by calculating the mg of protein required to produce 50% suppression.

Results: We found that myelin had much less suppressive activity than whole brain homogenate. Myelin had only 31% of the activity of whole brain homogenate. The most active sucrose gradient fraction was the intermediate density P2 fraction. This fraction, which contains mitochondria and cell membranes, had 2.3 times the activity of the whole brain homogenate, and 5.8 times the activity of myelin.

Conclusions: We conclude that myelin contains only a fraction of the immunosuppressive activity present in brain tissue. Thus, myelin may be less able to inhibit inflammation and may be uniquely susceptible to immune mediated damage. This observation may also explain why MS lesions in white matter have more inflammatory infiltrates than lesions in grey matter. This work supported in part by the Clayton Foundation for Research.

P233
Arundic acid (ONO-2506) prevents chronic progressive and relapsing-remitting EAE
K. Takiwaca, R. Tomioka, S. Kinoshita, M. Onuki, K. Shimazu, K. Sagawa, C. Koh, K. Nomura; Saitama Medical School (Saitama, JP); Saitama Medical Center (Saitama, JP); Ono Pharmaceutical Co. Ltd (Osaka, JP); Shinsyu University School of Health Sciences (Nagano, JP)

Arundic acid (ONO-2506) is a novel neurological agent that modulates the function of astrocytes. We evaluated the preventive effects of ONO-2506 in chronic progressive and relapsing remitting EAE (CP-EAE and RR-EAE). CP-EAE in C57Bl/6 and RR-EAE in NOD/IT mice were injected with MOG35-55 and pertussis toxin to obtain EAE animals. Animals were orally treated with 30mg/kg/day of ONO-2506 every day. RR-EAE animals treated with vehicle have showed chronic progressive clinical course with 3.4 of mean clinical score (mCS), whereas animals with ONO-2506 have showed mild neurological symptoms with 2.5 mCS at the day 30 after immunization. In the pathological examination, CP-EAE animals treated with ONO-2506 were decreased in the number of total demyelinating lesion compared with control EAE animals in brain and spinal cord. RR-EAE animals treated with vehicle have showed double peaks clinical course with 1.6 mCS per first peak and 2.0 mCS at second peak. Animals treated ONO-2506 every 60 days also have showed two peak clinical course, however, have revealed less neurological symptom with 0.2 mCS at first peak and 0.25 mCS at second peak. Animals treated ONO-2506 every 30 days have showed no first peak but had second peak with 0.66 mCS. In the pathological examination, RR-EAE animals treated
with ONO-2506 were decreased demyelinating lesions in brain and spinal cord as well as CP-EAE animals. Arundic acid was shown to have preventive effects for CP-EAE and RR-EAE.

Immunology - Part I

P234
High levels of soluble MIC molecules in serum are related to multiple sclerosis disease activity

Background: Major histocompatibility complex (MHC) class I chain related (MIC) molecules called MICA and MICB are known as non classical human leucocyte antigens (HLA) molecules that do not combine with beta-2 microglobulin, do not bind peptide and are not expressed on normal circulating lymphocytes. MIC proteins can be found expressed on intestinal epithelium, tumoral transformed or viral infected cell and engage the activating natural killer cell receptor NKG2D. This molecule is found on NK cells and on CD8+ alpha/beta and gamma/delta T cells which are known to be part of the cellular infiltrate of multiple sclerosis (MS) plaque. High soluble MIC molecules levels have been found as an immune evasion method in some cancers as well as a part of modulating immune activity during transplant rejections. This study examined whether soluble MICA and MICB levels correlate with disease activity in MS.

Methods: Soluble MICA (sMICA) and MICB (sMICB) levels were measured by ELISA (R&D, Abingdon, UK) in eighteen consecutive MS patients with clinical relapse under McDonald criteria and twenty-four patients with remitting MS. Also, eight healthy sera were measured for sMICA and sMICB levels. Results: No differences between serum sMICA levels among different MS groups or healthy controls was found. However a significant high sMICB serum levels in MS during relapses was found when compared with remitting MS or healthy controls levels (p<0.001). Conclusions: Soluble MICB molecules levels are correlated with inflammatory disease activity in relapsing-remitting MS. This could be part of a complementary activation of innate immunity by stress during inflammatory activity of relapses in multiple sclerosis lesions.

P235
MIC molecules and NKG2D receptor are expressed in multiple sclerosis plaques and they are partially induced by IL-15
J.L. Fdez-Morera, A. Tunon, S. Rodriguez-Rodero, A. Lopez-Vazquez, A. Astudillo, S. Gonzalez, C.H. Lahoz, C. Lopez-Larrea; Hospital Universitario Central Asturias (Oviedo, E); Universidad de Oviedo (Oviedo, E)

Background: MIC molecules are non-classical MHC class I antigens with highly limited tissue distribution under non-pathological conditions. Not capable of acting as a peptide-presenting molecule, their functions during viral infection or autoimmune diseases, such as arthritis rheumatism or celiac disease, have been associated to NKG2D recognition by NK cells, CD8+ and gamma/ delta lymphocytes. This study examined whether MIC and NKG2D expression are present in multiple sclerosis plaques and how can be MIC molecules partially induced by multiple sclerosis sera during relapses.

Methods: Human MICA promoter was cloned in to PGL2 plasmid, transfected in HeLa transfected cultures. Moreover MIC molecules were found to be expressed in the vicinity of multiple sclerosis plaques. Astrocytes, vascular endothelial cells, epidermidy endothelium and “foaming” activated macrophages were found positive stained for MIC molecules. Perivascular and periplaque infiltrating lymphocytes were also found positive for NKG2D staining.

Conclusions: Our results show that MICA expression can be induced by IL-15 that is known to be present in multiple sclerosis sera of patients during relapses. Moreover MIC molecules are expressed in the vicinity of multiple sclerosis plaques by different cell types and it receptor NKG2D is also expressed in the lymphocyte infiltrate in multiple sclerosis lesions. Those results show MIC-NKG2D interaction as a possible novel route in the pathogenesis in multiple sclerosis.

P236
ICOS gene haplotypes correlate with multiple sclerosis development and progression
C. Comi, L. Castelli, A. Chiocchetti, D. Galimberti, C. Fenoglio, R. Mesturini, G. Cappellano, E. Cerutti, M. Carecchio, M. Leone, F. Perla, E. Scarpini, F. Monaco, U. Dianzani; University “A. Avogadro” (Novara, I); University of Milan (Milan, I); Ospedale di Canzo (Canzo, I)

Background: T cell activation requires both the recognition of the antigen presented by MHC molecules on the surface of antigen presenting cells (APC) by T cell receptor and the contemporary costimulation of T cell accessory molecules by their ligands expressed by APC. These costimulatory signals are also crucial to drive T cell differentiation into effector T cells. ICOS is a costimulatory molecule belonging to the CD28 family. It is selectively expressed by activated T cells and binds a ligand belonging to the B7 family, i.e. B7th. Recent studies have highlighted an involvement of ICOS in autoimmune diseases. In ICOS-deficient mice, defective ICOS-mediated costimulation exacerbates development of experimental autoimmune encephalomyelitis. In humans, a possible role of ICOS has been suggested in Systemic Lupus Erythematosus and Rheumatoid Arthritis. Aim of the study: To investigate the role of the ICOS gene in MS development and course.

Patients and Methods: Genomic DNA was isolated from PBMCs of 421 patients with MS (156 m, 265 f), and 816 ethnically matched controls. Patients were enrolled from the MS Centers of University “Amedeo Avogadro”, Novara, and University of Milan, Italy. Results: The 3’ UTR of the ICOS gene was initially sequenced in 52 MS patients and 87 controls to identify variations in the Italian population. We then carried that haplotype-1 (1564 T, 1624 C, 2007 A, 0.07 G), and -2 (1564 C, 1624 C, 2007 A, 0.05 G) accounted for more than 98% of the total haplotype frequencies in both patients and controls. To assess whether haplotype-1 homozygosity is a protective factor for MS development, we analyzed SNPs +1564 that allows to discriminate haplotype-1 (T) form haplotype-2 and -3 (C) in 421 patients and 793 controls. Haplotype-1 homozygosity was significantly more frequent in controls than in patients and conferred an OR = 0.75 for MS development. We then compared disease course in haplotype-1 homozygotes and haplotype-2 or -3 carriers and found that haplotype-1 homozygotes displayed lower relapse rate than haplotype-3 or 2 carriers (p<0.05). We then analysed disease severity with the multiple sclerosis severity score (MSSS) and found that RR patients homozygotes for haplotype-1 had a milder disease compared to haplotype-3 or 2 carriers (mean MSSS 2.2 vs. 3.6, p<0.05). Conclusion: These studies suggest that ICOS gene haplotype-1 not only confers protection from MS development but also predisposes to a milder disease course.
A polymorphism in MHC2TA is strongly associated to MS in HHV-6A-infected Spanish patients: a gene-environment interaction
A. Mas-Fontana, R. Alvarez-Lafuente, A. Martinez, M. Garcia-Montojo, V. De las Heras, E. Urcelay, M. Bartolome, E. Gomez de la Concha, R. Arroyo; Hospital Clinico San Carlos (Madrid, E)

Introduction: Environmental and genetic influences are believed to cooperate to confer susceptibility to Multiple Sclerosis (MS). However, no systematical effort has been made to relate the genetic constitution of the patient with the specific environmental influences present in MS patients. One of the most important environmental influences described in MS is the presence of herpesviruses. More specifically, human herpesvirus 6A (HHV-6A) has been strongly associated to MS in a number of studies. It has been speculated that the inflammatory stress due to viral infection, and the subsequent release of pro-inflammatory cytokines as interferon-gamma, may induce the expression of HLA class II molecules in tissues that do not normally express those genes, e.g. oligodendrocytes. Objectives: We tried to evaluate the importance of a polymorphism in MHC2TA, the master gene regulating the expression of HLA class II genes, and its interaction with presence or absence of HHV-6A in a group of Spanish MS patients.

Methods: 104 MS patients from Madrid were analyzed both at the MHC2TA locus and by HHV-6A status in serum. The polymorphism studied is a G/C polymorphism (rs4774) located at the exon 11. It is located at the +1614 position changing glycine to alanine. A TaqMan Assay-on-Demand was used under conditions recommended by the manufacturer (Applied Biosystems) in a HT7900 fast real time PCR platform. HHV-6A genomes in serum were evaluated by quantitative real-time PCR analysis by TaqMan probes in a Rotor-Gene 3000 thermocycler. A control group of 405 healthy Spanish individuals also from the Madrid region was included for comparative purposes in the genetic analyses.

Results: The CITA +1614 genotype frequency was very different when MS patients with HHV-6A were compared with MS patients without this virus. The proportion of carriers of the minor allele (C) was higher in MS patients with HHV-6A in a group of Spanish MS patients. The polymorphism studied is a G/C polymorphism (rs4774) located at the exon 11. It is located at the +1614 position changing glycine to alanine. A TaqMan Assay-on-Demand was used under conditions recommended by the manufacturer (Applied Biosystems) in a HT7900 fast real time PCR platform. HHV-6A genomes in serum were evaluated by quantitative real-time PCR analysis by TaqMan probes in a Rotor-Gene 3000 thermocycler. A control group of 405 healthy Spanish individuals also from the Madrid region was included for comparative purposes in the genetic analyses.

Discussion: Our results provide for the first time evidence of an interaction between a genetic factor and the presence of HHV-6A in a group of Spanish MS patients. Our data suggest that hyper-expression of MHC class II molecules in virus-infected patient may lead to MS by an unknown mechanism.

Toll-like receptors gene expression in a murine model of multiple sclerosis: the effect of glatirameric acetate on EAE-induced mice
S. Haque, A. Qasim, A. Haque, L. Kasper; Dartmouth Medical School (Lebanon, USA)

Objective: To evaluate the effect of glatiramer acetate (GA) on the induction of Toll-like receptors (TLR) gene expression and TH1/TH2 signaling molecules in experimental autoimmune encephalomyelitis (EAE). Background: Within the CNS, activation of resident cells initiates an inflammatory cascade, leading to tissue destruction, demyelination, and neurologic deficit. CNS-resident microglia have been shown to express an array of different TLRs, depending on their state of activation. Toll-like receptor (TLR) recognizers pathogen-associated molecular patterns (PAMPs) that are expressed on infectious agents, and mediate the production of cytokines necessary for the development of effective immunity. GA is an approved drug for relapsing-remitting MS that has been shown to induce a Th2 polarization in these autoimmune conditions. In vivo administration of GA induces regulatory T cell properties.

Methods: We have studied the repertoire of cytokines (IL-4, IL-6, IL-10, IL-12, IL-17, IFNgamma, and expression of TLRs compared with naive mice. Cervical and lingual lymph nodes and brain tissues were obtained from EAE induced, CD25 depleted EAE induced mice and GA treated EAE mice. Expression was evaluated by immunocytochemistry and RT-PCR.

Results: GA treated EAE mice express high level of IL-10 mRNA and decreased IFNg in lymph nodes whereas only IFNg mRNA was increased in lymph nodes of control mice. Increased expression of TRL-9, TLR-2 and STAT4 genes were observed in both EAE induced and CD25 depleted EAE induced mice compared to GA treated mice and control mice.

Conclusion: These observations are consistent with the possible induction of innate immune responsiveness in the CNS during EAE induction and persistence. TLR9 or TLR2 signaling within the CNS may be responsible in part for mediating the inflammatory effect in EAE induced and CD25 depleted mice. Preliminary findings suggest that the message for these innate pattern recognition molecules are down regulated by exposure to glatirameric acetate. Supported by NIH grant 5-30608 and Teva Neuroscience.

Development of a new assay to detect murine and human, conformation dependent anti MOG antibodies using a human eucaryotic glycosylated construct
H. Schneider, M. Zocher, T. Ziemsens; TU Dresden (Dresden, D); Micromet (Munich, D)

Objective: Patients with a clinically isolated syndrome suggestive of a first demyelinating event are at high risk to develop a definite multiple sclerosis (MS). Currently available data are conflicting concerning the predictive value of antemyelin especially anti-myelin oligodendrocytic glycoprotein (MOG) antibodies. Our objective was the development of a new detection method for the pathogenic MOG antibodies using an eucaryotic, properly folded and glycosylated MOG construct which can be used for risk evaluation in patients with a first demyelinating event. Methods: An ELISA-based method was established using a MOG-Fc construct containing the extracellular domain of human MOG protein and the Fc domain of a human IgG1 antibody. We could demonstrate that the construct expressed in CHO cells was correctly folded and glycosylated. First we tested sera of wildtype mice and transgenic mice producing MOG-reactive antibodies and B cells. After detection of specific anti MOG antibodies in sera of transgenic mice we examined the relative amount of different Ig classes. This was followed by a screening of 25 sera of healthy controls, of patients with a first demyelinating event and of patients with definite Multiple sclerosis. Results: Using an eucaryotic human MOG-Fc construct we developed an ELISA based technique for the detection of conformation-dependent anti-MOG antibodies which is in contrast to the mostly applied assays. These most probably disease relevant antibodies can detected in the sera of transgenic mice producing MOG reactive B cells but not in the sera of wildtype mice. After immunization, different isotypes of MOG-antibodies could be detected. In men, low levels of anti MOG antibodies could be detected in less then 10% of human sera of patients with a first demyelinating event and in sera of MS patients. In addition, we compared the anti MOG-Fc antibody response with anti-rMOG and MOG peptide specific responses in mice and in men demonstrating remarkable differences depending on the used antigen. Conclusions: Our established method for the detection of conformation-dependent anti MOG antibodies is able to discriminate between murine sera positive or negative for anti-native MOG antibodies. In addition, MOG-reactive B cells and antibodies could be stained in the different tissues. The sensitivity and specificity of this test for detection of human anti MOG antibodies in well documented CIS patients is under further investigation.
Mannan-binding lectin related to CSF and MRI findings in optic neuritis and multiple sclerosis

V. Veyhe, A. Tsakiri, I. Laursen, J.L. Frederiksen; Glostrup University Hospital (Glostrup, DK); Statens Serum Institute (Copenhagen, DK)

Background: Mannan-binding lectin (MBL) plays an important role in the innate immune system. We examined the serum concentration of MBL in patients with multiple sclerosis (MS) and acute optic neuritis (ON). We studied if MBL was influenced by the number of days from onset to venous puncture and the disease severity.

Methods: The serum concentration of MBL in 138 adult patients with MS or ON and in 87 healthy donors was measured by a sandwich ELISA method. The patients were divided into 3 groups: monosymptomatic acute ON, acute ON as part of MS, and CDMS. Results: The mean concentration of MBL in patients (1.22 μg/ml, 95% confidence interval 1.03–1.42 μg/ml) did not differ significantly from healthy controls (0.97 μg/ml, 95% confidence interval 0.79–1.17). The results within subgroups did neither differ from each other nor from the donors. There were neither significant correlations between the concentration of MBL and interval from onset, nor with CSF and MRI findings. Nineteen (13.8%) of patients had a very low (<0.05 μg/ml) and 24 (17.4%) of patients a low (<0.1 μg/ml) concentration of MBL. Conclusions: The mean serum concentration of MBL did not differ from healthy controls, but 13.8% of patients had a very low and 17.4% a low value of MBL. The value of MBL was neither influenced by the number of days from onset to venous puncture nor to the disease severity judged by CSF and MRI findings.

Impaired energy metabolism and increased lipid peroxidation in multiple sclerosis

A. Petzold, M. Eikelenboom, G. Keir, G. Giovannoni, C. Polman, B. Utilehaug, A. Amorini, V. Di Pietro, B. Tavazzi, G. Lazzarino; Institute of Neurology (London, UK); VU Medical Center (Amsterdam, NL); University of Catania (Catania, I); Catholic University of Rome (Rome, I)

Background: The universal source of energy in living organisms is ATP. There is experimental evidence that the demethylated axon pays a metabolic penalty to maintain conduction. In this study we measured products of the purine and oxypurine pathways in the plasma and CSF to investigate indirectly whether there may be a general imbalance of the mitochondrial energy metabolism in MS leading to a deficit in ATP production. Method: Baseline serum and CSF samples from MS patients (18 RR, 21 SP, 9 PP) and serum samples from 30 healthy controls were analysed by HPLC for measures of lipid peroxidation, nitrosative stress and ATP catabolites. Patients were assessed clinically using the EDSS, MSSS, nine-hole PEG test and an ambulation index at baseline and at the 3-year follow-up visit. Results: Patients with MS had higher median plasma levels compared to controls for the following metabolites, b-pseudouridin (5.37 vs. 3.88 μmol, p < 0.01), cytosin (0.79 vs. 0.68 μmol, p < 0.05), hypoxanthine (11.08 vs. 3.39 μmol, p < 0.001), xanthine (3.81 vs. 2.18 μmol, p < 0.001), inosine (0.96 vs. 0.32 μmol, p < 0.001), uric acid (313.97 vs. 248.44 μmol, p < 0.001), malonyldeidehyde (0.16 vs. 0.001 μmol, p < 0.001) and total oxypurines (328.08 vs. 252.41 mmol, p < 0.001). There was a correlation between the CSF total oxypurine (r = 0.38) and purin (r = 0.48) levels and the degree of progression on the EDSS (p < 0.05 for each comparison). Conclusion: These findings suggest an increase in lipid peroxidation (increased MDA) alongside an imbalance between energy production and consumption (increased of ATP metabolites) in patients with MS. Because of the magnitude of some of these differences for the serum oxypurine compounds, we speculate that a peripheral source, such as the muscular tissue, may reflect an imbalance of the energy metabolism in MS. Finally, the correlation between the CSF oxypurine and purine levels with the increase of disability could reflect the energy penalty related to neuronal death and axonal degeneration.
P244

mRNA levels of CD46 and HHV-6 infection in multiple sclerosis patients
R. Alvarez-Lafuente, M. Garcia-Montojo, V. de las Heras, M. Bartolomé, R. Arroyo; Hospital Clínico San Carlos (Madrid, E)

Background: CD46 has been identified as a cellular receptor for human herpesvirus 6 (HHV-6). Recently, increased levels of serum CD46 have been found in multiple sclerosis (MS) patients in conjunction with the detection of serum HHV-6 DNA; furthermore, CD46 has been involved in a cell-cell fusion mechanism by which certain viruses could spread the infection from the periphery to the cells in the nervous system. Objectives: 1) To analyze the levels of expression of CD46 in MS patients of a Spanish cohort in comparison with healthy blood donors (HBD). 2) To study the possible association between the levels of expression of CD46 and HHV-6 DNA prevalences and viral load in blood and serum samples. Methods: Fifty four MS patients and the same number of HBD were recruited. Two blood and serum samples were collected from all the subjects. Total mRNA was extracted from peripheral blood mononuclear cells (PBMCs), and DNA was extracted from serum and PBMCs. The cDNAs were synthesized, and then analyzed by quantitative real-time PCR for the detection of CD46 transcripts; the expression of GAPDH, a house-keeping gene, was used for the calculation of the relative expression of CD46. DNA was analyzed to detect HHV-6 genomes by quantitative PCR; finally, a standard curve with known amounts of HHV-6 was performed to measure the viral load. Results: 1) CD46 expression: the 80% of MS patients had increased levels in comparison with HBD (p<0.001). 2) When we attempted to correlate CD46 expression with HHV-6 DNA prevalence, we found that the 70.9% of MS patients with high CD46 expression had HHV-6 in their PBMCs vs. 35.7% of MS patients with low CD46 expression (p=0.002). Furthermore, 21.4% of MS patients with increased levels of CD46 expression had HHV-6 genomes in their sera vs. 14.3% among MS patients with low levels of CD46 expression. 3) In the MS patients group with increased levels of CD46 expression (80%), we found higher HHV-6 viral loads among those patients with levels over the median value (71.3 copies/μg of DNA in blood, 210 copies/ml in serum) than in those patients with levels below the median value (47.9 copies/μg of DNA in blood, 123.5 copies/ml in serum). Conclusions: The expression of CD46 seems to be up-regulated in MS patients; this up-regulation appears to be associated with a higher prevalence and viral load of HHV-6 in blood and serum in a subset of MS patients, but further studies are needed to elucidate its role in the pathogenesis of MS.

P245

PCR for bacteria in multiple sclerosis cerebrospinal fluid
J.W. Lindsey; S. Patel; University of Texas-Houston (Houston, USA)

Objective: The objective of this study was to use a sensitive PCR method to test for the presence of seven different groups of bacteria in the cerebrospinal fluid (CSF) of multiple sclerosis patients. Background: The etiology of multiple sclerosis is currently unknown, but many features of the disease suggest an infectious cause. The clinical course, the inflammatory infiltrates seen on histology, and the bands in the cerebrospinal fluid are consistent with a recurrent or persistent infection. Similar diseases, such as acute transverse myelitis, acute disseminated encephalomyelitis, and Guillain-Barre syndrome, are linked to infections. And chronic infection with Treponema or Borrelia can cause neurologic disease. Methods: We designed nested sets of PCR primers specific for the 16S ribosomal DNA of spirochetes, campylobacter, mycoplasma, chlamydia, bartonella, mycobacteria, and streptococcus. Each set of primers is designed to amplify DNA from all members of the desired group without amplifying DNA from common laboratory contaminants. We extracted DNA from the CSF of 10 patients with relapsing-remitting MS, 10 patients with primary progressive MS, and 9 controls. We amplified the DNA with nested PCR, and visualized the PCR products on agarose gels. Each experiment included both MS patients, controls, and a saline negative control. We defined the sensitivity of our method using serial dilutions of known amounts of bacterial DNA. Results: The majority of the CSF specimens were negative for bacteria with all sets of primers. Three MS specimens and one control had a faint band of the correct size with the spirochete primers. Sequencing identified this PCR product as coming from a propionibacterium rather than a spirochete. Further experiments with primers specific for that sequence demonstrated that it was present at low concentrations in all specimens and is likely a laboratory contaminant. The sensitivity of the method was excellent. We could detect 10 copies or less of bacterial DNA per PCR reaction. This corresponds to a concentration of 200 bacteria per μl of CSF. Conclusions: Using a very sensitive PCR method, we were unable to find evidence for the presence of any of the seven tested groups of bacteria in the CSF of MS patients. This study was supported in part by the National Multiple Sclerosis Society, pilot award PP1194.

P246

Cerebrospinal fluid findings in Devic’s neuromyelitis optica
L. Melamud, L. Madalena, M.L. Facio, M.A. PizoloIta, O. Garcea, A.M. Villa; Hospital Ramos Mejia (Buenos Aires, RA); Hospital De Clinicas, FFBY, UBA (Buenos Aires, RA)

Objective: Devic’s neuromyelitis optica (NMO) associates optic neuritis and myelitis without other neurological signs. The prognosis is poor and no satisfactory treatment is known. The aim of our study is to describe the immunochemical characteristics of the cerebrospinal fluid (CSF) in patients with NMO. Material and Methods: We reviewed the medical records of all patients diagnosed with NMO who fulfilled the criteria proposed by Wingerchuk et al. (Neurology 1999; 53: 1107–1114) treated at the Ramos Mejia Hospital. We studied 30 CSF samples from 23 patients who had at least one lumbar puncture during the course of the disease. We analyzed total protein levels; CSF-serum albumin ratio and the IgG index. We also evaluated the presence of oligoclonal bands (OCBs) detected by isoelectric focusing or immunofixation. Fisher’s exact tests were used for dichotomous variables. 17 samples were obtained during an acute phase of the disease whilst 9 were taken in a period of clinical remission. Results: One patient underwent 3 lumbar punctures, 5 patients had two and 17 had only one CSF examination. Out of 30 samples, 17 (57%) were normal whilst 13 (43%) showed at least one abnormal finding. CSF/serum albumin ratio was abnormal in 7 samples, total protein levels higher in 9 and IgG index abnormal in 5 samples. Positive OCBs were found in 17% of all examinations. CSF examination was abnormal in at least one parameter in 9 out of 17 samples (53%) obtained in relapse and in 4 out 9 (45%) obtained during remission. Differences were statistically significant (p<0.05) only when IgG index was considered (45% during bout versus 0% in remission). Among the 6 patients who had more than one CPS, 2 shifted from elevated IgG index and positive OCBs in relapse to normal Index and negative OCBs in remission, one shifted from negative OCBs in relapse to positive in remission and the other 3 subjects did not show changes. Conclusions: The study showed that immunochemical characteristics of the CSF in patients with NMO were abnormal in 13 of 30 samples (43%). Positive OCBs were found in 17% of the CSF similar to previous reports. There was a decrease of the inflammatory pattern during remissions, especially when IgG index was considered. Compared with Multiple Sclerosis in which CSF findings are quite stable over time, in NMO the CSF abnormalities tend to disappear during remission in the majority of the cases.
**P247**

**Does oligoclonal IgM bands specificity predict clinical course and response to treatment in MS patients?**


**Objective:** The presence of oligoclonal IgM bands (OCMB), in particular those directed against myelin lipids, associates with an aggressive disease course in multiple sclerosis (MS). We aimed to study the evolution of MS depending on the different antigenic specificity of OCMB. The role of these antibodies in the decision of early treatment initiation and its influence in treatment efficacy were also studied. **Methods:** 75 MS patients were prospectively studied since clinical onset. The presence of OCMB and its specificity was determined and according to them, patients were assigned to different groups. The initial evolution was studied in all of them. Immunomodulatory treatment was started in patients with two relapses in the previous three years. We calculated the proportion of patients of each group who began treatment and when it was initiated. Treatment response was evaluated studying the relapse rate (number of relapses per year) and the progression index (PI) (EDSS score increase per year) after 72 months of follow-up. After 72 months of follow-up, only 25 ML—patients had started immunomodulatory treatment at 36 months of follow-up. 9 showed OCMB that did not recognize those lipids (M+I+), 9 showed OCMB that did not recognize those lipids (M+I−) and 48 had no OCMB (M−). Patients were followed during 70.75±3.045 months. After a year of follow-up, the clinical course of the M+I+ group was worse than that in the other two (p = 0.005 and p = 0.003 when the number of relapses was determined and p = 0.017 and p = 0.027 when the EDSS was analyzed). There were no differences between M+I− and M− groups. Consequently, both groups were merged in a single group named ML−. All M+I+ patients had started immunomodulatory treatment at 36 months of follow-up. After 72 months of follow-up, only 25 ML− had started it (p < 0.0001). Before treatment, the PI was higher in M+I+ (0.66 ± 0.18) than in treated ML− (0.19 ± 0.95) groups (p = 0.02). Treatment reduced significantly the relapse rate in both groups. It also diminished the PI in treated ML− patients (p = 0.01). There were no differences in the PI of treated ML− patients before and during treatment and in that of untreated ML− patients, these values were always low.

**Conclusions:** An early treatment initiation may be helpful in M+I+ patients because they show a rapid increase of the disability since disease onset. Conversely, our results strongly suggest that the occurrence of two relapses in the three previous years is an appropriate criterion to adequately select those M− patients that will benefit of immunomodulatory treatment.

**P248**

**Reference values for standard cerebrospinal fluid examinations in multiple sclerosis. Results from 99 healthy volunteers**

U. Wurster, R. Stuchan, A. Windhagen, H.F. Peterit, F.M. Leweke; Neurology-Medical School (Hannover, D); Virology-Medical School (Hannover, D); Neurology-University Cologne (Cologne, D); Psychiatry-University Cologne (Cologne, D)

**Background:** Cerebrospinal fluid (CSF) analysis is an integral part of the diagnostic process of multiple sclerosis (MS). Oligoclonal bands (OB) have a sensitivity of 98% for definite MS, but are also found in infectious or inflammatory CNS diseases. The occurrence of CSF antibodies against measles (M), rubella (R) and varicella zoster (Z) virus was suggested as a method with higher specificity for MS. Difficulties in interpretation arise, when CSF abnormalities are encountered in patients with no objective signs for neurological disease. **Objective:** It is often implicated that healthy subjects do not show any CSF alterations, but almost no work exists to substantiate this notion. A psychiatric research project offered an opportunity to establish reference values for the standard CSF parameters used in MS in 99 healthy volunteers of a similar age group. **Methods:** 15 ml CSF was gained from 48 women (27.1 ± 5.9 years) and 51 men (28.3 ± 5.4 years). Albumin and IgG were estimated by nephelometry and OB were analysed by isoelectric focusing on polyacrylamide gels with silver staining. Positive results were confirmed by IgG immunofixation. Viral antibodies against M, R, Z, mumps (Mu) and herpes simplex (H) virus were quantitated by ELISA and an antigen index (AI) was calculated. **Results:** Slight pleocytosis (max. 7.0 cells/μl) occurred in 3 men. With age—corrected cut—offs 7 men and 2 women displayed slight alterations (max. 10.3) of the CSF/serum albumin quotient. No person had an IgG index >0.700, but 2 had a marginal IgG synthesis of 4.1% and 6.1% in the Reiber graphs. 4 OB were found in only 1 subject, 4 had 2 unique CSF bands and 3 displayed a single OB. Mu AI (2.1–13.8) were found in 7, H in 5, Z in 2 and M in 1 person. 4 of the individuals with at least 1 OB also showed an elevated AI. **Conclusion:** An unexpected number of CSF abnormalities (from 3% with pleocytosis to 9.1% with a raised albumin quotient or 9.4% with elevated mumps AI) were observed in 99 apparently healthy volunteers. However, most of the alterations were subtle and would disappear after minimal adjustments of currently used cut—off values. The frequency of OB depends heavily on the chosen limit of bands: 1% with a minimum of 3 OB, 5% with 2 OB and 8% with 1 OB. Although elevations of AI were found in up to 9.4% (mumps), classical combinations of MRZ were not seen. OB and raised AI may represent an anamnestic reaction from former clinically unapparent events.

**P249**

**Highly differentiated CD8+ T cells have different characteristics in the CSF than in the blood**

S. Jilk, M. Schlep, J. Kleber, G. Le Goff, G. Pantaleo, R.A. Du Pasquier; CHUV (Lausanne, CH)

**Introduction:** We have previously shown that highly differentiated CD8+ T cells (THD) were preferentially recruited in the CSF of MS patients as compared to other neurological diseases (OND). CD8+ THD cells are usually considered as cytotoxic T cells. Here, we examined the perforin content of these CSF—enriched CD8+ THD cells. We also looked at the expression of CD82, a co—stimulatory molecule which is important for the activation of T cells during antigen presentation. **Material and Methods:** We enrolled 80 patients undergoing a lumbar puncture, including 21 with definite MS, 25 with possible MS (PoMS), and 34 with other neurological diseases (OND). To analyse the phenotype and activation level of T cells, paired samples of peripheral blood mononuclear cells (PBMC) and CSF cells were stained for CD3, CD4, CD8, perforin (33 patients), or CD4, CD8,CCR7, CD45RA, CD28 molecules (47 patients) and then analyzed using a flow cytometer. **Results:** We did not find any difference in CD28 expression in T cells between MS and OND patients. The progression to the highest degree of differentiation [CCR7−/CD45RA+ (effector stage)] was associated with a decrease of CD28 expression in PBMC, which was more pronounced for CD8+ (14%) than CD4+ T cells (71%, p < 0.05). Among effector CD8+ T cells, the expression of CD28 was higher in the CSF (50%) than in the PBMC (14%, p < 0.05) whereas it was not the case for CD4+ T cells (71% in PBMC versus 89% in CSF, p > 0.05). We found a significant increase in PBMC CD8+ T cell perforin content in OND versus MS patients (12% versus 3%, respectively, p < 0.01) which was not dependent on the presence of inflammatory events in OND patients. This difference was abolished in the CSF. In peripheral blood, CD4+ T cells of MS and OND contained very little perforin (0.2%). By contrast, CD8+ THD cells (CCR7−) had a high amount of perforin (8%), both in CD28+ and CD28− T cells. In the CSF however, the amount of perforin decreased dramatically, even if there was a concomitant recruitment of THD cells. **Conclusion:** CD8+ THD cells in the CSF seem to have different characteristics than in peripheral blood. They express a higher level of CCR7 in parallel with a decrease of perforin content. This profile does not correspond to regular CTL. We are currently examining if these cells could be suppressor T cells. This work was supported by grants from the Swiss
National Foundation 3200B0-104262 and PP00B–106716 and from the Swiss Society for Multiple Sclerosis to RADM.

P250

Morphometric study of oligoclonal bands
M.I. García-Sánchez, E. Gómez-González, M.A. Gamaro, J.E. Iglesias, M.D. Patrano, M. Lucas, R. Suárez, L. Dimca, G. Izquierdo; Hospital Virgen Macarena (Seville, E); University of Seville (Seville, E)

Introduction: The presence of immunoglobulin G oligoclonal bands (OCB) in cerebrospinal fluid (CSF) of a patient, indicates a high percentage (90–95%) possibility of suffering from multiple sclerosis (MS). The role of OCB in developing the disease is still unknown, but is currently considered the most important complementary laboratory test in the diagnosis of MS. Several factors are necessary to perform an exhaustive and contrasting study of OCB: a high sensitivity and specificity technique, a large cohort of patients to obtain statistically reliable results and a normalising system of OCB. Our laboratory uses a high sensitivity and specificity technique (91.6 and 95.8 respectively), we have a data base of 1,350 CSF samples to date, and is developing morphometric software whose prototype results are presented in this abstract. Objectives: To validate morphometric prototype software as an application for the study of OCB present in the CSF of patients with MS. With it, we intend to demonstrate the reproducibility of results compared to those visually attained by a group of neurologists and medical staff as observers. The final purpose is to evaluate the capacity of OCB as a signal of the development of this disease. Patients and Methods: A pilot application was tested with 10 pairs of OCB+ randomly chosen, and without being previously diagnosed, patient samples. The determination of OCB is done by isoelectrofocusing, transfer to nitrocellulose membranes and immunodetection by human anti-IgG antibodies. After developing, the membrane is digitised and archived for its later use. The observational study was carried out by giving the digitised membranes to five neurologists, three people unrelated to neurology, and one laboratory staff member involved in determining these types of samples. The same images were input into the software program being studied and the results were analysed. Results: Disparity of results acquired in the observational study confirmed the subjectivity of the OCB visual study and suggests the need to implement a plan for standardising OCB as a first step towards a more in depth investigation of them. The software provides high reproducibility and reliability in definitive band profiles for analysis.

P251

Software prototype for morphometric analysis of the oligoclonal bands
E. Gómez-González, M.I. García-Sánchez, J.E. Iglesias, G. Izquierdo; University of Seville (Seville, E); Hospital Virgen Macarena (Seville, E)

Introduction: At present time, Multiple sclerosis (MS) is the disability neurological disease of greatest impact in young people, having a high-priority affection in women. Diagnosis is not easy in most of the cases, being necessary to use all possible strategies to exclude other pathologies and to search for possible predictive biomarkers of the evolution in a disease whose etiology is still unknown. It is of fundamental importance in the diagnosis, the study of the cerebrospinal fluid (CSF) and particularly the detection of the oligoclonal bands (OCB). The OCB studies have been made to consider OCBs as biological markers of evolution of the disease but no references are found on the normalization of the images and their analysis at the moment. We think such normalization and processing is necessary since the visual study of the OCB is completely subjective. Objectives: To design, develop and put into operation an application (software) program to analyze digital images of oligoclonal bands, extracting quantitative information from them, and to implement tools of analysis of these results that allow to characterize the presence of relative maxima and minima, characteristic sequences or patterns that may be statistically related to clinical data of the patients. Method: For each case, using the developed program of analysis, two partial images are processed: a control band (serum) and an analysis band (CSF). To each image, specific algorithms based on statistical and morphologic analysis are applied to detect, locate and characterize the presence of strips in the bands. A statistical analysis is used to characterize dispersion of the values of each parameter for cases with the same pathology and to quantify their expected differences to cases corresponding to other pathologies. This analysis may also define new parameters for the characterization of the strips. Result: Initial tests made so far using the pilot program, allow to process images of OCB with a minimal or no intervention of the user. This implies that results of the optical digital analysis and morphometry of OCBs show a high reproducibility. Additionally, a method of image registration is also being developed to improve robustness and reliability of the processing.

P252

Sulfatide is shedded into the CSF in multiple sclerosis patients and their healthy siblings
S. Haghhi, A. Lehmann, E. Turkowski, A. Turkowski, P. Fredman, O. Andersson; Institute of Clinical Neuroscience (Goteborg, S); Institute of Internal Medicine (Goteborg, S)

Background: MS is associated with breakdown of myelin membrane lipids, two of which are sulfatide and galactosylceramide (GalCer). A network of adhesion molecules, proinflammatory cytokines, and chemokines amplifies the local inflammation in MS. We investigated the levels of myelin membrane lipids breakdown products in the cerebrospinal fluid (CSF), in the MS patients and their healthy siblings. Glycosphingolipids galactosylceramide and its sulphated form, sulfatide, molecules have been shown to be potential antigens and to have opposite effect on cytokine production. The present study also correlates CSF cytokine and glycosphingolipid levels in MS patients and their healthy siblings.

Method: We investigated 47 sibling pairs, each with a patient with clinically definite MS and one healthy sibling including 9 healthy siblings with more than 2 oligoclonal bands in the CSF (MS immunopathic trait), and 50 unrelated healthy controls. Results: Increased CSF levels of sulfatide were found not only in MS patients but also in the siblings. The CSF GalCer levels did not differ between these 3 groups. Serum IgM antibody levels were increased in the MS group as well as in the sibling group. We found increased level of TNF-alpha in CSF in the MS patients and the siblings group. There was a positive correlation between GalCer and IL-6 in the subgroup of siblings with MS immunopathic trait. Conclusion: The results of the present study reveal that shedding of glycosphingolipids to the CSF and an inflammatory response against glycosphingolipids occurs in MS patients. Glycosphingolipids may have immunomodulatory consequences and primarily antigen in MS pathogenesis and also may provide valuable tools for tracking disease activity. The relative importance of glycosphingolipids compared with myelin protein epitopes as early antigens needs to be evaluated in further studies.

P253

CSF regulatory T cells of patients with clinical isolated syndrome suggestive for MS correlate with MRI pathology

Objective: Multiple sclerosis (MS) is a heterogeneous inflammatory disease. However, CD4+ autimmune T cells seem to play a major role in its pathogenesis. Mounting evidence suggests that CD4+
CD4+ regulatory T cells (Treg cells) may be able to protect brain tissue from autoimmune inflammation. In MS, clinical outcome and predictive markers are hardly established, but MRI lesion load seem to be a negative one. Here we correlated cerebrospinal fluid (CSF) Treg cells of patients with clinically isolated syndrome with the initial MRI lesion load.

**Patients and Methods:** 14 untreated and otherwise healthy consecutive patients with first clinical event suggestive for MS (CIS), CSF pleocytosis and positive oligoclonal bands underwent brain MRI according to established sequence protocols for MS. CSF cells and peripheral blood lymphocytes (PBL) were analysed flowcytometrically, using monoclonal antibodies for CD4, CD8, and CD25. Means of relative amounts of Treg cells of PBL and CSF were compared and total MRI lesion load were correlated with the ratio of both (PBL/Treg (CSF)). Two-sided Mann-Whitney-U test and correlation coefficient were performed. p < 0.025 was considered significant after Bonferroni-adjustment (0.05/2 = 0.025).

**Results:** Means of relative amounts of Treg cells differed significantly (p < 0.001) between PBL (45.3 ± 12.9% of all CD4+ T cells) and CSF (23.0 ± 13.1% of all CD4+ T cells). The individual ratio of Treg (PBL)/Treg (CSF) correlated positively with the number of MRI lesions (r = 0.72; r2 = 0.52; p < 0.0003).

**Conclusions:** Treg cells are significantly less present in the CSF than in the blood of CIS patients. Additionally, their absence in the CSF correlates positively with increased MRI pathology. Because MRI lesion load is a surrogate marker for inflammatory activity, central nervous system lack of Treg cells may suggest a higher risk for MS conversion in CIS. Furthermore, our data may give another indirect hint for a possible protecting role of Treg cells in the pathogenesis of multiple sclerosis in general.

**P254**

**Characterisation of cell populations in cerebrospinal fluid of patients with multiple sclerosis**

B. Kuenz, A. Lutterotti, R. Ehling, C. Gneiss, M. Khalil, F. Deisenhammer, T. Berger, M. Reindl; Insbruck Medical University (Insbruck, A).

**Introduction:** Recent studies on the immunopathogenesis of multiple sclerosis (MS) indicated a crucial role of B-cells, plasma cells and their products. B-cells were detected in the cerebrospinal fluid (CSF) of patients with MS and other neurological inflammatory diseases (IND), but were largely absent in non-inflammatory neurological diseases (OND). Moreover, it has long been known that an increased intrathecal immunoglobulin synthesis is observed in more than 90% of MS patients, reflected by increased IgG indices and oligoclonal IgG bands (OCB). **Objectives:** The aim of this study was to analyze CSF cell populations in patients with clinically isolated demyelinating syndromes (CIS; n = 25), relapsing-remitting (RRMS; n = 8) and primary progressive (PPMS; n = 6) MS in comparison to IND (n = 16) and OND (n = 23) in order to determine differences in the distribution of B-cell subsets and to address their role in neuroinflammation and MS disease progression. **Methods:** CSF was obtained by standard diagnostic lumbar puncture and CSF cells were immediately stained with fluorochrome-labeled antibodies to human leukocyte surface antigens. The lymphocyte subpopulations in CSF were then analyzed using three-color flow cytometry on a BD FACScan with Cell Quest software. **Results:** We found a significantly increased number of CSF B-cells (CD19+CD138−) in CNS inflammation (CIS, RRMS, PPMS and IND). CSF plasma cells (CD19+CD138+) and CSF plasma blasts (CD19+CD138−) were significantly increased in CIS and RRMS, and were thus more specifically associated with bout-onset MS. Furthermore, we could demonstrate a highly significant positive correlation of CD19+ and CD138+ CSF cells with the total number of CSF leukocytes as well as with intrathecal IgG and IgM production. **Conclusion:** Our results confirm previous studies on the specific accumulation of B-cells (CD19+) and plasma cells (CD138+) in the CSF of MS patients, thus providing further evidence for their complex role in the neuroinflammatory pathogenesis of MS.

**P255**

**Differential immunomodulation profile in multiple sclerosis patients suffering viral or parasitic infection**


Epidemiological and clinical observations suggest that viral infections may introduce a bias immune responsiveness in Multiple Sclerosis (MS) patients, which in turn triggers disease exacerbations. Conversely, striking inverse correlation occurs between parasite infections and autoimmune diseases. In this investigation, clinical and radiological findings were monitored in MS patients, together with T cell responses during viral or parasitic infections. MS attacks occurring 2 weeks prior to onset and up to 5 weeks after a viral infection were considered temporally related infections (TRI). Twenty-three MS patients suffering a viral infection, and 12 MS patients presenting parasite infection were studied. Increased risk of relapse was observed during temporally related virus infections in MS patients (Odds ratio 3.2; p < 0.0001). Moreover, the number of Gd-enhancing lesions was greater during exacerbations overlapping TRI, compared to those unrelated to viral infection. In contrast, chronic exposure to parasites in MS patients was associated with a significant reduction in the number of exacerbations, as well as in the number of T2 and Gd-enhancing MRI lesions, when compared to non-infected MS patients. Numbers of IFN-γ, TNF-α, and IL-12 secreting cells, were higher in PBMC collected during exacerbations associated to viral infections than during stable disease or exacerbations not associated with infections. In parasite infected MS patients on the other hand, MBP-specific responses showed a significant increase in IL-10 and TGF-β, and a decrease in IL-12 and IFN-γ secreting cells, compared with uninfected MS individuals. In addition, viral antigen (Ag) stimulation induced maximal myelin-Ag specific T cells effector response, at concentrations 20 to 30 times lower than native Ag alone. Moreover, MBP-specific T cell clones from parasite-infected patients were characterized by a cytokine profile similar to Th3 and Th1 T cell subsets, and cloning frequency of CD4+CD25+FoxP3+ T cells was substantially increased in parasite infected MS patients, compared both to uninfected and viral infected MS individuals. Overall, these observations suggest that viral infections can increase PBMC response to myelin-Ag, causing a Th1-like response and increasing risk of relapse, whereas parasite infections do the opposite, inducing regulatory T cells and disease remission.

**P256**

**Immunological profile of patients with multiple sclerosis associated fatigue**

K. Strassburger, M. Kumar, S. Vago, E. Kreuzfelder, V. Limnouros, N. Putzki; University Clinic Essen (Essen, DE).

**Background:** The fatigue syndrome is one of the most frequent and most disabling symptoms in multiple sclerosis. Little is known about its underlying pathology. Fatigue does not correlate with disability or lesion load on conventional MRI. Previous studies suggested that immune factors may play a causative role in the development of fatigue. **Objective:** To compare the counts of leukocytes and their subpopulations as well as frequency and function of CD4+CD25+ regulatory T cells (Treg) in untreated MS patients with and without the fatigue syndrome. **Results:** 44 untreated age, gender and EDSS matched MS patients (63% n MS, 27% sp MS) were investigated (22 with fatigue, Fatigue Severity Score > 5 in fatigue patients, mean age 38 years, mean disease duration 7 years, mean EDSS 3.6). Routine blood examinations were normal. No significant differences in the absolute and relative counts of leukocytes, granulocytes, monocytes and natural killer cells were found. Frequencies of lymphocytes and their subsets as CD4+,CD8+, CD4+CD25+, CD4+HLA-DR+, CD4+CD8+HLA-DR+, CD4+CD8+CD25+, and CD4+HLA-DR+ and HLADR expression on monocytes were not significantly different. Although T regulatory lymphocytes showed a slightly increased

*Multiple Sclerosis* 2006; 12: 51–5228

www.sagepub.co.uk

Downloaded from msj.sagepub.com by Shula Edelkind on October 1, 2010
frequency in both groups compared to healthy controls. Treg function was similarly impaired after stimulation with Myelin Basic Proteins. 

**Conclusion:** We compared a variety of quantitative and qualitative immunological parameters to identify differences between MS patients with and without fatigue. The immunological profiles did not show any differences in the two groups. We conclude that other than immunological differences are responsible for the fatigue syndrome in MS. Further investigations of i. e. cytokines and chemokines are currently undertaken to further elucidate the aetiology of this phenomenon.

P257

**Hypothalamos-pituitary-adrenal axis dysregulation in multiple sclerosis: clinical, MRI and immune correlates**

C. Hiesen, S. Gold, U. von der Mark, I. Fischer, C. Reich, C. Sauger, T. Kucinski, C. Otte, K. Schulz; Universitätsklinikum Hamburg-Eppendorf (Hamburg, D); MS Center, UCLA School of Medicine (Los Angeles, USA)

**Background:** The functional status of the Hypothalamo-Pituitary-Adrenal (HPA) axis might be of relevance in the control of MS. HPA hyperactivity is the most consistent clinical finding, however, makers of diminished HPA responses have been reported in severe MS. Hyperactive responses in the dexamethasone ( Dex)-CRH test which might reflect attenuated negative feedback of the glucocorticoid (GC) system correlate with disease severity and cognitive deficits and may predict disease progression. 

**Methods:** Based on a brief screening test of processing speed, we compared cognitively impaired (CI, n = 25) and cognitively preserved (CU, n = 25) RR and SPMS patients. Neuropsychological tests as well as multimonial MRI, Dex-CRH suppression test and peripheral sensitivity of CD4 and CD8 cells to Dex in vitro were performed. 

**Results:** Patients with either hypo- (n = 11) or hyperactive (n = 2) dysregulation had lower scores on several neuropsychological tests compared to patients with a normal HPA response in the Dex-CRH test. Verbal memory and divided attention showed significant differences to patients with normal regulation (p = 0.015 and 0.016). None of the MRI markers (GD lesions number, T2 lesion number, atrophy and spectroscopy) correlated with Dex-CRH test results or tests of dexamethasone sensitivity of immune cells in vitro. Baseline Interferon-gamma production of CD4+ cells however was correlated with ACTH and to a lesser degree with cortisol measures of the Dex-CRH test (r = -0.41, p = 0.007 and r = 0.28, p = 0.07), indicating a higher IFN production by immune cells obtained from patients with stronger HPA responses. Furthermore, there was an association between Gd+ lesions on MRI and a diminished peripheral dexamethasone sensitivity (r = 0.30 up to r = 0.38, p = 0.014 up to p = 0.052). 

**Conclusion:** Neuropsychological deficits and HPA dysregulation seem to share pathophysiological pathways leading to an inverse U-shaped relation between HPA axis activity and cognition in MS. We found no evidence for a correlation of central HPA regulation and MRI as well as immune markers. Peripheral Dex sensitivity seems to be negatively associated with acute inflammatory brain lesions (Gd enhancing lesions). These findings supports the concept of peripheral GC insensitivity in active MS.

P258

**Paraoxonase activity in plasma of patients with multiple sclerosis: preliminary results**

H. Bartosik-Psujek, A. Jamroz-Wisniewska, J. Beltowski, K. Rejdak, Z. Stelmasiak; Medical University of Lublin (Lublin, PL)

**Background and objectives:** Multiple sclerosis (MS) is a chronic disabling condition of unknown etiology that is thought to be influenced by environmental factors. Increasing evidence supports a role for oxidative stress in the inflammatory processes and in the pathogenesis of MS. Paraoxonase (PON) 1 is an antioxidant enzyme associated with high-density lipoproteins (HDL) that protects low-density lipoproteins (LDL) and HDL against oxidative modification. The purpose of this study was to evaluate plasma PON1 activity in patients with MS. 

**Subjects and Methods:** Sixty seven people with MS according to McDonald criteria and 31 healthy individuals matched for sex and age, agreed to participate in the study. The MS group comprised 22 men and 45 women; mean age was 34.4 ± 8.0 years (range 20–51), mean disease duration was 7.6 ± 5.9 years (range 1–30). Depending on clinical symptoms of MS, the MS-patients were divided into relapsing-remitting MS (36 patients) and progressive MS (31 patients). In all patients we examined plasma PON1 activity towards paraoxon, towards phenyl acetate (i.e. arylesterase activity), towards paraoxon in the presence of 1 mM NaCl buffer and lipid profile. 

**Results:** We found statistically significant differences in arylesterase activity of PON1 in patients with progressive type of MS compared to the control group (161.54 ± 42.11 U/ml vs. 122.75 ± 32.61 U/ml; p < 0.001). There were no statistically significant differences in other results between groups. 

**Conclusions:** Our study shows that in patients with progressive type of MS there is low PON1 arylesterase activity. We conclude that oxidative stress is involved in the development and the progression of MS.

P259

**Expression of activity-dependent neuroprotector in the immune system: relevance to multiple sclerosis**

M. Braitch, A. Robins, C.S. Constantinescu; The University of Nottingham (Nottingham, UK)

**Background:** Activity dependent neuroprotector (ADNP) has been identified as a induced neuroprotective molecule enhancing neuronal survival. ADNP is induced by VIP, a neuroprotective peptide, in astrocytes and protects neurons against damage through a variety of mechanisms. VIP is protective against EAE, the animal model of MS. ADNP is an 828 amino acid peptide which is expressed in the cerebellum, hippocampus and cerebral cortex. Recently it has been shown that ADNP is also neuroprotective in EAE. We have recently found a reduced expression of ADNP in peripheral blood mononuclear cells (PBMC) of MS patients using microarray analysis. This finding prompted us to investigate expression of ADNP in cells of the human immune system. 

**Methods:** PBMC from healthy volunteers were surface stained with antibodies CD4 (T helper cells), CD3 (Tcells), CD25 (marker of activation and Treg), CD19 (B cells) and CD14 (monocytes) and intracellularly stained with an ADNP antibody. Quantitative PCR was used to measure ADNP expression in T cells from relapsing remitting MS patients (n = 60) and healthy matched controls (n = 20). 

**Results:** We confirmed that ADNP mRNA is reduced in PBMC from MS patients compared to controls. 

**Conclusions:** ADNP is abundantly expressed in the immune system and it is reduced in PBMC in MS. This may reflect a reduced neuroprotective capacity in MS. Supported by University Hospital Nottingham.
MS. ADNP is a 828 amino acid peptide which is expressed in the cerebellum, hippocampus and cerebral cortex. Recently it has been shown that ADNP is also neuroprotective in EAE. We have recently found a reduced expression of ADNP in peripheral blood mononuclear cells (PBMC) of MS patients using microarray analysis. This finding prompted us to investigate expression of ADNP in cells of the human immune system. Methods: PBMC from healthy volunteers were surface stained with antibodies CD4 (T helper cells), CD3 (Tcells), CD25 (marker of activation and Treg), CD19 (B cells) and CD14 (monocytes) and intracellularly stained with an ADNP antibody. Quantitative FACS was used to measure ADNP expression in T cells from relapsing remitting MS patients (n = 60) and healthy matched controls (n = 20). Results: We confirmed that ADNP mRNA is reduced in PBMC from MS patients compared to controls. 1 x 10^6 human PBMC were isolated and surface stained with CD4 (T cell), CD3 (T cell) CD4+CD25+ (Regulatory T cell) CD19 (B cell) and CD14 (monocytes) antibodies to characterize the cells. Conclusion: ADNP is abundantly expressed in the immune system and it is reduced in PBMC in MS. This may reflect a reduced neuroprotective capacity in MS. Supported by University Hospital Nottingham.

P261

**Serum levels of soluble CD14 correlate with disease activity in multiple sclerosis patients and are increased by interferon-beta treatment**

A. Lutterotti, B. Kuertz, M. Khalil, V. Greddler, R. Ethling, C. Greiss, F. Deisenhammer, T. Berger, M. Reindl; Innsbruck Medical University (Innsbruck, A)

Background: There is increasing evidence that, besides adaptive immunity, the innate immune system plays a substantial role in the induction of autoimmune diseases. Recently the innate immune receptor CD14 was shown to be upregulated in the brain of multiple sclerosis (MS) patients and CD14 expression correlated with disease activity in experimental allergic encephalomyelitis (EAE). CD14 is a receptor expressed on monocytes, macrophages and neutrophil granulocytes. A soluble form of CD14 (sCD14) can be detected in serum or plasma. Elevated serum levels of sCD14 have been reported in MS patients but also in other inflammatory diseases of autoimmune etiology. Aims: Since there is increasing evidence that CD14 may be involved in the immunopathogenesis of MS we were interested whether serum levels of sCD14 correlate with the clinical course, disease activity and disease severity in 165 MS patients (96 RR, 50 SP, 19 PP). As a control group we analyzed 22 patients with other neurological disease and 50 healthy controls. To further evaluate whether immunomodulatory therapies, such as interferon-beta, have an influence on sCD14 levels we analyzed prospectively collected serum samples at baseline and one year in an independent cohort of 50 RR-MS patients. Methods: Serum levels of soluble CD14 (IBL, Hamburg, Germany) were detected by enzyme linked immunosorbent assay according to the manufacturers instructions. Results: Serum levels of sCD14 were higher in MS patients compared to healthy controls (p < 0.001) and OND (p < 0.001). Within the MS group sCD14 levels were selectively increased in bout-onset (RR+SP) MS compared to PPMS (p < 0.001). When correlating sCD14 levels with clinical disease activity we found sCD14 levels were significantly increased in MS patients with stable disease (RR+SP) compared to patients with an acute relapse (RR+SP; p < 0.01) or progressive disease (SP+PP; p < 0.05). Interferon-beta treatment increased sCD14 serum levels in MS patients after one year of treatment, whereas there was no significant increase of sCD14 levels in patients receiving other immunomodulatory treatments or patients without therapy. Conclusion: In summary we report evidence that serum sCD14 levels are increased in MS and correlate with disease activity in MS patients. Further studies are now needed to elucidate whether sCD14 might serve as a useful marker of disease activity or treatment response in relapsing MS patients.

P262

**Prognostic value of serum anti-myelin antibodies in early multiple sclerosis as measured by a myelin flow cytometry assay**

J.M. Nielsen, E.C.W. Breij, C.H. Polman, D.A.M. Heijnen, T. Korteweg, R. Vloet, L. van Winsen, R.M.J. Uitdehaag, F. Barkhof, C.D. Dijkstra; University Medical Centre (Amsterdam, NL)

Background: Little is known about factors that predict disease progression in individual MS patients. Recent data suggest that the presence of anti-myelin oligodendrocyte glycoprotein (anti-MOG) and anti-myelin basic protein antibodies (anti-MBP) predict a shorter time to relapse in patients with a clinically isolated syndrome (CIS). Increased serum levels of antibodies to a range of different myelin antigens have been shown in different subgroups of MS patients, although no single antigen was specifically associated with MS. We developed a flow-cytometry assay that detects antibodies directed against whole human myelin, including conformational and posttranslationally modified myelin antigens. Using this assay we previously demonstrated increased anti-myelin IgG levels in approximately fifty percent of patients with established MS [1]. Goals: To determine the prognostic value of the myelin flow cytometry assay in a group of early relapsing patients in relation to clinical and magnetic resonance imaging (MRI) parameters. Methods: We tested baseline sera of 74 consecutive early MS patients included in a longitudinal follow up study and 40 healthy controls. For analysis clinical parameters of patients with anti-myelin antibody levels in the lowest were compared to the highest tertile and increased to non increased levels. Increased levels were defined as levels above 2.5 times standard deviation of those of healthy controls. Patients were followed prospectively with regular MRI and clinical evaluation. Disease progression was measured with the expanded disability status scale (EDSS) and multiple sclerosis functional composite (MSFC). Results: 33 patients had a CIS and 41 patients relapsing remitting MS. Median follow up time was 2.8 years. Increased antibody levels for IgM were present in 11% and for IgG in 15% of patients, when compared to healthy controls. For IgM or IgG antibodies there was no difference in time to next relapse, change in MSFC or EDSS when comparing the lowest to the highest tertile. Patients with increased levels of IgM and/or IgG had slightly less EDSS progression and less often used disease modifying therapy, but these differences did not reach statistical significance. Conclusion: Preliminary analysis of a limited number of patients suggests no prognostic value in terms of time to next relapse, EDSS and MSFC over time. MRI and clinical data of the extended follow up will be presented.

Reference


P263

**Multiple sclerosis and autoantibodies**

N. Çömez İmaz, B. Türkoglu, C. Orken, M. Gençer; Haydarpaşa Numune Education and Research (Istanbul, TR)

Immunopathogenesis in multiple sclerosis is heterogeneous. Autoimmune mechanisms triggered by environmental factors has been considered to be effective in genetically susceptible individuals. In this study we investigated the prevalence of autoantibone parameters in CIS and their influence on disease progression and EDSS scores. Fifty MS patients who had been followed-up in our clinic and known to have no autoimmune or systemic illness were enrolled in this study. The diagnosis was made according to Mc Donald criteria and EDSS scores were evaluated in all patients. In all patients Anticardiolipin (ACA), antinuclear (ANA), antithrombin (AGA), antiTPO and antidualNA antibodies were searched in the serum. Antiendomysial antibodies (AEA) were assayed to exclude Celiac disease in patients who had positive AGA. The prevalence of
these antibodies in MS patients and their interaction with age, gender, disease course and EDSS scores were assessed statistically. Fifty-four percent of the patients were relapsing-remitting type MS. AGAIgG and AGAIgA were positive in 30% and 12%, respectively. 6 of the 15 patients who had positive AGAIgG also had positive AGAIgA. AEs were negative in all these patients. Anti-TPO were positive in 38% either 89.5% of them had another autoantibody. Of these patients were using IFN-. ACAIG and AcALM were positive in 22% and 24%, respectively. ACAIG values were significantly high in patient group compared to reference group (p = 0.000). In 22% of patient group AcALM values were above 11 IU. AcALM levels were ≥ 10 in 24% of patient group and 4% of reference group. The difference was statistically significant (p < 0.05). Meanwhile all these patients had mildly positive AcALM. 5 of the patients had both positive ACAIG and AcALM. ANA and antids DNA were both 2% positive. There was no significant difference between study and reference groups in terms of gender, age of onset, course of disease and EDSS scores. (p > 0.05).

In conclusion, high levels of autoantibodies could be found in MS patients without any clinical finding of an autoimmune disease. Sometimes it might be a challenge to differentiate MS from other autoimmune diseases. The positivity of systemic autoantibodies in MS is a non-specific finding without any clinical significance. These findings may only serve to interpret the immunopathogenesis of MS. The essential of MS diagnosis is still Mc Donald criteria.

P264

Autoantibodies against alpha-Fodrin are associated with relapses of multiple sclerosis

E. Wiesemann, S. Markmann, T. Witte, A. Windhagen; Medical School Hannover (Hannover, D)

Alpha-Fodrin is abundantly expressed in salivary glands but also in other tissues including synapses. Antibodies against alpha-Fodrin are directed against a 120-kDa neosynten that is released by caspase 3 during apoptosis. Alpha-Fodrin IgG and IgA antibodies are observed with increased frequency in Sjögren’s Syndrome and other autoimmune diseases including Multiple Sclerosis (MS) and systemic lupus erythematosus (SLE). The goal of our study was to examine antibodies against alpha-Fodrin in patients with MS in relation to their clinical disease activity. Serum was collected from 99 patients with relapsing remitting or relapsing progressive MS who were clinically stable and 27 patients with acute relapses who were treated with high dose intravenous steroids (here serum was obtained before and after treatment). Both untreated patients and patients treated with interferon-beta or Gilartimamocetate were included in the study. Serum samples were frozen at −20°C and later tested for both IgG and IgA alpha-Fodrin antibodies by ELISA as well as antinuclear antibodies (ANA). Sera were considered positive if they had values ≥ 15 U/ml for both IgG and IgA ELISA. Out of the 99 stable MS patients 6 (6.06%) had increased IgG and/or IgA anti-alpha-Fodrin concentrations compared to 10 out of 27 patients (37.04%) with relapses (p < 0.001). In all patients antibody titers decreased after steroid therapy. In 26 patients (clinically stable) serum was obtained repeatedly over a period of one year (at 0, 1, 3, 6 and 12 month). The antibody concentrations in these patients remained stable. Testing for ANA in 77 patients with MS showed ANA ≥ 1:320 in 23.4% of patients and in 49.4% ANA ≥ 1:160. No correlation was observed between anti-alpha-Fodrin and ANA titers. Although the pathophysiological role of alpha-Fodrin in MS has yet to be determined our results suggest that antibodies against alpha-Fodrin can be considered as a marker of disease activity at least in a subgroup of patients with MS. It is unclear whether increased titers of alpha-Fodrin antibodies can be considered as markers of increased/chronic apoptosis or reflect immune activation. Longitudinal studies are planned to investigate whether alpha-Fodrin antibodies are associated with other activation markers or correlate with disease subtypes.

P265

Focusing on relevance of anti-infectious antibodies in multiple sclerosis

P. Stourac, J. Bednarova; Faculty Hospital Brno (Brno, CZ)

Background and Goals: Multiple sclerosis (MS) is an immune-mediated, chronic demyelinating disease causing the significant neurological disability. The various specific anti-infectious (anti-measles, rubella, varicella zoster and toxoplasma) and non-infectious antibodies were reported in serum and cerebrospinal fluid of MS patients. Such findings pose a serious difficulties as concerns their relationship to the hypothetical etiology of MS and clinical significance. The elucidating the relevance of anti-infectious antibodies is also strenghten in endemic areas of neuroborreliosis (NB) where as 10% of population could be anti-borrelia antibodies positive and with respect to some clinical aspects of these two entities. The goal of this study is to report the frequency of intrathecal synthesis of specific IgG antibodies against Borrelia (B.) burgdorferi in well defined MS, neuroborreliosis and other neurological diseases (OND) cohorts of patients. Methods and Results: We investigated a cohort of 85 patients: 40 patients were diagnosed as multiple sclerosis, 30 patients were diagnosed as neuroborreliosis and 15 patients had other neurological diseases. Serum and cerebrospinal fluid samples were analysed in each patient. For the detection of specific antibodies the diagnostic kit of Test-Line Company, Clinical Diagnostics Ltd, Czech Republic (ELA Borrelia garinii IgG) was used. The intrathecal synthesis of specific anti-bodies was evaluated as specific antibody index (AI) according to Reiber’s method, where absolute values of absorbances were converted to arbitrary units (AU). Values above 1.4 indicated positive intrathecal synthesis. In the cohort of MS patients the intrathecal synthesis of specific IgG antibodies against B. burgdorferi was detected in 23% of patients with AI in the range 1.8 – 7.8 Intrathecal synthesis of specific IgG antibodies against B. burgdorferi was detected in 90% NB patients with AI in the range 1.5 – 33.0 Low anti-borrelia AU in MS patients reflects polyspecific immune response against B. burgdorferi compared to high anti-borrelia AU in NB patients that indicate immune response against B. burgdorferi as the causative antigen in NB. Conclusions: We report positive intrathecal synthesis of specific IgG antibodies against B. burgdorferi in 23% of MS patients. We conclude that specific intrathecal synthesis against infectious agents in MS is a part of polyspecific immune response in chronic autoimmune disease and does not represent an etiological relevance to MS.

P266

Development of multiple sclerosis after vaccination against hepatitis B: a prospective study based on clinical and MRI features and HLA haplotypes

S. Ozakkbas, E. Ildman, B. Yulug, B. Pakoz, H. Bahar, Z. Gulay; Dokuz Eyal University (Izmir, TR)

The aetiology of multiple sclerosis (MS) is still not fully understood. Infectious agents are thought to play a role in the development of this multifactorial disease. There are cases reported occurring after both plasma-derived and recombinant hepatitis B vaccines. In this study we compared a group of 11 MS patients who developed first clinical symptoms after hepatitis B vaccination (Group I) with 71 MS patients that have never been vaccinated against hepatitis B and were negative for hepatitis B serology (Group II), and 20 healthy blood donors (Group III). Group I and Group II were also compared on the basis of magnetic resonance imaging (MRI) data, not only in the first or index relapse but also in the follow-up period. For comparison, T2 hyperintense lesions, Gd-enhanced lesions and T1 hypointense lesions (black holes) were calculated in the first relapse, in the second MRI which was performed 2 years after the presentation, and in the fifth year. Mean age was 27.75 (19–39) in Group I, 30.16 (18–50) in Group II and 34.4 (18–50) in Group III. Mean
Abstracts

attack rate after 2 years was 1.5 in Group I, and 1.63 in Group II. Mean Expanded Disability Status Scale (EDSS) score after 2 years was 1.31 in Group I, and 1.89 in Group II. Although there were more Gd-enhanced lesions in Group I than Group II, it was not statistically significant (n = 3.12 and n = 2.64, respectively, p = 0.075). This trend disappeared in the second and third year MRI (p = 0.13 and p = 0.26, respectively). It was not demonstrated any difference on the basis of neither T2 lesions nor T1 hypointense lesions between Group I and II, respectively. It was not demonstrated any difference on the basis of the diffusion of soluble molecular tracers as well as the migration of immune cells and monocytes from relapsing-remitting multiple sclerosis patients correlated with disease activity.

P267

Statins restrict leukocyte migration and promote human blood-brain barrier characteristics

I. Ifergan, K. Wosik, R. Cayrol, H. Kebir, P. Duquette, A. Prat; CHUM (Montreal, CAN)

Multiple Sclerosis (MS) is characterized by development of multifocal demyelinating lesions disseminated in time and space throughout the central nervous system (CNS). Deregulation of the blood-brain barrier (BBB) and transendothelial migration of activated leukocytes are among the earliest cerebrovascular abnormalities seen in MS brains and parallel the release of inflammatory cytokines/chemokines. Evidence for the anti-inflammatory effects of statins within the CNS arose from studies demonstrating that statins improve clinical signs of Experimental Autoimmune Encephalomyelitis (EAE), the animal model of MS. While the effects of statins on immune cells are well characterized, the impact of statins on the BBB is not known. In this study, we demonstrate that Lovastatin and Simvastatin induce a 50–60% reduction in the diffusion rates of bovine serum albumin (BSA) and [14C]-sucrose across human BBB-Endothelial Cells (ECs) in vitro through abrogation of isoprenylation processes, but independent of the expression of the tight junction molecules Occludin, VE-Cadherin, JAM-1, ZO-1 and ZO-2. Simvastatin and Lovastatin were pre-treating SH-SYS cells with 2 microM of TO901317 for 24 hours partially counteracted the extent of neuronal death induced by both 50 mg/l (p < 0.001) and 100 mg/l (p < 0.05) of 7-ketocholesterol. Discussion: Over-expression of LXRBeta in PBMCs from MS patients strongly suggests the involvement of this transcription factor in MS pathogenesis. The observed inhibitory effects of agonist-specific stimulation of LXRs on T cell activation, IFN gamma secretion, and on 7-ketocholesterol-induced neurodegeneration may open novel therapeutic avenues in MS.

P269

pSTAT1, pSTAT3 and T-bet expression in peripheral blood mononuclear cells from relapsing-remitting multiple sclerosis patients correlated with disease activity

G. Frisullo, F. Angelucci, M. Caggiula, V. Nocti, R. Iorio, A.K. Patanella, C. Sanmuccia, M. Mirabella, P.A. Tonalli, A.P. Batocchi; Catholic University of Rome, Rome, Italy

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS). The etiopathogenic mechanisms underlying the development of MS are still not completely understood but it is considered to be a T helper 1 (Th1) cell-mediated autoimmune disease. T-bet has been identified as a key transcription factor for the development of Th1 cells and the induction of IFN-g production. T-bet is induced during T cell activation by the IFN-g-signal transducer and activator of transcription (STAT)-1 signalling pathway. In this study we found an up-regulation of T-bet and pSTAT1 in peripheral blood CD4+ T cells and monocytes from relapsing-remitting MS patients in relapse as compared to patients in remission and to healthy subjects. The increased expression of pSTAT1 strongly correlated with T-bet expression in CD4+, CD8+ and monocytes from patients in relapse and was associated with an increased production of IFN-g by peripheral blood mononuclear cells (PBMC). pSTAT3 was also up-regulated in CD4+, CD8+ and monocytes from patients in relapse and was associated with an increased production of IL10 but not IL6. pSTAT1, pSTAT3 and T-bet expression strongly correlated with Gd-DTPA enhanced lesions at brain and spinal cord Magnetic Resonance Imaging. Our data show for the first time that there is an up-regulation of type 1 immunity-correlated transcription factors such as STAT1 and T-bet in peripheral blood subpopulations of MS patients in active phase of disease. The evaluation of T-bet and pSTAT1 expression in peripheral blood CD4+, CD8+ T cells and monocytes could be used as a marker of disease activity in RRMS.
Parent of origin effect on risk for multiple sclerosis in a genetic isolate in the Netherlands

I.A. Hoppenbrouwers, F. Liu, Y.S. Aulchenko, B.A. Oostra, C.M. van Duijn, R.Q. Hintzen; ErasmusMC (Rotterdam, NL)

Background: Multiple sclerosis (MS) is a complex disease, resulting from genetic as well as environmental factors. Parent of origin effects may influence the risk for MS. Objectives: To investigate parental relationships between MS patients, for whom extensive genealogic information was available. Methods: This study is part of a larger research program named Genetic Research In Isolated Populations (GRIP), in the South West of the Netherlands. We ascertained 24 MS patients according to standard diagnostic criteria. For these patients, genealogic information was extensively collected based on church records, which resulted in a single pedigree containing 2471 individuals in 19 generations. A total of 36466 pair-wise connections were identified between these patients via 796 ancestors. Among these connections, we counted the shortest connections, and compared the resultant distribution with the expected distribution under no parent of origin effect. Results: Among a total of 814 shortest connections, 333 (41%) were maternal, 98 (12%) paternal-maternal, and 383 (47%) were paternal-paternal (chi square 2df, p = 0.038, OR = 2.05, 95% CI = 1.03 - 4.08). Conclusions: The 188ST autoimmunity risk allele was not associated with MS as a whole. However, in the subset of patients with the benign course the frequency of the risk allele was similar to the ones described in other autoimmune diseases (from 10% in SLE up to 18% in RA). Confirmation/replication of this result in a larger sample would support the hypothesis that benign MS may have a distinct etiopathogenesis from the more severe MS forms.

Genetics/transcriptomics

P270

The role of PTPN22 1858T autoimmunity risk variant in a Portuguese multiple sclerosis population

C. Pereira, A. Martins da Silva, D. Pinto, A. Bettencourt, P. Costa, B. Koelman, B. Martins da Silva; Institute of Biomedical Sciences Abel Salazar (Porto, P); Santo Antonio Hospital (Porto, P); University Medical Centre (Utrecht, NL); National Health Institute Dr. Ricardo Jorge (Porto, P)

Background: Multiple Sclerosis (MS) is a heterogeneous chronic inflammatory disease of the CNS with several features of autoimmunity, including the presence of autoantibodies. A functional variation within the PTPN22 gene (1885C>T), which encodes a protein tyrosine phosphatase involved in the negative regulation of T-cell activation, has been implicated in the development of several autoimmune diseases, especially those with a humoral component. Until now, the 1885T allele has not been found associated with MS. Objective: In this study we aimed to evaluate the role of the 1885T allele in the susceptibility to MS in Portuguese patients and to correlate it with disease severity. Patients and Methods: A total of 237 MS patients and 279 ethnically-matched controls were studied. To take disease severity into account, 113 patients with disease duration of at least 10 years were divided into 3 groups: 45 patients were considered to have benign MS (EDSS ≤3), 35 non-benign MS (EDSS >3, ≤6), and 33 aggressive (EDSS ≥7). Genotyping of the 1885C>T PTPN22 polymorphism was performed using a TaqMan® assay. Results: The PTPN22 1885T allele frequency in the overall MS population was the same as in the controls (6.3% vs. 7.0%). When the time course of the disease was considered, we found an increased frequency for this allele within the benign subgroup (13.3%, p = 0.038, OR = 2.05, 95% CI = 1.03 - 4.08). Conclusions: The 1885T autoimmunity risk allele was not associated with MS as a whole. However, in the subset of patients with the benign course the frequency of the risk allele was similar to the ones described in other autoimmune diseases (from 10% in SLE up to 18% in RA). Confirmation/replication of this result in a larger sample would support the hypothesis that benign MS may have a distinct etiopathogenesis from the more severe MS forms.

P272

Genetic association of chromosome 11p15 with multiple sclerosis in the Northern Irish population

H. Aheen, S.A. Hawkins, C. Graham; Belfast City Hospital (Belfast, UK); Royal Victoria Hospital (Belfast, UK)

Introduction: A non-formal meta-analysis of the genetic linkage and association studies of multiple sclerosis (MS) identified 36 potential susceptibility loci in European populations. Examination of 25 of these loci revealed association with MS in 15 candidate genomic regions in the Northern Irish population. The remaining 13 regions relied mainly on evidence from linkage studies in multiplex families and sib-pairs, extended over long genomic distances and were reserved for future analysis. The objective of this study is to analyze some of the regions showing linkage with MS in several populations. Methods: All participants are Caucasians of N. Irish descent. Patients (n = 426) were diagnosed with clinically definite relapsing remitting MS as per Poser criteria, with a mean Kurtzke expanded disability severity score (EDSS) of 5.7. The control group (n = 578) consisted of anonymous blood donors, spouses or friends of MS patients (unrelated to MS patients) and laboratory personnel. Genetic analysis was carried out by genotyping four microsatellite markers in potential susceptibility regions on chromosomes 3p14, 9q21, 11p15 and 17q25, using multiplex polymerase chain reaction (PCR). PCR products were fractionated and allele sizes identified. Allele frequencies for each marker in patients and controls were compared statistically by performing a chi-squared test using the association analysis program Clump2. Results: The microsatellite marker D11S1318 on chromosome 11p15 showed a significant difference in allele frequencies between cases and controls (p = 0.002). The other three markers did not show association with the disease. Conclusions: Chromosome 11p15 has been identified as one of the regions showing linkage with MS in several populations including American, Canadian, Scandinavian and Sardinian. Furthermore, it has been identified in linkage studies of other autoimmune diseases such as type 1 diabetes mellitus, autoimmunity thyroid disease and Systemic lupus erythematosus (SLE). This suggests a general role of this region in susceptibility to human autoimmune diseases. The current study confirms the importance of chromosome 11p15 in multiple sclerosis.

P273

A second MHC susceptibility locus for multiple sclerosis

T.W. Yeo, P.L. De Jager, S.J. Sawcer on behalf of The International Multiple Sclerosis Genetics Consortium

The Major Histocompatibility Complex (MHC) region has been associated with multiple sclerosis susceptibility for over 30 years. Presently, the only established multiple sclerosis susceptibility is the HLA-DRB1 locus. A combination of tight linkage disequilibrium and high degree of polymorphism has confounded efforts to determine the number of susceptibility loci within the MHC. Using a combination of microsatellites, Single Nucleotide Polymorphism (SNP) and Human Leukocyte Antigen (HLA) typing, we performed two parallel screens of the MHC in 25 families with multiple sclerosis. In the first screen, we genotyped 33 microsatellites and 5 classical HLA loci (HLA-A, -B, -C, -DRB1 and -DQB1) in
480 UK trio families. In the second screen of 480 UK and 480 US trio families, 78 coding SNPs and 2 HLA loci (HLA-DRB1 and -DQB1) were genotyped. In each screen we identified residual association with susceptibility beyond any effects attributable to the well established DRB1*1501 risk haplotype. In both screens this residual association signal was strongest in the region of the HLA-C gene. By extending analysis of the S classical loci in 721 independent sporadic UK cases, and utilizing data from 3660 previously HLA typed UK control individuals, we have refined this secondary association and show that it is partly due to allelic heterogeneity at the HLA-DRB1 locus, but also reflects an independent effect from the HLA-C locus. Specifically, the HLA-C*05 allele, or a variant in tight LD with this, appears to have a protective effect in multiple sclerosis (p = 3.3 x 10^(-7)). Functionally, HLA-C interacts with killer cell immunoglobulin-like receptors (KIRs), suggesting that variations in KIR genes might also influence susceptibility. This possibility is supported by the modest evidence of linkage observed at the KIR gene cluster at chromosome 19 in our recent high density screen for linkage in multiple sclerosis. Our data also suggests that the MHC II and DRB1 effects are additive, consistent with the involvement of two independent pathways in the pathogenesis of multiple sclerosis.

**P274**

**Role of the FcRL3 gene on multiple sclerosis pathogenesis**

E. Urcelay, M. Bartolomé, A. Mas, V. De las Heras, A. Martínez, E. Gómez de la Concha, R. Arroyo; Hospital Clínico San Carlos (Madrid, E)

**Background and Aims:** Receptors for the Fc portion of Ig, FcγRs are widely expressed on cells of the immune system, modulating cellular and humoral functions. These classical receptors influence inflammatory processes as shown in FcγR-Md-deficient mice models and also demonstrated in humans. Similarly to these FcγR-Md, the FcγRIII-5 homolog genes map to the human chromosome 1q21–23 region and encode proteins that share similar extracellular Ig-like domains and cytoplasmic regions with consensus motifs suggesting inhibitory or activating signaling function. The FcγRs proteins are preferentially expressed by B cells and belong to a phylogenetically conserved family. A functional variant in the FcγRIII (FcγRIII) gene was found to affect NFκB binding and was associated with several autoimmune diseases in Japan. The FcγRIII receptor differs from the FcγR-Md in the simultaneous presence of both inhibitory and activating cytoplasmic sequences, which could imply an additional functional complexity. We pursued to analyze for the first time the effect of the described functional variant at −169 of the FcγRIII gene in multiple sclerosis (MS) risk. In order to estimate haplotypes within this gene, other FcγRIII promoter polymorphism at −110 was studied too. **Methods:** Case-control study performed with 400 Spanish MS patients and 507 healthy subjects. Genotyping was ascertained by using TaqMan assays-on-demand on a 7900HT Sequence Analyzer following manufacturer suggestions (Applied Biosystems, CA, USA). Haplotypes were inferred with the expectation-maximization algorithm implemented by the Arlequin software. **Results:** As shown in Japan for other autoimmune diseases, a significant difference was observed in the distribution of genotypes at −169 FcγRIII between the Spanish MS patients and healthy controls (p = 0.03; χ^2 = 6.99). However, the putative risk allele described in Japan, −169C, showed a protective effect for MS (C vs. T: p = 0.012; OR = 0.70 and CC vs. TC + TT: p = 0.013; OR = 0.64). No difference was observed for the other variant at −110 FcγRIII, but the haplotypes inferred with both markers evidenced one susceptibility haplotype, −169T/−110G (p = 0.026; OR = 1.24). **Conclusions:** A susceptibility haplotype was found when Spanish MS patients and controls were compared, supporting the role of the FcγRIII gene in MS predisposition. The etiological allele −169C FcγRIII showed a protective role in MS in our population, suggesting a different mechanism underlying susceptibility for MS.

**P275**

**Involvement of MHC2TA gene on multiple sclerosis risk in the Spanish population**

A. Mas, V. De las Heras, A. Martínez, M. Bartolomé, E. Urcelay, E. Gómez de la Concha, R. Arroyo; Hospital Clínico San Carlos (Madrid, E)

**Background and Aims:** MHC class II molecules are cell-surface glycoproteins of critical importance to the adaptive immune response, as they present peptides to antigen receptors of CD4-positive T cells. The expression of the MHC class II genes is exquisitely regulated almost exclusively by the class II transactivator, CIITA, according to a strict cell-type profile. A polymorphism (−168A/G, rs3087456) in the type III promoter of the MHC2TA gene was originally associated with increased susceptibility to multiple sclerosis (MS) in a Northern European population. However, no evidence of association of this MHC2TA variant with the disease could be subsequently detected in Germany. In view of these apparently contradictory results, we aimed at testing the aforementioned SNP and another G/C change located in exon 11 (nt1614 from coding sequence, rs4774), in order to analyze the haplotypic pattern within this MHC2TA gene in our Spanish MS cohort. **Subjects and Methods:** A case-control study was performed with 396 MS patients and 405 healthy controls from the Madrid area. Genotyping was ascertained by using TaqMan assays-on-demand on a 7900HT Sequence Analyzer following manufacturer suggestions (Applied Biosystems, CA, USA). Haplotypes were inferred with the expectation-maximization algorithm implemented by the Arlequin software. **Results:** No independent association with this autoimmune disease was found for either polymorphism. However, when haplotypes were compared between MS patients and controls a significant difference in their overall frequency distribution was observed (p = 0.04; χ^2 = 8.14), evidencing one protective (−168A/1614G, p = 0.09; OR = 0.81) and one risk (−168G/1614G, p = 0.01; OR = 1.72) haplotypes, in accordance with the results seen in our Spanish population for another autoimmune disease as rheumatoid arthritis (−168A/1614G, p = 0.005); OR = 0.68 and −168G/1614G, p = 0.005; OR = 1.87). **Conclusion:** The MHC2TA gene seems to influence predisposition to this autoimmune disease, MS. The −168A/G promoter polymorphism is not an etiological variant per se, but a genetic marker of susceptibility/protection haplotypes.

**P276**

**HLA allele association and multiple sclerosis in Portuguese patients**

M.E. Rio, B. Lima, S. Tafuilo, F. Mendes, H. Alves; Hospital Sao Joao (Porto, P); Centro de Histocompatibilidade do Norte (Porto, P)

**Introduction:** Multiple Sclerosis (MS) is a demyelinating disease of the Central Nervous System (CNS), and a leading non-traumatic cause of disability in young adults. MS is a complex disease, in which several environmental factors act together in a genetically susceptible individual. It is thought to be a cell-mediated autoimmune disease of the CNS. Many studies have tried to find the genes implicated in the pathogenesis of the disease. The strongest association between HLA and MS is found with the haptotype HLA class DR2 (DRB1*1501, DQA1*0102, DOB1*0602). **Methods:** 200 patients with MS from the north of Portugal. 69.5% were females, with a mean age of 43.5 years. The patients were genotyped for DRB1*181 of these where also genotyped to A*B*C, and 43 of these where also genotyped to DQB1*. Genotyping was done by molecular biology technics. DNA was extracted from peripheral blood and PCR-SSP and PCR-Reverse Hybridization methods have been used. χ^2 test was used to compare allelic frequencies between these patients and 678 controls of the same region. Patients where separated to DRB1*15 positives and DRB1*15 negatives, was tested independence of sex with these groups by Shula Edelkind on October 1, 2010msj.sagepub.comDownloaded from www.sagepub.co.uk
P277
Multifactor dimensionality reduction reveals gene-gene interactions associated with multiple sclerosis susceptibility in African Americans
D. Brassat, A. Motsinger, S. Caillier, H. Eritch, L. Steiner, K. Walker, B. Cree, L.F. Barcellos, M. Pericak-Vance, S. Schmidt, S. Gregory, S.L. Hauser, J. Haines, J. Oksenberg, M. Ritchie; University of Toulouse III (Toulouse, F); University of Vanderbilt (Nashville, USA); University of California (San Francisco, USA); Roche Molecular Systems (San Francisco, USA); Roche Molecular Systems (Alameda, USA); UC Berkeley (Berkeley, USA); Duke University Medical Center (Durham, USA); Vanderbilt University Medical Center (Nashville, USA)

Multiple sclerosis (MS) is a common disease of the central nervous system characterized by inflammation, myelin loss, gliosis, varying degrees of axonal pathology, and progressive neurological dysfunction. Multiple sclerosis exhibits many of the characteristics that distinguish complex genetic disorders including polygenic inheritance and environmental exposure risks. Here, we used a highly efficient multilocus genotyping assay representing variation in 34 genes associated with inflammatory pathways to explore gene-gene interactions and disease susceptibility in a well-characterized African-American case-control MS data set. We applied the multifactor dimensionality reduction (MDR) test to detect epistasis, and identified genes associated with MS susceptibility in African Americans. Of the 34 genes studied, 7 were found to be significantly associated with MS in African Americans. These genes include IRF4, OAS1, TRAIL, MAP3K3, MX1, IL-10, and CTLA4. We assessed association between gene polymorphisms and clinical outcome by means of a multiple logistic regression analysis. Positive associations, functional analysis was performed to determine if the polymorphisms of interest could influence mRNA expression and protein activity in 100 healthy controls. Genomic results and correlation with mRNA and protein levels will be presented. In conclusion, we found that polymorphisms in ISG influence protein activity in healthy controls and response to IFNβ treatment in MS patients. These results could be used as a predictive factor of response to IFNβ or for defining a subgroup of patients with a sustained response to IFNβ, a step toward personalized medicine.

P278
Polymorphisms in interferon-induced genes help to predict response to interferon-beta therapy in multiple sclerosis
D. Brassat, M. Comabella, S. Baranzini, S. Caillier, P. Villoslada, C. Arnaud, C. Cristini, M. Claeys, X. Montalban, J. Oksenberg; University of Toulouse III (Toulouse, F); Hospital Universitari Vall d’Hebron (Barcelona, E); University of California (San Francisco, USA); University of Navarra (Pamplona, E)

The aim of this study was to determine the pharmacogenomic effects of eight Interferon stimulated genes (ISG) genes in multiple sclerosis (MS). Two hundred sixty one patients naïve to immunotherapy and starting IFNβa treatment were recruited, and prospectively followed-up for 2 years at specialized MS Centers in Barcelona, Pamplona, and Toulouse. Ninety patients met our clinical criteria for positive response to IFNβ treatment and ninety for non-response. Patients with intermediate response were excluded (81 patients). Thirteen SNPs in eight ISG were selected as candidates for this study (IRF4, OAS1, TRAIL, MAP3K3, MX1, IL-10, CTLA4). We assessed association between gene polymorphisms and clinical outcome by means of a multiple logistic regression analysis. Positive associations, functional analysis was performed to determine if the polymorphisms of interest could influence mRNA expression and protein activity in 100 healthy controls. Genomic results and correlation with mRNA and protein levels will be presented. In conclusion, we found that polymorphisms in ISG influence protein activity in healthy controls and response to IFNβ treatment in MS patients. These results could be used as a predictive factor of response to IFNβ or for defining a subgroup of patients with a sustained response to IFNβ, a step toward personalized medicine.

P279
Association of HLA-DRB1*15 allele group and the DRB1*1501 allele with multiple sclerosis in a highly genetically diverse Brazilian population
A.A. Barreira, D.G. Braun, C. Sansaloni, E.A. Donad; Medical School of Ribeirão Preto -USP (Ribeirão Preto, BR)

The main contribution to susceptibility to multiple sclerosis (MS) is the presence of major histocompatibility complex genes, particularly the class II alleles, in various populations from distinct ethnic backgrounds. The HLA-DRB1*1501 allele is strongly associated with MS in North American and North European Caucasian patients, while the DRB1*1501, DRB1*1503 and DRB1*1504 alleles are over-represented in Sardinians and Turkish patients. Considering that the gene pool of Brazilian population is characterized by the contribution of genes originating from European, Afro-American and Native American individuals, the evaluation of MHC class II alleles in this population is of particular interest in understanding the role of these markers in a highly genetically diverse population. We evaluated HLA-DRB1 genes in an admixed Brazilian population. A total of 135 consecutive patients presenting clinically defined relapse-remitting MS diagnosed according the Poser criteria were typed for HLA-DRB1 allele groups. A total of 1241 blood and bone marrow donors were also typed for HLA class II allele groups. HLA-DRB1 alleles were typed employing PCR-amplified genomic DNA hybridized with sequence-specific primers using commercial kits. Statistical analysis was performed using the two-tailed Fisher’s exact or Chi-square tests, correcting the p-value when necessary, according to the number of specificities tested. The relative risk (RR) and the etiological (EF) and preventive fraction (PF) were also calculated. The frequency of the HLA-DRB*01 alleles were decreased in patients compared to controls, conferring an RR = 0.46 and a PF = 0.31. On the other hand, the HLA-DRB1*15 allele group was over-represented in MS patients compared to controls (p-value 0.0001) conferring an RR = 2.1 and an EF = 0.21. Compared to the controls, most MS patients exhibiting the DRB1*15 allele group also possessed the DRB1*1501 allele (p = 0.0072), which conferred an RR value = 2.1 and an EF = 0.15. The HLA-DRB1*1501 allele has been associated with MS in several Western European populations, corroborating the association of these alleles even in a highly genetically diverse population. Considering that DRB1*1501 is absent in Native Brazilians and present in a low frequency in Afro-Brazilian, it is plausible to suppose that this susceptibility gene was introduced in Brazil by the Western European gene pool.
the Norwegian Sami population, corresponding to a low prevalence of MS in the Sami compared to the rest of Norway. The Sami MS patients shared the same clinical characteristics as other Norwegian MS patients (n=312), and four of them carried the MS-associated HLA DR15-DQ6 haplotype. HLA typing of Norwegian Sami controls (n=200) compared to non-Sami Norwegian controls (n=293) showed however a significant reduction of the DR15-DQ6 haplotype in the Sami. In conclusion, the low frequency of the DR15-DQ6 haplotype among Sami may contribute to the low frequency of MS in this population.

P281

The role of SH2D2A gene polymorphisms in multiple sclerosis

Å.R. Lorentzen, C. Smestad, P.O. Ekstrøm, A.B. Oturai, E. Åkesson, J. Saarelä, K-M. Myhr, B.A. Lie, E.G. Celius, P.S. Sørensen, J. Hiltiert, A. Sparkland, H.F. Harboe; University of Oslo (Oslo, N); Uluedal University Hospital (Oslo, N); The Norwegian Radium Hospital (Oslo, N); Copenhagen University Hospital (Hvidinge, S); University of Køpino (Køpino, FIN); University of Bergen (Bergen, N); Rikshospitalet University Hospital (Oslo, N)

The SH2D2A gene at chromosome 1q22 encodes the T-cell specific adaptor protein (TSD), which is expressed in activated T-cells. Recent studies indicate that TSD may have a regulatory role in cellular activation of T- and NK-cells. We have previously reported that homozygosity for short alleles (GA13-16) of a dinucleotide repeat polymorphism in the promoter region of the SH2D2A gene is associated with multiple sclerosis (MS) as well as juvenile rheumatoid arthritis among Norwegians. In the present study we could not confirm the SH2D2A promoter polymorphism association in a new Norwegian MS cohort. Ten percent of MS patients (n=316) and six percent of controls (n=277) were found to be homozygote for GA13-16 alleles, even though an overrepresentation of short alleles in MS patients was found also in this Norwegian cohort. Only minor differences in SH2D2A GA13-16 allele frequencies between patients and controls were found in a large Nordic sample set consisting of Danish, Swedish and Finnish cases and controls (in total 1488 MS, 1390 controls). A single nucleotide polymorphism (SNP) in the SH2D2A exon 3 resulting in a non-synonymous change at amino acid position 52 (S to N substitution) was also genotyped in Norwegian MS patients (n=340) and controls (n=533). A weak association with the AA genotype (MS 0.16; controls 0.11; OR 1.52, p uncorr 0.04) and the GA13-16-A haplotype was found (MS 0.28, controls 0.23, OR 1.17, p uncorr 0.02). In conclusion, the SH2D2A GA repeat polymorphism that was previously found to be associated with MS, could not be confirmed in a large sample set of Nordic MS patients and controls. However, a weak association with a SNP in exon 3 was found in the Norwegian cohort. Further mapping by genotyping of other SNPs in the SH2D2A gene region is ongoing as well as clinical subgroup analysis.

P282

A91V variation of the perforin gene in patients with multiple sclerosis

C. Comi, G. Cappellano, A. Chiocchetti, D. Galimberti, C. Fenoglio, E. Cerutti, L. Castelli, R. Mesturini, M. Carecchio, M. Leone, E. Scarpini, F. Monaco, U. Dianzani; University “A. Avogadro” (Novara, I); University of Milan (Milan, I)

Background: Perforin is a protein stored in cytolytic granules of CD8+ cytotoxic T cells (CTL) and natural killer (NK) cells, that is released on the target cell upon its recognition by the cytotoxic cell. Perforin polymorphizes on the target cell membrane and forms pores allowing entry of granzymes, that trigger apoptosis of the target cell by cleaving caspases. Mutations of the perforin gene have been associated with about 30% of cases of familial hemophagocytic lymphohistiocytosis (HLH), a rare life-threatening immune deficiency that occurs in infants and young adults. The presence of A91V substitution in the perforin gene has been shown to decrease perforin function by altering its conformation, impairing its cleavage to the active form, and increasing its degradation. A deficient perforin function may therefore impair immune surveillance, thus predisposing to autoimmunity. Data from our group show that patients with autoimmune lymphoproliferative syndrome display a significantly higher frequency of A91V substitution.

Aim of the study: To evaluate whether the A91V variation of perforin may predispose to MS development. Patients and Methods: Genomic DNA was isolated from PBMCs of 408 MS patients (352 m, 56 f), diagnosed according to McDevitt et al criteria and 816 ethnically matched healthy controls. Patients were enrolled from the MS Centers of University “Amedeo Avogadro”, Novara and University of Milan, Italy. All patients and controls were unrelated, Caucasian and Italian. Exon 2 of the perforin coding region was amplified using standard PCR conditions, and the identification of single nucleotide polymorphisms was performed by sequencing PCR products. Results: The genotypic distribution of A91V variation did not deviate significantly from the Hardy-Weinberg equilibrium in both patients and controls. The A91V variation was carried by 61 MS patients (51 heterozygotes and 5 homozygotes) and 75 controls (69 heterozygotes and 3 homozygotes). Its allelic frequency was significantly higher in MS patients than in controls (7.5% vs. 4.6%, p<0.01) and it conferred an OR=1.68 (95% CI: 1.17–2.41). Conclusion: The increased frequency of A91V substitution in the perforin gene confers a higher risk of developing MS, probably through a deficient clearance of autoreactive T cells. Further data are needed to functionally characterise the role of perforin deficiency in MS pathogenesis.

P283

The association of human leukocyte antigens with disease severity and prognosis in patients with multiple sclerosis

S. Özakbas, E. Idiman, G. Koceshasanogullari, M.A. Öktem; Dokuz Eylul University (Izmir, TR)

The most important genetic factor that predisposes Multiple Sclerosis (MS) is the human leukocyte antigens (HLA) class 2 which are encoded on the short arm of chromosome 6. In this study, our purpose was to assess the correlation between HLA genotype and demographic and clinical aspects in patients with MS. We also aimed to determine the correlation of HLA genotype with disease prognosis and severity. 149 clinically definite MS patients (102 women) were included in the study. The mean age was 38.36. Most of patients (77 patients, 81.7%), has relapsing-remitting (RR) course. 43 patients (28.8%) have secondary progressive (SP) MS and 29 patients (19.5%) have primary progressive (PP) MS. 178 healthy people were disposed as the control group. HLA-DR15 was more frequent in the MS group compared with the control group (gene frequency: 0.548; OR: 8.95; p=0.00012). There was no difference neither in the RRMS and the SPMS subgroups. There was no HLA-DR15 frequency difference between PPMS subgroup and the control group. HLA-B8 and HLA-DR3 were more frequent in the PPMS subgroup than the RRMS, and the SPMS subgroups. The mean attack frequency was tending to be lesser in the first 2 years of the disease in the HLA-DR15 positive subgroup (0.67) than the HLA-DR15 negative subgroup (0.75) (p=0.046). The attack severity was lesser in the HLA-DR15 positive subgroup. EDSS scores of the HLA-DR15 positive subgroup were better than the HLA-DR15 negative subgroup (p=0.003) at the fifth year of the disease (EDSS scores were 4.01 and 5.13 respectively). The mean attack severity of HLA-DR15 positive subgroup was still lesser at the fifth year. In conclusion, these findings can be interpreted as HLA-DR15 is a very important allele and probably a good prognostic factor. The data obtained from

Multiple Sclerosis 2006; 12: S1–S228 www.sagepub.co.uk
this study ascertained that the HLA-DR15 is frequent in Turkish MS population. Our data also showed that our MS population has similar genotypic features with the other western populations. The similarity of HLA profile of RRMS and SPMS showed that these MS groups have similar genotypic features.

P284

IP-10 haplotypes and multiple sclerosis: association and correlation with clinical course

D. Galimberti, D. Scalabrini, C. Fenoiglo, C. Comi, M. De Riz, E. Venturrelli, E. Brighina, M. Piola, F. Cortini, C. Lovati, C. Mariani, F. Monaco, N. Bresolin, E. Scarpini, University of Milan, Ospedale Maggiore (Milan, I); University of Eastern Piedmont (Novara, I); University of Milan, Ospedale Sacco (Milan, I)

Interferon-gamma-inducible Protein-10 (IP-10) levels are increased in cerebrospinal fluid of Multiple Sclerosis (MS) patients with symptomatic attacks of inflammatory demyelination, supporting a role for this molecule in MS pathogenesis. Two hundred-twenty six patients with Multiple Sclerosis (MS) and 235 controls were genotyped for G/C and T/C Single Nucleotide Polymorphisms (SNPs) in exon 4 of IP-10 gene. Haplotypes were tested for association and correlated with clinical variables. The two SNPs studied were in complete linkage disequilibrium. None of the haplotypes determined was associated with MS. However, GGTT wild type haplotype carriers had a Progression Index (PI) significantly lower than non carriers (P = 0.016). Furthermore, among patients who had a bout onset of the disease, the time occurred between onset and second episode was significantly longer in GGTT carriers (P = 0.021). Lastly, considering SP-MS patients, the time occurred between the initial RR form and the subsequent worsening to SP was longer in this group (P = 0.08). Therefore, the GGTT haplotype of the IP-10 gene is not a susceptibility factor for the development of MS, but is likely to influence the course of MS, possibly contributing to slower the progression of the disease.

P285

Is TNFA-376A SNP a population specific genetic risk marker of the susceptibility to develop multiple sclerosis?

M. Kauffmann, D. Gonzalez Moron, N. Fernandez Liguori, G. Sandoval, A. Miezchenko, O. Garces, A. Villa; Hospital Ramos Mejia Universidad de Buenos Aires (Buenos Aires, RA); Hospital Ricardo Gutierrez (Buenos Aires, RA)

Background and aims: Multiple sclerosis (MS) is a complex genetic disorder in which only alleles of the major histocompatibility complex has reproducibly been associated with the disease in molecular epidemiology studies. Two hundred-twenty six patients with Multiple Sclerosis (MS) and 235 controls were genotyped for G/C and T/C Single Nucleotide Polymorphisms (SNPs) in exon 4 of IP-10 gene. Haplotypes were tested for association and correlated with clinical variables. The two SNPs studied were in complete linkage disequilibrium. None of the haplotypes determined was associated with MS. However, GGTT wild type haplotype carriers had a Progression Index (PI) significantly lower than non carriers (P = 0.016). Furthermore, among patients who had a bout onset of the disease, the time occurred between onset and second episode was significantly longer in GGTT carriers (P = 0.021). Lastly, considering SP-MS patients, the time occurred between the initial RR form and the subsequent worsening to SP was longer in this group (P = 0.08). Therefore, the GGTT haplotype of the IP-10 gene is not a susceptibility factor for the development of MS, but is likely to influence the course of MS, possibly contributing to slower the progression of the disease.

P286

The p150 subunit of dynactin gene in multiple sclerosis

C. Munch, R. Meyer, P. Linke, T. Meyer, A.C. Ludolph, J. Haas, B. Henner; Jewish Hospital (Berlin, D); Charite, Humboldt University (Berlin, D); University of Ulm (Ulm, D); Heinrich-Heine-University (Dusseldorf, D)

Multiple sclerosis (MS) is a multifactorial disease in which mostly unidentified genetic factors in conjunction with environmental agents affect its clinical expression. Neurodegeneration has emerged as a significant contributor to CNS lesions in MS. Genetic susceptibility of the neuron may determine the degree of ensuing neurodegeneration after an inflammatory attack. We have recently described mutations in the p150 subunit of the molecular motor dynactin (DCTN1) in amyotrophic lateral slerosis (ALS) and fronto-temporal dementia (FTD) that may predispose different neuron types to degeneration. Given the common features of neurodegeneration in MS, ALS and FTD, we raised the question whether genetic variants in the DCTN1 gene may constitute a risk factor for MS. We conducted a DCTN1 mutation analysis in 192 patients with MS (96 patients with relapsing-remitting MS and 96 with primary progressive MS) and the same number of unrelated controls. In MS, no mutations in the DCTN1 gene have been found. Three novel heterozygous mutations (R532L, T1249I, I196V) of the DCTN1 gene were detected in four controls. We conclude that DCTN1 may not contribute to the genetic background of neurodegeneration in MS. Our findings support the notion that the DCTN1 gene is highly heterogeneous. DCTN1 sequence alterations are also found under normal conditions and their pathogenetic relevance is far from being completely understood.

P287

Genetic polymorphism in type III promoter of the major histocompatibility complex class II transactivator gene is associated with susceptibility to multiple sclerosis

T. Mihalova, S. Eyre, A. Barton, J. Bowes, N. Rukin, M. Stone, M. Boggild, C.A. Young, A. Frier, P. Hohan, J. Worthington, R. Strange, C.P. Hawkins; North West Molecular Genetics Group, Manchester and Keele University, UK

Objectives: Multiple sclerosis (MS) is a chronic inflammatory neurological condition of complex aetiology, where susceptibility is significantly regulated by the major histocompatibility complex (MHC) class II genes. MHC plays an important role in T-cell dependent immunity and inflammatory response. MHC expression is controlled by MHC class II transactivator (MHC2TA) gene. Type III (MHC2TAIII), situated on chromosome 16p13, is the principal
MHC2TA promoter employed by human activated T-cells. A single nucleotide polymorphism (SNP) in the 5′flanking region of MHC2TAII (rs3087456) was reported to be associated with rheumatoid arthritis, multiple sclerosis and myocardial infarction in a Swedish population. Our study was designed to test for association between the MHC2TAII region and MS susceptibility in the previously described SNP and four additional SNPs in a UK population.

Methods: Five SNPs spanning the 5′flanking region of MHC2TAII were selected: SNP1: G/C-2217 (rs5701209); SNP2: T/G-6959 (rs66498114); SNP3: T/C-5473 (rs6416647); SNP4: C/T-4595 (rs7404672) and SNP5: A/G-168 (rs3087456). Genotyping was performed in 474 Caucasian MS cases and 775 controls, using a Sequenom MassArray platform. Estimated haplotype frequencies were generated using the EM algorithm on HelixTree and compared between cases and controls. Genotype and haplotype frequencies were compared between cases and controls using logistic regression. Results: We detected no significant differences between genotype frequencies in MS cases and controls. SNP4: C/T detected no significant differences between genotype frequencies.

P288

UCP2 and mitochondrial haplogroups as a multiple sclerosis risk factor

D. Otaegui, A. Saenz, J. Ruiz-Martinez, J. Olascouga, A. Lopez de Munain; Hospital Donostia (San Sebastian, E); Hospital Mendaro (Mendarker, E)

A recent paper noted an SNP (rs660339) located in the promoter area of UCP2 gene as a MS risk factor. UCP2 is a member of the mitochondrial proton transport family that uncouples proton entry in the mitochondrial matrix from ATP synthesis. In this study we analyze this SNP and the mitochondrial haplogroups in our MS population (with a cluster of Basque-origin people). 166 patients were recruited from the Neurology Departments of Hospitals in the region of Gipuzkoa (Basque Country, Northern Spain). The patients were diagnosed with MS according to the McDonald criteria. As controls, 372 blood samples from anonymous healthy donors were used.

Methods: The UCP2-SNP and Mitochondrial haplogroups characterization was performed by PCR-RFLP according to a previously described protocols. Variance analysis, chi 2 test, Fisher statistic, standard allelic odds ratio and logistic regression analysis were done by SPSS and MSAS software.

Results: The average age of the patients was 43.2 ± 12.7 yrs; and 31.7 ± 10.17 yrs at onset and 4.79 ± 2.97 MSSS value. The average age differs between MS-Basque and Other origin patients (p = 0.004), as does the age at onset (p = 0.003) and the MSSS average (p = 0.007). Rs660339 alleles reveal differences between patients and controls (p = 0.038). G/G genotype is an MS risk factor (p = 0.017; OR: 1.62) and gives a lower MSSS average (4.04 vs. 5.15, p = 0.039). Haplogroups are distributed differently between the Basque and Other origin samples; U haplogroup is more frequent in Basque (p = 0.007) and Y is more frequent in the Other-origin group (p = 0.004). None of the mitochondrial haplogroups are related with MS. The data show no relation between the haplogroups and rs660339. Discussion: Ethnic origin seems to have a slight influence on MS, the onset appears to occur later in Basque-origin patients with a better progression, underlining the importance of genetic background. G/G genotype is associated with lower UCP2 expression.

P289

Macrophage migration inhibitory factor: first association study with multiple sclerosis

A. Martinez, V. De las Heras, M. Bartolome, A. Mas, E. Urcelay, E. Gomez de la Concha, R. Arroyo; Hospital Clinico San Carlos (Madrid, E)

Background: The cytokine macrophage migration inhibitory factor (MIF) participates in fundamental events in innate and adaptive immunity. Raised MIF concentrations within the serum, plasma or tissue have been found in several diseases with a chronic inflammatory basis like rheumatoid arthritis, colitis, pancreatitis... Elevated levels of MIF were also reported in the cerebrospinal fluid of patients with multiple sclerosis (MS). Moreover, the in vivo blockade of MIF ameliorates acute experimental autoimmune encephalomyelitis by impairing the homing of encephalitogenic T cells to the central nervous system. MIF has been implicated in the pathogenesis of autoimmune disorders such as arthritis, glomerulonephritis and lupus. All these evidence impelled us to investigate for the first time whether polymorphisms in the MIF gene are involved in susceptibility to MS. The MIF promoter polymorphism located at -173G/C creates an AP4 binding site affecting gene expression in a cell-specific way. The analysis of another functional promoter polymorphism, a CATT tetranucleotide repeat at position -794, allowed us to study the haploptic association of the MIF gene with this neurodegenerative disease in the Spanish population. A risk haplotype (CAAT)/-173C has been previously described in other autoimmune diseases.

Subjects and Methods: A case-control study was performed with 412 Spanish MS patients and 534 healthy controls of the same ethnicity. Genotyping was ascertained by using TaqMan assays-on-demand on a 7900HT Sequence Analyzer for the -173G/C variant and the microsatellite was discriminated in an AbiPrism 3100 automatic sequencer following manufacturer suggestions (Applied Biosystems, CA, USA). Haplotypes were inferred with the expectation-maximization algorithm implemented by the Arlequin software.

Results: Carriers of the -173C allele, of the (CAAT)7 allele or of the haplotype containing both markers did not show significantly increased predisposition to overall MS (p = 0.17, p = 0.16 and p = 0.2, respectively). Stratification for the HLA-DRB1-1501 susceptibility factor evidenced significant difference when carriers of the (CAAT)7 allele were compared with healthy controls (p = 0.05; OR = 1.53). The risk haplotype (CAAT)/-173C was also significantly increased in 1501-positive patients vs. controls (p = 0.046; OR = 1.54).

P290

Phage display minilibraries to study the epitopes involved in multiple sclerosis

A. Cortini, S. Bembich, C. Maggiore, M. Zorzon, G. Pizzolato, R. Marzari, P. Edoni; University of Trieste (Trieste, I)

Multiple sclerosis (MS) is considered the prototype inflammatory autoimmune disease of the central nervous system (CNS). The most extensively studied putative autoantigens are components of CNS myelin (myelin basic protein MBP, proteolipid protein PLP, myelin oligodendrocyte glycoprotein MOG). The autoantibodies in MS recognize both linear and conformational epitopes, but at present the conformational epitopes of myelin proteins have not been identified. For example, in MS, the T-cell receptors of autoactive T lymphocytes recognize various peptides of the MBP, and, in EAE, the anti-MOG antibodies recognize only conformational epitopes.
Furthermore, the progression of MS is accompanied by the decline of primary T-cell autoreactivity and by the concurrent emergence of neo-autoreactivity (epitope spreading). We have setted a system to construct phage display libraries of epitopes encoded by single gene as tool to investigate the immune response in MS patients. With our system it is possible to make minilibraries from single gene through the fragmentation of the gene of interest with nucleases protection by means of archibacteria histones; the different and randomly produced epitopes derives from real open reading frames (ORF) and the controlled fragmentation generates both linear and conformational epitopes. In fact, the use of a proprietary phagemid (ORF) and the controlled fragmentation generates both linear and randomly produced epitopes derives from real open reading frames (ORF) and the controlled fragmentation generates both linear and conformational epitopes. We have generated minilibraries from MBP, MOG, and PLP genes. The dimension of these libraries are from 2 × 10^3 to 2 × 10^5. The sequencing analysis demonstrated that about 90% of clones were real ORF and that the library clones covered all the gene length. To test the system a selection of MBP minilibrary was made against the commercial monoclonal MAB82-99 and only clones containing the epitope recognized by the antibody were identified. These minilibraries are using in selection with sera and CSF from MS patient chosen in a period of single immune attack and absence of clinical treatment. The aims of this project is to provide a genetic dissection of the humoral immune response in MS, offering the possibility to identify the epitopes involved, to identify pre and subclinical condition of patients who are developing the disease, to follow the evolution of immune response, and, finally, to hypothesize novel treatment strategies such as specific antigen-tolerizing therapy.

**P292**

**Whole genome SNP association studies in multiple sclerosis**

H. Abderrahim, J. Wojcik, F. Martinelli-Boneschi, F. Esposito, J. Yaoang, J. Hiltet, T. Wells, G. Comi; Serono Pharmaceutical Research Institute (Geneva, CH); San Raffaele Hospital (Milan, I); CHU Rennes (Rennes, F); Karolinska University Hospital (Stockholm, S); Serono International S.A (Geneva, CH); San Raffaele Hospital (Milan, I)

The genetic component of MS etiology is believed to result from the action of allelic variants in several genes, each with small individual effects, according to the restricted polymorphism model. Genetic association studies are an effective approach for detecting the effects of such common variants with modest effect. Our aim was to identify susceptibility genes or markers that are associated to MS in three independent Caucasian populations of Relapsing Remitting, Secondary Progressive and Primary Progressive patients. We enrolled and typed 3 different collections: one from Sweden (323 MS patients and 368 healthy controls), one from France (396 patients and 384 healthy controls), and one from Italy (361 cases and 234 healthy controls). An additional study was performed on a pure primary progressive population (176 cases). Prior to association studies, all cases and controls were genotyped for a limited number of unlinked genetic markers (100) in order to assess genetic homogeneity in each population. No stratification effect was detected. We then performed whole genome association studies using the Affymetrix 500K Single Nucleotide Polymorphism (SNP) genotyping assay in each of the 4 collections. Genotyping has been completed on these collections. A range of statistical tests (univariate, Mantel-Haenszel, pBlasT, NNBC) were performed to identify the genes associated with the disease, either as causative or in linkage disequilibrium with the markers used (SNPs). In addition, the False-Discovery Rate has been assessed to adjust for multiple testing issue. The validation of these results is currently ongoing, it includes pathway analysis, high density genotyping, and functional analysis.

**P293**

**FOXP3 gene polymorphism in multiple sclerosis**

N. Yıldız, N. Isık, I. Aydin, A. Unsal, G. Dreskeneli; Goztepe Educational Hospital (Istanbul, TR); Istanbul University (Istanbul, TR)

Multiple sclerosis (MS) is widely believed to be an autoimmune disorder. It is well recognized that both genetic and environmental factors play an important role in the pathogenesis of MS. Immune pathogenesis of MS centers on CD4+ T lymphocytes. FOXP3 represent a key factor in the development and function of the CD4+ regulatory T cells which express the interleukin 2 (IL-2) receptor alpha chain, CD25 and are effective in regulation of both the adaptive and innate immune system. FOXP3 gene is localized on the X chromosome encoding ‘scurfin’, which binds to the IL-2 promoter and the granulocy–monocyte colony-stimulating factor enhancer near the “nuclear factor of activated T cell” (NFAT) site. FOXP3 repress these genes, thus reducing IL-2 production by CD4+ T cells. Functional alteration of FOXP3 gene expression may promote the development of autoimmune diseases, which is observed in type 1 diabetes and autoimmune thyroiditis. Decreased CD4+CD25 regulatory T cell effector function was also reported in MS patients. In this study, we screened a non-synonymous coding single nucleotide polymorphism (rs2232369) of human FOXP3 gene in 148 MS patients and 102 age and sex-matched controls and evaluated its association with susceptibility or course of the disease. The distribution of this polymorphism, which has not been screened in other populations before, revealed that the polymorphic allele only detected in only 3% of the control group. We observed no evidence of genetic association between the FOXP3 polymorphism and MS. But further studies of other polymorphisms has no, or only a negligible effect on MS susceptibility in the Spanish population, results also confirmed in some other populations.
P294

Genetic analysis of perforin gene polymorphisms in multiple sclerosis
M. Caminha, R. Martin, A. Navarro, C. Morgtulo, R. Goertschles, E. Julita, X. Montalban, M. Comabria; Vall d’Hebron University Hospital (Barcelona, E); Universitat Pompeu Fabra (Barcelona, E)

Background: The etiology of multiple sclerosis (MS) remains unclear, although it is considered to result from the interaction of environmental factors in genetically susceptible individuals. A recent genome study has provided evidence that chromosome 10q22.1 may contain candidate genes for MS. We conducted an initial screen focused on this locus and identified the perforin as a candidate gene for MS susceptibility. The protein encoded by this gene is one of the main cytolytic proteins of cytolytic granules, and it is known to be a key effector molecule for T-cell- and natural killer-cell-mediated cytosis. Objective: To perform, by means of single nucleotide polymorphisms (SNP), a case-control study of the perforin and apply an indirect approach to infer at risk haplotypes from genotypes of tested individuals. Methods: We genotyped 3 SNPs (SNP 1 located in exon 2, SNP 2 in intron 1, and SNP 3 in the 5’ untranslated region) spanning 5.4 Kb of the perforin in 512 healthy controls (HC) and 420 MS patients [323 relapsing remitting (RRMS)+ secondary progressive (SPMS), and 97 primary progressive (PPMS)], by using the 5’ nuclear assay (Taqman®). All SNPs were stated to have minor allele frequencies >0.10. Thermal cycling and end-point PCR analysis were performed on an ABI PRISM® 7900. Statistical tests to determine allele and genotype frequencies, linkage disequilibrium (LD) between markers and at risk haplotype estimations were performed applying the SPSS, Arlequin and PHASE software. Results: When allele and genotype frequencies were compared between MS patients and HC, SNPs 2 and 3 were found to be associated with MS (p<0.05). When the case and control groups were stratified by gender, association was observed only in the male population. In addition, haplotype reconstruction revealed risk haplotypes strongly correlated with male patients with the primary progressive form of MS. Conclusion: These findings support the hypothesis that individual polymorphisms within the perforin gene (or their combined haplotypes) may influence genetic predisposition for MS, especially in male patients with PPMS. Functional studies are currently in progress to investigate differential gene expression patterns related to the risk haplotypes found in PPMS patients.

P295

Influence of HLA DR2 on progression from clinically isolated syndrome to clinically definite multiple sclerosis
V. De las Heras, M. Bartolome, A. Man, A. Martinez, E. Urcelay, E. Gomez de la Concha, R. Arroyo; Hospital Clínic San Carlos (Madrid, E)

Background: The Barkhof criteria of magnetic resonance imaging (MRI) is one of the most important predictor for conversion to “clinically definite” multiple sclerosis (CDSM) after a first isolated, well-defined neurologic event consistent with demyelination (closely defined isolated syndrome—CIS—). The human leukocyte antigen (HLA)-DR2 allele is the best genetic predisposition factor of MS susceptibility. Objective: We studied the influence of HLA-DR2 on progression of CIS to CDMS in accordance with MRI results. Methods: Observational retrospective cohort study. Eligible subjects were patients who had a first isolated, well-defined neurologic event consistent with demyelination (monofocal or multifocal), and who had submitted for follow-up at MS Unit since then up to five years at least. All patients had studied with MRI and classified in accordance to Barkhof criteria (three or more, and less than three criteria). Statistical analysis: survival analysis with Kaplan-Meier test adjusted by MRI results. Results: It was included 54 patients: 76% women (41). The more frequent CIS was sensitive (27.8%), optic neuritis (20.4%), brain stem (18.5%) and spinal cord syndrome (13%). Median time to second relapse (and therefore to CDMS) was 1.29 years (IQR 2.52). MRI results: 59.6% had three or more Barkhof criteria; 40.4% had two or less. HLA-DR2 was present in 20 (37%) of the 54 patients. Survival analysis for time to CDMS: There was no difference for HLA-DR2 adjusted for Bharkoff criteria. Conclusions: We did not found any association of HLA haplotype DR2 with an increased odds for conversion to CDMS in a shorter time. This results could be influenced for the less prevalence of DR2 haplotype in Spanish population. Other limitations of our study were: the low number of cases and the retrospective nature of design.

P296

The role of glucocorticoid receptor gene polymorphisms in disease progression in multiple sclerosis
L. van Wijnen, T. Hooper-van Veen, E.F.C. van Rossum, J.W. Koper, F. Barkhof, C.H. Polman, B.M.J. Uitdehaag; VU Medical Center (Amsterdam, NL); Erasmus MC (Rotterdam, NL)

Background: Multiple sclerosis (MS) is a chronic inflammatory and demyelinating disease of the central nervous system, in which unknown environmental factors are thought to trigger disease in genetically susceptible persons. Glucocorticoids (GC) play an important role in controlling chronic inflammatory diseases like MS, and are often used in the treatment of these diseases. Endogenous cortisol may also play a role in the disease course and even in susceptibility. In healthy individuals and in patients with MS, differences in GC sensitivity have been described. Polymorphisms in the glucocorticoid receptor (GR) gene may contribute to these differences, and thereby influence the inflammatory response. Aim: To investigate the role of three polymorphisms (N363S, ER22/23EK and the Bcl I C/G of the GR gene in disease course and susceptibility to MS. Methods: For three polymorphisms in the GR, genotypes have been determined in DNA from of 257 patients with clinically definite MS recruited from the MS Centre at the VU medical centre (VUMc) Amsterdam and 178 healthy controls. Regression analysis was used to investigate the influence of these polymorphisms on onset type and age of onset, and MRI parameters for lesion volume and atrophy. We corrected for the presence of the two other polymorphisms, gender, disease duration, onset type of the disease and use of interferon beta (IFNb). To investigate whether clinical disease progression was influenced by these polymorphisms, Cox regression analysis was performed. We corrected for onset type and use of IFNb. To analyse the effect of carriage of ER22/23EK, N363S, or the Bcl I variant G on disease susceptibility, odds ratios were calculated. Results: InER22/23EK-carriers, time to EDSS 6 was significantly shorter (median 98 months, CI 95% 67 to 129), compared to non-carriers (median 204 months CI 95% 168 to 240), indicative of a more progressive disease. Moreover, carriers of the ER22/23EK allele had a higher T1 lesion load compared to non-carriers (median value 3.1 cm^3 versus 2.1cm^3). This difference remained significant after correcting for gender, disease duration, onset type and use of IFNb (p=0.024; 95% CI of the difference: 0.59 cm^3 to 8.3 cm^3). Genotypes were equally distributed in HC and MS patients. Conclusion: Our study indicates that ER22/23EK, a polymorphism in the gene encoding for the GR, may be associated with a more aggressive disease course in MS.

P297

Common transcriptional signature in the clinical forms of multiple sclerosis: the role of downregulation of apoptotic genes
E. Brini, P. Martini, V. Barbui, R. Farlan, C. Ferrandi, G. Coni, T. Wells, M. Mariani, G. Martino, P.F. Zaratin; San Raffaele Scientific Institute (Milan, I); Serono Research Institute (Rockland, USA); RBM/Serona (Colleretto Gisacco, I); Serono Pharmaceutical Research Institute (Geneva, CH)

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system. Genes expressed in peripheral blood mononuclear cells (PBMCs) are involved in the onset and development of different
clinical forms of MS. In the present study we have investigated a comprehensive gene expression profile of PBMC isolated from Relapsing-Remitting (RR), Secondary Progressive (SP), Primary Progressive (PP) MS patients and healthy controls (CTRL) by using microarray that carry out probes for 45000 genes, representing the whole human genome and analyzed them with advanced computational methods. We have identified 1572 genes differentially modulated (total/unique) in RRMS/CTRL (934/668); SPMS/CTRL (279/19) and PPMS/CTRL (344/49). We did not find any differences between SPMS and PPMS, but we did find a significant difference between RRMS and the other MS forms. Downregulated genes greatly outnumbered the upregulated genes in MS. 187 were found to be in common in RRMS in stable remission, SPMS and PPMS and consistently up- or down-regulated in comparison to PBMC from healthy controls. A selection of 96 genes (out of 187) based on Ingenuity software functional pathways relevant to MS led to RT-PCR validation of 75 genes that implicate underlying processes involved in MS pathogenesis. In particular 29 cell death/apoptotic genes were found to be consistently downregulated in all the clinical forms. It is interesting to note that MS is reported to involve mechanisms that abrogate apoptosis of autoreactive T cells therefore the above genes may have implications for the design of new therapies for MS.

P298

Proteomic-based profiling of biological fluids in multiple sclerosis
M. Johnson, K. Theil, R. Raffaj, A. Stanley, J. Peng, D. Thompson, R. Banks; Leeds General Infirmary (Leeds, UK); St. James’s University Hospital (Leeds, UK)

Proteomic approaches allow simultaneous analysis of hundreds to thousands of proteins/peptides, facilitating discovery of potential markers of activity and pathogenesis in multiple sclerosis (MS). It is recognised however that in addition to the technological challenges in such studies where the relative abundances of different proteins may range up to 10^{12} fold, the reliability of the results is crucially dependent on the sampling methods used. Using cerebrospinal fluid (CSF) and blood samples we have examined the effects of pre-analytical and analytical factors on the profiles obtained so that recognition however that in addition to the technological challenges in such studies where the relative abundances of different proteins may range up to 10^{12} fold, the reliability of the results is crucially dependent on the sampling methods used. Using cerebrospinal fluid (CSF) and blood samples we have examined the effects of pre-analytical and analytical factors on the profiles obtained so that recognition of the above genes may have implications for the design of new therapies for MS.

P299

Time-course differential gene expression patterns of peripheral blood mononuclear cells from interferon-beta 1a (IM) treated multiple sclerosis patients disclose potential therapy related biomarker genes
R. Goertches, D. Koczan, M. Ernst, P. Serrano-Fernandez, S. Moeller, U. Zettl; University of Rostock (Rostock, D)

Applying high density microarray technology to find new prognostic and diagnostic markers in peripheral blood cells after therapeutic intervention opens great perspectives regarding responsiveness or patient subclassification and has an ethical impact on the acceptance of cost-intensive treatment. Interferon beta (IFNB) therapy in patients with MS has proven efficacious, but appears suboptimal in terms of therapy responder rate. A systematic time-course microarray experiment and comprehensive gene expression analysis of sets of genes from IFNb1a treated MS patients will provide a rational basis for optimized or novel therapeutic strategies aimed at modulation of the disease course. It is intended to detect and confirm surrogate biomarkers for therapeutic intervention-related molecular candidates in human PBMCs, to infer mechanism(s) of action affiliated to the effect(s)/pathways of applied MS drug, and to discern RNA-signatures derived from (non-)responders to treatment. Employing the Affymetrix HGU133 set (33 000 genes), we perform an ongoing full genome study monitoring 20 patients receiving IFNb1a (i.m., once weekly) over a period of 24 months. Samples were collected before first treatment (t0), after 1 and 4 weeks, 1 year and will be drawn at future dates. Extensive analysis of generated longitudinal data was realized, accounting for within-subject correlation of longitudinal gene expression data. Comparing later time points versus t0, dynamic expression changes in individual RNA profiles could be recognized. 115 genes showed increased or decreased expression levels in minimum 75% of the patients. Between ascertainment genes were 5 IFN modulated, 20 immune response related, 5 identified in host defense and 3 in inflammatory responses. Besides of modulating interferon responsive genes, IFNb1a may also alter the stability and coordination of cell components, as 30 genes were implicated in protein and lipid metabolism, cell adhesion and signaling, cytoskeletal (re-)arrangement and the ubiquitin pathway. The expression changes of these genes could be an important ancillary diagnostic tool.

P300

R92Q mutation in the TNFRSF1A gene in two multiple sclerosis patients with flu-like symptoms and fatigue
T. Kumpfel, L.A. Hoffmann, R. Hohlfeld, P. Loks; Institute of Clinical Neuroimmunology (Munich, D); Institute of Clinical Chemistry (Munich, D)

Background: Tumor necrosis factor receptor-associated periodic syndrome (TRAPS; MIM 142680) is an autosomal dominant auto-inflammatory disorder caused by mutations in the TNFRSF1A gene on chromosome 12p13. It is usually characterised by episodes of inflammation with fever, abdominal pain, myalgia, exanthema, arthralgia/arthritis, and ocular involvement. However, the phenotypic spectrum can vary significantly and has broadened in the past years. Cases: We describe two patients with relapsing remitting multiple sclerosis (MS), who were treated with interferon-beta 1a and reported additional symptoms such as arthralgia, myalgias, and fatigue. These complaints gradually became worse and occurred in episodes (lasting 3–4 days) independently of IFN-beta injection. Detailed past medical history revealed that these symptoms were already present periodically before the onset of MS. Several rheumatologic investigations had been performed without any pathological findings. An autoinflammatory syndrome was suspected and molecular genetic testing for TRAPS indeed revealed a heterozygous c.362G>C replacement in exon 4 of the...
P031
Genome-wide association study using high-density SNP arrays on pooled genomic DNA from relapsing-remitting multiple sclerosis patients and healthy controls
M. Comabella, G. Craig, M. Caminá, M. Tintore, J. Río, N. Téllez, X. Montalban, R. Martin; Vall d’Hebron University Hospital (Barcelona, E); TGen (Phoenix, USA)

Background: A large body of evidence supports the idea that multiple sclerosis (MS) is a complex disorder resulting from an interaction between an inherent genetic susceptibility and environmental exposures such as viral infections. Regarding the genetic background, only genes of the HLA-class II region have consistently been associated with MS, and it has been estimated that they may confer up to 50% of the genetic risk. Genome-wide case-control association studies are a powerful approach to identify genes that predispose to genetically complex disorders. The current development of dense, genome-wide genotyping technologies allows, for the first time, a hypothesis-free approach to unravel the genetics of complex disorders such as MS. Objective: To identify genes that confer susceptibility to MS by means of a genome-wide case control association study using high-density single nucleotide polymorphisms (SNPs) arrays.

Methods: DNA from 250 relapsing-remitting (RR) and secondary progressive (SP) MS patients and 250 sex-matched healthy donors was pooled and nine pooling replicates for cases and nine pooling replicates for controls were hybridized to high-density SNP arrays (Affymetrix GeneChip Mapping 500K). SNPs were ranked based on statistical tests that incorporated both relative allele signals (RAS)-1 and RAS-2, SNP variability between pools, and allelic frequencies. Top scoring SNPs were selected for validation by means of individual genotyping in a confirmatory and independent cohort of cases and controls comprised of DNA samples from 100 RRMS and SPMS patients and 100 controls.

Results and Conclusions: Genome-wide association study using SNP arrays in MS patients and healthy controls lead to a number of candidate genes that conferred susceptibility for the disease. The use of high-density genotyping technologies in association studies may prove to be a powerful tool to uncover the genetic component of complex disorders such as MS.

P032
Regulation of genes for adhesion molecules in central nervous system glial cell cultures by mixtures of cytokines
R. Lisau, B. Bealmear, L. Niedokoska, J. Benjamins, B. Yao, S. Lund; Wayne State University (Detroit, USA)

Adhesion molecules are important in development and function of the Central Nervous System (CNS), including migration and matura- tion of neurons and glia and axon (Ax)-glial cell interactions. Little is known about the effects of cytokines (CY) present in the CNS in multiple sclerosis (MS) on glial cell expression of adhesion molecules. We have previously described CY-induced early gene regulation of adhesion molecules important in immune and vascular system cells (Mult Scler 12: 149, 2006), and now extend our analysis to other adhesion molecules implicated in glial and Ax function. We incubated mixed rat CNS glial cell cultures with CY mixtures typical of Th1 and Th2 lymphocytes and monocyte/macrophages (M/M). The mixture for Th1 CY consisted of rat recombinant (r)IL-2, r-IFN-γ, and rTNF-α; for Th2 CY, rIL-4, rIL-5, rIL-10, porcine TGF-b1 and rG-CSF; and for M/M CY, rIL-1α, rIL-1β, rIL-6, rIL-12p40 and rTNF-α. We then examined gene expression at 6 hours (early gene expression) employing Affymetrix microarray gene chips, concentrating on several adhesion molecules known to be important for CNS cells. In these screening experiments we considered increases or decreases of ≥2 fold in gene expression compared to control cultures as significant.

We find changes in expression of genes for several proteoglycan proteins. Expression of the gene for syndecan 4, a heparin sulfate binding proteoglycan core protein, was upregulated by Th1 and M/M CY and downregulated by Th2 CY. Genes for other proteoglycans were also affected with upregulation for glypicans 3 by Th2 CY, for serglycin by Th1 CY and downregulation of perlecian by Th1 CY. Changes in expression of genes for adhesion molecules that are members of the immunoglobulin superfamily were also observed, including upregulation of Tenascin C by Th2, upregulation of neutrotinmin by M/M and Th2 CY, downregulation of nidogen (entactin) by M/M and Th2 CY and of contactin 4 (Ax-associated adhesion molecule) by Th2 CY. The expression of message for claudin-7, a tetraspan protein involved in tight junction formation, was modestly downregulated by Th2 CY; this protein is expressed on CD4 cells, and downregulated in tumor progression, but not previously identified in glia. Changes in expression of the proteins controlled by these genes have the potential to affect cells important in neuronal and oligodendroglial function and thus regulate both the evolution of lesions in MS and also the protection and repair of neurons/Ax and oligodendrocytes/myelin.

P033
Cannabinoid receptor type 2 expression in relapsing-remitting multiple sclerosis
P. González Pérez, A.J. Sánchez, V. García, E. Ramí, A. García-Merino; Clínica Puerta de Hierro (Madrid, E)

Introduction: Two types of cannabinoid receptors, CB1 and CB2, have been identified so far. CB1 receptor is widely distributed in the central nervous system, whereas CB2 is mostly present in immune cells. In both systems, these receptors seem to be involved in complex regulatory functions. Cannabinoids are known to suppress or alleviate the animal model of MS through complex and ill defined mechanisms. The presence of CB2 receptors in immune cells raises as well the possible implication of the endocannabinoid system in the modula- tion of the altered immune response that exists in multiple sclerosis (MS). No information is available on the possible role of cannabinoids as immunomodulators in MS. In this report we analysed the expression of CB2 receptors in peripheral blood mononuclear cells (PBMCs) from patients undergoing a relapse, and 3 months later.

Methods: Blood was drawn from 17 patients with relapsing remitting (RR) MS, devoid of immunomodulator or immunosuppressant therapy and undergoing a clinical relapse, before the institution of steroid therapy (sample 1) and 3 months later (sample 2). Total RNA was isolated from PBMCs by RNasy Mini kit (Quiagen) and transcribed into cDNA using GeneAmp gold RNA PCR Core Kit (PE Biosystems). PCR amplification was performed using primer sets specific for human CB2 and the situation dehydrogenase complex (SDHA); SDHA was used as the housekeeping gene. The LightCycler FastStart DNA MasterPlus SYBR Green I Kit (Roche Diagnostics) was used for the amplification and detection of CB2 by real-time RT-PCR. To refer results, expression values were normalised according to those of the housekeeping gene; a quotient between results of samples 1 and 2 was established.

Results and Conclusions: In 15 patients no significant differences were observed between samples corresponding to relapses and those taken 3 months apart. In one patient, the CB2 mRNA expression was
significantly higher during relapse, and in another patient, it was higher during remission. Our results do not suggest a clear involvement of the CB2 receptors in the modulation of the clinical relapses in MS; however, further studies may be needed to draw definite conclusions.

P304
Polymorphisms of the human cannabinoid receptor gene (CNR1) in multiple sclerosis
P. González Pérez, P. Ortiz, A.J. Sánchez, R. Arroyo, E. Ramil, V. De las Heras, E. Urcelay, A. García-Merino; Hospital Clínico Puerta de Hierro (Madrid, E); Hospital Clínico San Carlos (Madrid, E)

Two types of cannabinoid receptors, CB1 and CB2, have been identified so far. CB1 receptor is widely distributed in the central nervous system, whereas CB2 is mostly present in immune cells. CB1 receptor gene (CNR1) is the only receptor accessible to genotyping thanks to a microsatellite polymorphism (AAT)n with nine alleles. In humans, CNR1 is located on chromosome 6 at 6q14-q15. The involvement of CB1 receptors in the regulation of critical neurotransmitters such as glutamate, their regulation by corticosteroids and their modulation in the course of experimental allergic encephalomyelitis, raises the possibility of a role as modifiers of multiple sclerosis (MS) expression. Methods: Genomic DNA from 133 MS patients, 90 with relapsing remitting (RR) and secondary progressive (SP) MS and 43 with the primary progressive (PP) variety, and 92 healthy controls (HC) was obtained by DNAzol, and analysed by PCR. The amplified PCR products were separated in polyacrylamide gels.

Results: The commonest allele found in the two groups was number 4. It was present in 34% of the HC, and in 24% of the MS patients. Allele 4 frequency was significantly higher in HC than in PP-MS patients (34 vs. 15%; OR: 2.92, 95% CI 1.44 – 6.01; p = 0.001) and was significantly higher in RR+SP – MS than in PP – MS patients as well (28 vs. 15%; OR: 2.22, 95% CI 1.08 – 4.61; p = 0.018). Allele 8 frequency was significantly higher in PP-MS patients than in the RR+SP varieties (34.9 vs. 19%; OR: 2.3, 95% CI 1.24 – 4.28; p = 0.004) and was higher in PP-MS patients than in HC (34.9 vs. 25%; OR: 1.5, 95% CI 0.86 – 2.8; p = 0.12). Considering the possible influence of the length of the repeats on gene regulation, alleles were divided according to their length into a short group (<5) and a long group (>5), which produced three genotypes: one heterozygous (5/5) and two homozygous (5/5; >5/5) genotypes. PP-MS patients and HC showed a significant difference in the frequency of the >5/5 homozygous genotype (62.8 vs. 38%; OR: 2.75, 95% CI 1.22 – 6.23; p = 0.007) and in the frequency of the <5/5 >5 homozygous genotype (32.6 vs. 52.2%; OR: 2.26, 95% CI 0.99 – 5.18; p = 0.033). RR+SP-MS patients and HC had a similar allele and genotype frequencies.

Conclusions: Using the AAT polymorphism of the CNR1 gene, a significant association was found in allele and genotype frequencies in PP-MS patients when compared to HC. No significant differences were seen in the allele or genotype distribution between RR+SP-MS patients and HC.

Disease modifying therapy

P305
Combined therapy with mitoxantrone and methylprednisolone in multiple sclerosis
P. Mihanacca, Cristina M. Brisc, N. Hanavi, Ciprian Brisc; University of Oraطة (Oraطة, RO)

Background: The aim of our study is to compare the therapeutic effect of mitoxantrone (MX) combined with methylprednisolone (MP) and on the other hand, the therapeutic effect of MP as mono-therapy upon multiple sclerosis (MS). Methods: The study was done on a batch of 15 patients hospitalized into the Clinic of Neurology II of Oraطة with secondary progressively multiple sclerosis (SPMS), who received a unique dose of 12 mg/square meter of MX IV at each 3 months plus 0.5 mg of MP IV for 5 days also at each 3 months. In parallel other 15 patients interned in the same clinic were administered 0.5 mg of MP IV, for 5 days a 3 months interval, as mono-therapy. The duration of therapy at each batches lasted for 21 months. The patients with MS had an increased activity of the disease, which fulfilled the Mc Donald’s criteria for a clinically manifested disease and who had residual neurological deficiencies. The score containing the Expanded Disability Status Scale (EDSS) was monitored at each 3 months and the number of lesions at Nuclear Magnetic Resonance (NMR) at the beginning of the therapy and after 21 months of therapy at both batches. Results: During the 21 months therapy period patients who received MX in combination to MP had an obviously decreased trimester average of EDSS than the ones who were administered only MP, and the EDSS modifications related to the very moment of initializing the therapy were significantly less at the group of patients who followed the combined therapy, in comparison to the ones that used only MP as a mono-therapy. At MNR 75% of the patients from the group that used a combined therapy were found with no new lesions in comparison to 25% of the patients using MP as single therapy, over a 21 months interval. Conclusions: The results obtained in our study based on the clinical observations and MNR prove that treatment with MX in combination with MP is significantly more efficient than the therapy with MP as mono-therapy. MX plus MP reduce the neurological disability, the frequency of relapses and induces in important decrease of new lesions appearing, which indicates a direct effect upon the demyelization of the central nervous system. The trimester mono-therapy with MP induces fewer modifications upon the SPMS.

P306
Intravenous synthetic peptide MBP8298 significantly delayed disease progression in a class II-defined cohort of patients with progressive multiple sclerosis
I. Catz, K. Warren, L.Z. Ferenczi, M.J. Krantz; University of Alberta (Edmonton, CAN)

Objective: To assess the clinical efficacy of MBP8298 administered IV to patients with chronic progressive MS. Background: MBP8298 is a synthetic peptide that corresponds to residues 82–98 of human MBP, a sequence that is the immunodominant target of both B-cells and T-cells in MS patients with HLA haplotype DR2. IV administration of MBP8298 according to the principle of high dose tolerance induction resulted in long term suppression of anti-MBP autoantibody levels in cerebrospinal fluid of a large fraction of progressive MS patients. Design and Methods: 500 mg of MBP8298 was administered IV every 6 months, in a 24 month double blind placebo controlled Phase II clinical trial in 32 patients with progressive MS. Clinical efficacy was assessed by changes in EDSS scores. Results: Contingency analysis of results from all patients at 24 months showed no significant difference between MBP8298 and placebo treatments (n = 32, p = 0.29). Subset analysis by HLA-DR haplotype showed a statistically significant benefit of MBP8298 treatment in patients with HLA haplotypes DR2 and/or DR4 (n = 20, p = 0.01). Long term treatment and follow-up of patients in this responder group showed a median time to progression of 78 months for MBP8298 treated patients compared to 18 months for placebo treatment (Kaplan-Meier analysis, p = 0.004). Anti-MBP autoantibodies that reappeared in the CSF of one patient at 36 months, while under treatment with MBP8298, were not reactive with the MBP8298 peptide in vitro. Conclusions/relevance: The identification of a responder subgroup (62.5% of the patients in this study) enables a more efficient design for a large confirmatory clinical trial of MBP8298. Patients with certain other less common HLA-DR haplotypes may also respond to this treatment.
P307
Severe lymphopenia and opportunistic infection as a complication during a so-called harmless alternative therapy for multiple sclerosis
V. Desestret, C. Renoux, R. Mariginer, C. Confavreux, S. Vukusic; Hôpital Neurologique Pierre Wertheimer (Lyon, F)

We report a patient who developed severe iatrogenic side-effects while taking a so-called alternative therapy as a treatment for MS. A 33-year-old woman presented in December 2005 with a respiratory failure secondary to a Pneumocystis carinii bilateral pneumopathy. Her respiratory condition required mechanical ventilation for two months. During this period, she developed a right flaccid hemiplegia and an aphasia, which was reported to a left deep median cerebral artery stroke. Blood testsings revealed a severe lymphopenia (100 cells/mm$^3$) involving CD4$^+$ and CD8$^+$ T-cells altogether, without HIV infection. She had a history of clinically definite MS, with at least 6 relapses involving the brainstem and the optic nerve. CSF evaluation was positive for oligoclonal bands. The first and only MRI scan performed in 1995 showed a single lesion in the medulla, suggestive of an inflammatory lesion. She had been treated with azathioprine from 1995 to 2001. Since then she turned to so-called alternative therapies. In June 2005, she started to take SOVITA and VITORAL, a treatment developed and diffused by Yangos Solomides, which is a non-labelled remedy distributed under the counter. An extensive check-up, including viral serologies and a myelogram, failed to find an explanation to the severe lymphopenia. The patient had not been exposed to any environmental toxic agent. She had not taken any other drug but the Solomides treatment since 2001. Solomides treatment is an inhomogeneous preparation that contains some potentially toxic agents, such as urethan, which is known to be lymphotoxic and can induce profound and long lasting lymphasting. Patients who take those drugs are not advised to have any biological follow-up. In our patient, the severe, uncontrolled lymphocytic depletion lead to a life-threatening opportunistic infection. It is clear if the stroke can be related to the drug or not. Patients suffering from chronic diseases like MS, that can not be cured, often turn to so-called alternative therapies considered harmless. They are therefore usually not discouraged by their neurologist to continue the treatment. Our case demonstrates that those treatments may sometimes be dangerous and that doctors should be more cautious about them.

P308
Inflammatory demyelinating events associated with antitumour necrosis factor treatment
A. Fromont, J.F. Maillefert, D. Audry, T. Moreau; University Hospital (Dijon, F)

Background: Tumor Necrosis Factor alpha (TNF-a) an inflammatory cytokine has been implicated in certain inflammatory diseases including Multiple Sclerosis (MS), Rheumatoid Arthritis (RA) and Crohn disease. Anti-TNF therapy for RA has been associated with monophasic inflammatory CNS demyelination and possibly could worsen known MS. Objective: We report two new cases of inflammatory CNS demyelinating event induced by anti TNF therapy. Case report: A 42 year old woman with a 5 year history of RA presented with right ocular pain and decreased vision in her right eye (4/10). She has been treated with Humira for almost one month with dramatic efficacy. Visual evoked responses were altered with low amplitude on her right optic nerve. MRI of brain made four months later was normal. The second case is a 43 year old man presented with paroxysmal dysarthria and dizziness. He has been diagnosed with normal. The second case is a 43 year old man presented with right optic nerve. MRI of brain made four months later was normal. He has a history of anti-TNF therapy. Sometimes diagnosis of MS is made on the basis of polyphasic occurrence of neurologic events and MRI findings independent of anti-TNF cessation. Patients who develop neurologic symptoms while on any anti-TNF medication should be monitored closely with frequent MRI.

P309
Efficacy of mitoxantrone and intrathecal triamcinolone acetonide treatment in chronic progressive multiple sclerosis patients
K. Hellweg, T. Mueller, C. Lukas, S. Schimrigk; St. Josef Hospital (Bochum, D)

Treatment approaches are rare for chronic progressive patients with multiple sclerosis. Objective was to evaluate the clinical benefit of repeat intrathecal application of the sustained release steroid triamcinolone acetonide or the administration of mitoxantrone in two similar cohorts of chronic progressive patients with multiple sclerosis in an open label fashion. EDSS scores significantly decreased after the first six intraspinall triamcinolone acetonide injections, which were performed every third day, and then remained stable. Walking distance significantly increased and did not reduce until the end of the one year lasting trial period. Mitoxantrone treatment did not improve the EDSS score, however no further significant deterioration appeared. Walking distance did not significantly decrease. Both treatment regimes were safe, the patients experienced nearly no side effects. Triamcinolone acetonide application provided a clinical benefit, whereas mitoxantrone administration prevented further worsening of multiple sclerosis symptoms. We stress, that only specialists with a broad experience of intraspinall triamcinolone acetonide application and mitoxantrone administration should perform both kinds of therapy only after a careful information and risk-benefit evaluation in cooperation with the patient. Future trials will show the efficacy of combination of both treatment approaches in chronic progressive patients with multiple sclerosis.

P310
Comparison of the efficacy of interferon-beta 1-b and azathioprine in secondary progressive multiple sclerosis: results of a 3-year open controlled prospective study
N.S. Oztekin, M.F. Oztekin; SB Diskapi Hospital (Ankara, TR)

Background: Both Interferon beta-1b and azathioprine (AZA) are effective in reducing relapse frequency in RR MS. However some patients’ response to interferon beta 1-b is poor and some deny to usejections for a long period of time. On the other hand no prospective study has compared the efficacy of the two drugs in secondary progressive Multiple Sclerosis (SPMS). Objective: The aim of the study is to assess the relative efficacy of these two drugs in patients with secondary progressive multiple sclerosis and compare the results with control group. Method: 39 patients with secondary progressive MS were enrolled to the study and all of them were recruited among secondary progressive MS patients receiving IFNB1-b treatment. Patient selection criteria for the study was two or more relapses requiring corticosteroid treatment or deterioration by at least 0.5 points on EDSS while on IFNB1-b in the preceding 13 years in patients whose response was good to IFNB1-b stay on their regimen. The remaining 26 patients whose response was inadequate was assigned either to receive either AZA (n=16, 50 mg t2-d, oral) or non treatment (n=10). Mean EDSS of the patients were 5.5. Safety was assessed in terms of adverse reactions and laboratory measures graded according to WHO toxicity scale. Efficacy is determined by changes in relapse rate and MRI results. Results: All the patients completed the
study period. Two patients in the AZA treated group had mild lymphopenia which totally recovered with lowering of dose. Annual relapse rate was 0.84 in IFNB-1b group, 1.27 in the AZA group and 1.49 in the non treated group and the results were not statistically significant (p = 0.005). There was a significant trend for EDSS increase in the AZA and untreated groups (p = 0.0045) Total lesion load measured by MRI unchanged in the IFNB1-b and AZA treated groups whereas increased in the untreated group at the 12th, 24th and 36 months of the study period. Conclusion: The results of this 3 year prospectively controlled study revealed that IFNB1-b has a prominent effect in reducing relaps rate, disability, MRI lesion load and activity compared to AZA treated and control groups in patients with secondary progressive MS. AZA treatment requires strict clinical and laboratory monitoring during treatment and found to be less effective compared to IFNB1-b treated group although the patients were recruited among the patients with poor response to IFNB1-b.

P311
Analysis of reasons for DMD therapy discontinuation
R. Talah, M. Valis, M. Talabova; University Hospital (Hradec Kralove, CZ)
Multiple sclerosis (MS) is a chronic inflammatory autoimmune disorder of the central nervous system (CNS) with not clearly understood causes. Therapy of MS should be always complex and systemic, appropriate for the stage and form of the pathologic process. The most important role in the therapy of the relapse/ remittent form of the disease is played by disease modifying drugs (DMD). Materials and Methods: We retrospectively analyzed patients with the R/R form of MS who discontinued therapy with DMDs in a 10 year period (1996–2005). In this period treatment of 303 patients was started in the MS centre. In the same time period a total of 23 patients, i.e. 7.6%, discontinued the treatment. We compared the incidence of relapses and relapse rate and also the degree of disability using the EDSS score. We discuss various causes of DMD therapy discontinuation. Results: The median age of the 23 patients who discontinued the DMD therapy (14 women, 9 men) at the beginning of therapy was 41 years (25–57), duration of disease 1–19 years, median = 5, and the degree of disability evaluated using the EDSS was 1.5 – 4.0, median 3. The number of disease attacks used for indication of DMD therapy, i.e. in the past 2 years, was 5.0, relapse rate 2.5. The DMD therapy of this group of patients lasted on average 30 months (1–96 months). During therapy patients suffered from 0–5 relapses, median = 2, and there was a progression of disability in this time period (median >30 months) to EDSS = 4.5, i.e. increase by 1.5. Discussion: The most common cause of discontinuation of DMD therapy was its lack of effect manifested by secondary progression of the disease. The total percentage of DMD therapy discontinuation was 7.6% of patients. From the clinical point of view, progression of disability was detected: on average by 1.5 EDSS over 30 months. Also the frequency of disease relapses was on average 2 over the studied period. Very interesting was the group of 5 patients (21.8%) who discontinued the therapy on their own request due to psychological problems with the required drug injections or due to doubts about the efficacy of the treatment. Conclusion: The analysis of 303 patients with R/R form of MS treated with DMDs over the period of 10 years showed treatment discontinuation in 7.6% mostly (in 65.3%) due to lack of effect, permanent occurrence of relapses and disability progression.

P312
Higher serum uric acid levels after treatment with interferon-beta 1b (Betaferon®)
G. Tonchev, S. Miletic Drakalic, Z. Knezevic; Clinical Hospital Centre Kragujevac (Kragujevac, CS)
Background: Interferon beta is a safe and efficacious treatment for relapsing multiple sclerosis (MS). On the other hand there is some evidence that uric acid, as scavenger of peroxynitrite, is involved in MS pathology and that increasing of serum uric acid levels may have beneficial therapeutic effect. Objective: The aim of this study is to investigate serum uric acid levels in MS patients before and after one year treatment with interferon beta-1b. Methods: Blood samples from 26 MS patients (6 male and 20 female; mean age 33.46 ± 7.89 years; mean duration of disease 6.38 ± 3.9 years; mean EDSS 1.79 ± 0.63) before and after interferon beta-1b treatment (months 0, 6 and 12) were analyzed. Serum uric acid were measured using a quantitative enzymatic assay (Elitech diagnostic, Sees, France). Results: We observed significantly linear trend (p = 0.024) in increasing serum uric acid over the time. MS patients had significantly increased serum uric acid levels on month 12 (after one year treatment), than those on month 0 (beginning of treatment) (250.11 ± 69.38 μmol/l vs. 212.27 ± 67.99 μmol/l; p = 0.029, Wilcoxon Mann-Whitney U-test). We did not found significantly differences in serum uric acid levels between month 0 and month 6 (p = 0.341) and between month 6 and month 12 (p = 0.170). We also observed significantly decreasing in annual relapse rate after one year of Betaseron treatment (0.75 ± 0.3 before treatment vs. 0.27 ± 0.6 after treatment; p = 0.000, T-test). Conclusions: These results implicate that one of the beneficial effect of interferon beta 1b in MS might be based on the elevation of uric acid as a natural scavenger of peroxynitrite.

P313
Rational treatment algorithms for relapsing multiple sclerosis
J. Herbert; NYU Hospital for Joint Diseases (New York, USA)
Background: Rational algorithms have not yet been developed which stratify treatments by MS subtype. This is due in part to disease heterogeneity, poor predictive ability, and the absence of a classification system which differentiates patients according to disease severity. Objective: To devise rational treatment algorithms for relapsing Multiple Sclerosis (MS). Method: In these proceedings, a new classification system has been proposed for MS, based on disease severity (following Roxburgh et al., Neurology, 2005). Six subpopulations are defined, of equal prevalence in the reference population. Severity assignments are based on single cross-sectional Expanded Disability Status Scale (EDSS) measurements made at least one year after disease onset. Based on this classification, two possible treatment algorithms are proposed and compared. They differ only in degree of intervention; thus, algorithm 2 is framed shifted by one step towards more aggressive treatment. Results: Using treatment algorithm 1, patients with MS severity Grade 1 would require no treatment; with Grade 2A, treatment with Avonex, Betaseron, Copaxone, or Rebif (ABCR); with Grade 2B, ABCR + pulse steroids (PS) or pulse IVIg; with Grade 3A, ABCR + PS or pulse IVIg + oral immunosuppressant; with Grade 3B, ABCR + PS or pulse IVIg + parenteral immunosuppressant, OR Tysabri alone; and with Grade 4, ABCR + PS + parenteral immunosuppressant + plasmapheresis, OR Tysabri alone. Alternatively, treatment algorithm 2 shifts the treatment recommendations towards a more aggressive treatment modality. Using treatment algorithm 2, patients with MS severity Grade 1 would require treatment with ABCR; with Grade 2A, ABCR + PS or pulse IVIg; with Grade 2B, ABCR + PS or pulse IVIg + oral immunosuppressant; with Grade 3A, ABCR + PS or pulse IVIg + parenteral immunosuppressant, OR Tysabri alone; with Grade 3B, ABCR + PS + parenteral immunosuppressant + plasmapheresis, OR Tysabri alone; and with Grade 4, ABCR + PS + parenteral immunosuppressant + plasmapheresis, OR Tysabri alone. Conclusion: Subpopulations of MS patients may be defined after one year’s duration and assigned to different treatment cohorts based on predicted disease severity. The stratification subtypes suggested here for defining rational therapeutic algorithms and for the design of therapeutic trials comparing homogeneous subpopulations to determine optimal treatment interventions.
P314

Neurological consequences of delay treatment in early relapsing-remitting multiple sclerosis. A five-year follow-up study

B. Casanova, I. Bosca, A. Pascual-Lozano, C. Valero, F. Coret; Hospital Universitari La Fe (Valencia, E); Hospital Arnau de Vilanova (Valencia, E); Hospital Clinic Universitari (Valencia, E)

Objective: To study if there are differences in the disability at five-year of multiple sclerosis patients when interferon treatment is initiated earlier. Method: Patients with a first event suggestive of multiple sclerosis were recruited. In patients visited between January 1996 to December 1998 (group A) a minimum of one year after a stablished diagnosis according the Poser criteria (CDMS) must be accomplished to begin treatment with interferon. And in patients visited from January 1999 to April 2001 (Group B) this year of delay as CDMS was eliminated. We have analysed the impact over the disability at year five of the application of these criteria. Results: 63 patients accomplished the criteria to begin treatment; 28 in the group A, and 37 in the group B. Mean time to CDMS was similar in both groups (11.0 months). Mean time to begin treatment was 16.0 and 7.3 months (group A and B). Total time under IFNB was 33.0 and 40.7 (group A and B). The final median EDSS for patients treated in the group A was 3.0 vs. 2.0 in patients treated in the group B (p < 0.0001). The time to reach an EDSS of 3.0, was lower for patients of the group B (Hazard ratio 7.4, p = 0.0003). Conclusions: Earlier treatment had a favorable impact over the disability in the first five years of evolution of RRMS.

P315

Betaainterferons in the treatment of multiple sclerosis: a naturalistic survey

H.M. Kuusisto, R. Soikkeli, T. Laukkaula, I. Eloranta; Tampere University Hospital (Tampere, FIN); Tampere University (Tampere, FIN)

Background: Controlled clinical trials have shown that beta-interferons are effective and moderately well tolerated in the treatment of multiple sclerosis (MS). The annual costs of the treatment are, however, substantial. To date, reports of the efficacy and tolerability of beta-interferons in unselected MS patient populations are scarce. Objectives: To evaluate retrospectively the efficacy and tolerability of beta-interferons in an unselected MS-cohort. Subjects and Methods: The data was collected of 96 consecutive patients with relapsing-remitting MS (RRMS) treated at the Tampere University Hospital, Finland between 1996–2003. Results: During beta-interferon treatment, the annual relapse rate declined in 66% of the patients. The total number of relapses was reduced by 58% compared to the time prior to the treatment. Adverse effects were experienced by 80% of the patients. Altogether 28% of the patients switched to another beta-interferon product during the follow up. The main reason for change of a product was lack of efficacy. Conclusions: Beta-interferons are efficacious and well tolerated by most of the RRMS-patients. However, one third of the patients seem to obtain no benefit from the treatment and adverse effects can be common. Thus, the effects of beta-interferon treatment should be carefully monitored and the value of the therapy should be always individually assessed.

P316

The Global Adherence Project – A multicentre observational study on adherence to disease-modifying therapies in patients suffering from relapsing-remitting multiple sclerosis

V. Devonshire, Y. Lapiere, R. MacDonell, C. Ramo Tello, F. Patti, P. Fontoura, L. Suchet, R. Hyde, I. Balla, B. Kieseier, E. Frohman on behalf of the GAP Study Group

Background and Objectives: Adherence is a key component in the therapy of chronic diseases. The Global Adherence Project (GAP) is the largest global, observational study that has evaluated real-world adherence rates to approved disease-modifying therapies (DMTs) for relapsing-remitting multiple sclerosis (RRMS), factors influencing adherence, quality of life and level of cognition and depression. Design/Methods: Eligible RRMS patients were >18 years old and on their current DMT for at least six months. 179 sites across 22 countries recruited patients in this retrospective paper-based survey. Neurologists completed a practice-related survey and patients completed a patient survey plus the Multiple Sclerosis International Quality of Life Questionnaire (MusiQoL) and the Multiple Sclerosis Neuropsychological Screening Questionnaire (MSNQ, in English-speaking countries). Non-adherence was defined as missing at least one DMT injection or changing dose within 4 weeks prior to the survey. Results: 2646 patients treated with Avonex, Rebif, Betaferon/Betaseron or Copaxone had a mean age of 40 years, 73% were female, median disease duration was 6 years, and median time on treatment was 32 months. Overall, 25.3% of patients reported non-adherence. The non-adherence rate was significantly lower for Avonex (15.0%) than for Rebif 22 mcg (22.0%, p = 0.012), Rebif 44 mcg (27.3%), Betaseron/Betaseron (30.9%), and Copaxone (34.2%), all p < 0.0001. The most common reason for non-adherence was forgetting to administer the injection (50.6%). Other univariate factors that affected adherence included duration of current DMT (p = 0.0017) and duration of disease (p = 0.0004). Adherent patients had higher MusiQoL scores in 7 of 9 dimensions (indicating better QOL; p < 0.05), fewer cognitive problems (MSNQ, p = 0.0002) and fewer problems with injection-site reactions (p < 0.01) than non-adherent patients. Full multivariate analyses will be conducted on all primary factors that impact adherence. Conclusions: Several univariate factors including current DMT used, duration of current DMT and disease duration affected adherence to therapy. Adherent patients reported better quality of life, less cognitive impairment and fewer problems with injection site reactions than non-adherent patients.
Comparative effect of modafinil on fatigue in multiple sclerosis and stroke

A. Brioschi, S. Gramigna, F. Staub, M. Schluep, J.-M. Annoni; CHUV (Lausanne, CH)

Background: Fatigue is a common and debilitating complaint in various neurological diseases. Its differential pattern still needs to be characterised, and its effect on the response to medication comparatively determined. Objectives: 1. Does primary neurological fatigue respond to adrenergic therapy (modafinil)? 2. Comparison of the efficiency of modafinil on subjective fatigue in 2 different neurological populations with similarly mild functional and neurological impairments, in the absence of depression (DSM-IV criteria): relapsing-remitting multiple sclerosis (MS) patients (Expanded Disability Status Scale, EDSS <4, Rankin <2), and post-stroke patients (National Institute of Health Stroke Score, NIHSS <4, Rankin <2). Methods: We enrolled prospectively 19 patients 12 months after a stroke (mean age 51.2, SD 12.3; mean NIHSS 1, SD 0.94), and 18 MS patients (mean age 43.3, SD 10.1; mean EDSS 2.31, SD 0.71) in an open study with a ADA design. The Fatigue Assessment Instrument scale (FAI) was performed at baseline, after 3 months of modafinil treatment (initial daily dose of 50 mg for 1 month, then increased to 100 – 200 mg/day), and after a washout period of 1 month. Three of the 4 FAI factors (severity, specificity, psychic impact) were analysed. Cognition (learning, executive functions, attention), mood (Hospital Anxiety and Depression Scale [HAD]) and somnolence (Epworth scale) were also assessed, and correlated to FAI scores (t test). Results: 6 MS and 5 stroke patients dropped out due to modafinil side effects (mainly headaches, abdominal pain, hypertension and anxiety). Modafinil improved significantly FAI severity scores (p = 0.0007). Subgroup analyses showed that MS patients were responsible for this favourable effect. However, when individual data were analysed within the stroke subgroup, patients with brainstem or thalamic lesions were prone to ameliorate their FAI severity scores with modafinil. Other FAI factors were not significantly modified, and improvement in FAI severity scores had no effect on cognitive performances, Epworth or HAD scores. Conclusion: Psychostimulants such as modafinil may be effective on subjective primary fatigue of cerebral origin, at least in MS patients. However, in stroke the efficiency of this therapy may depend on the lesion site, such as strategic structures responsible for cerebral activation.

Interferon-beta enhances fatigue but not mood disorders in early multiple sclerosis

S. Staniott, C. Raffieux, L. Bruggimann, J.-M. Annoni, M. Schluep; CHUV (Lausanne, CH)

Background and purpose: In multiple sclerosis (MS), mood disorders and fatigue are frequent and disabling symptoms associated with increased neurological handicap and a poorer quality of life (QoL). The effect of disease modifying therapies such as interferon beta (IFNB) on mood and fatigue in MS is still controversial. The aim of the present study was to measure fatigue, anxiety and depression comparatively in patients in the early phase of MS with and without IFNB, and its influence on handicap and QoL. Methods: We enrolled 134 patients (42 males, mean age 35.8) with a diagnosis of confirmed MS (n = 110) or possible MS (n = 24) according to McDonald’s criteria. Mean disease duration was 2.3 years and mean Expanded Disability Status Scale (EDSS) score was 1.7 (range 1 – 2.5). Twelve patients were receiving antidepressive drugs, 58 were treated with IFNB while 76 had no disease modifying therapy. Patients were evaluated using the Hospital Anxiety and Depression scale (HAD), a psychiatric interview (DSM-IV criteria), the Fatigue Assessment Instrument (FAI), and two questionnaires assessing handicap (London Handicap Scale [LHS]) and QoL (SEP-59). Parametric and non parametric tests were used for statistical analysis. Results: On the HAD, 52.2% of the patients had a significant score for anxiety and 17.1% for depression but only 8/134 patients reached the diagnostic criteria for mood disorders using the DSM-IV criteria. On the FAI, 54.4% of the patients presented a significant score of fatigue on the severity subscale. A trend to an association was found between IFNB treatment and fatigue (p = 0.05) while it was not significant for anxiety (p = 0.9) or depression (p = 0.1). The risk ratio (RR) for fatigue was also higher (RR = 1.34, 95% CI [0.99, 1.82]) than the RR for anxiety (RR = 0.98, 95% CI [0.71, 1.36]) or depression (RR = 0.57, 95% CI [0.25, 1.30]). Finally, IFNB had no significant impact on handicap or QoL. Discussion: IFNB influenced mildly the severity of fatigue, while the occurrence of anxiety and depression was independent of IFNB in this early MS patients’ group. This supports the idea of an absence of link between IFNB and the occurrence of mood disorders at least in patients with low EDSS score and a short MS duration. However, IFNB was linked to increased fatigue in this undiagnosed MS population, stressing its potential role as an independent negative factor.

Autoimmune hepatitis and multiple sclerosis. Is it a serendipity?

M. Rodríguez, B.G Giraldez, P. Bellas, D. Muñoz, J. Gómez-Alonso; Xeral-Cies (Vigo, E)

Background: Nearly one third of the patients with Multiple Sclerosis (MS) treated with Interferon beta (IFN b) develop abnormal liver function tests. Autoimmune hepatitis (AIH) has been rarely reported as secondary to IFN b 1a, and in 4 untreated MS patients. We report here an unusual case of AIH in a MS patient on IFN b treatment, but not secondary to the drug. Report: A 38-year-old woman with a 5-year history of relapsing-remitting MS (RRMS) had been on IFN b 1b therapy for 2 years (Betaseron) without relapses. As she wished to become pregnant, therapy was discontinued. She had a two-month-miscarriage, and 15 days later a spinal relapse. She was then restarted on Betaseron (8 million SC doses on alternate days). In January 2003, three weeks later, she was admitted for abdominal pain, jaundice and cholicr uririne. There was no history of alcohol or drug intake except for IFN b. Laboratory tests showed increased hepatic enzymes and total bilirubin of 5.8 mg/dl. Hepatitis panel and thyroid function tests were normal. Autoimmunity tests were also negative. Abdominal echography was normal. After Betaseron cessation, AST and ALT decreased about 100 U/L. With the diagnosis of acute hepatitis secondary to IFN b, we decided ambulatory control of hepatic function. In April 2003, she became pregnant again. When she was two-month-pregnant, she was readmitted for elevation of transamases coinciding with a new miscarriage. Laboratory tests showed positive ANA (1:40). Hepatic biopsy was suggestive of AIH. The AIH diagnosis was made according to the International Criteria. Treatment with azathioprine and steroids was started, with normalization of hepatic function tests. In April 2003, she became pregnant again. When she was two-month-pregnant, she was readmitted for elevation of transamases coinciding with a new miscarriage. Laboratory tests showed positive ANA (1:40). Hepatic biopsy was suggestive of AIH. The AIH diagnosis was made according to the International Criteria. Treatment with azathioprine and steroids was started, with normalization of hepatic function tests. She has had no new relapses. Discussion/Conclusion: Hepatotoxicity during IFN b treatment in MS has been largely documented. However, it is surprising that the association between AIH and MS has been poorly reported. It could be explained because in the event of altered hepatic function, IFN b is the first suspected causative factor. We think that our patient has RRMS and AIH triggered by miscarriages, and not secondary to IFN b, therefore IFN b cessation was probably unnecessary. Our case suggests that: (1) Even in MS patients receiving IFN b, AIH may be a coexisting disease. (2) The combination of azathioprine and corticosteroids may be a good therapeutic alternative in patients with MS and other autoimmune diseases.
ineffective in some patients and alternate therapeutic strategies are desired. In this study, we investigated whether periodical immunoadsorption plasmapheresis (IAPP) could prevent relapses in RR-MS. **Material and Methods:** IAPP (Immusorba TR-350, Asahi-kasei medical, Japan) was carried out on eight MS patients who had many relapses. These eight patients had either relapsed more than 1.2 in a single year or more than 2.0 over a 6 month period just before treatment. The patients with RR-MS have received three or four times of IAPP treatment during a year. Peripheral blood lymphocyte subsets and plasma levels of cytokines before and after IAPP were analyzed for indices of immunological abnormality in RR-MS. Results: The average annualized relapse rate decreased significantly from 2.0 before IAPP to 0.6 in the first year and 0.2 in the second year after IAPP (p < 0.05). A significant decrease in active CD4+ T cells and helper-inducer T cells was observed after IAPP as compared with before IAPP (p < 0.001 and 0.005, respectively), whereas a significant increase in natural killer (NK) cells was observed after IAPP (p < 0.005). In addition, plasma levels of inflammatory cytokines (IL-2, IFN-gamma, and TNF-alpha) did not change significantly after IAPP, whereas those of anti-inflammatory cytokines tended to decrease (IL-4, p < 0.05; TGF-beta, p = 0.079; IL-10, ns). Conclusion: These results suggest that periodical IAPP could correlate immunological abnormality in MS and could reduce relapses in RR-MS. Therefore, IAPP may be an efficacious therapy for RR-MS patients relapsing frequently and poorly responsive to IFN-beta.

**P322**

**Plasma exchange for acute central nervous system inflammatory demyelinating disease: French nation-wide study**

C. Giannesini, P. Chillet, J.M. Korach, E. Roullet on behalf of the PEMS French Study Group

**Background:** Plasma Exchange (PE) is an effective treatment for severe acute central nervous system inflammatory demyelinating diseases, including neuromyelitis optica (NMO) (Weinshenker B, Ann Neurol 1999; 46:878). In MS, efficacy seems associated with specific immunopathological patterns (Keegan M, Lancet, 2005; 366:579). Experience from this treatment is limited to small series, mostly from specialized MS centres. In France, some 80% of patients treated by PE are recorded in a national register, allowing large-scale studies. **Objective:** To report on the preliminary results of a nationwide, retrospective evaluation of PE in acute attacks of demyelinating inflammatory disease in France. **Methods and Patients:** We selected patients from this register with a diagnosis of MS, NMO, acute demyelinating myelopathy (ADM), and acute disseminated encephalomyelitis treated between 1999 and 2005. All patients had been evaluated by neurologists, and were treated in intensive care units or haemapheresis centres. We reviewed medical records for 1) pre-treatment and high dose IV MP as treatment for severe attacks of CNS demyelination.

**P323**

**Drop outs analysis: 10 years follow-up in a cohort of relapsing-remitting multiple sclerosis patients in Argentina treated with interferons or glatiramer acetate**

A. Carra, P. Onah, G. Luzetic, M. Burgos, E. Crespo, J. Hulian, A. Lopez, V. Sinay, C. Vrech; Hospital Británico (Buenos Aires, RA); Instituto de Neurociencias de Rosario (Rosario, RA); Hospital San Bernardo (Salta, RA); Hospital Municipal de Bahía Blanca (Bahía Blanca, RA); Hospital San Roque (Córdoba, RA); Hospital Frances (Buenos Aires, RA); Sanatorio Allende (Córdoba, RA).

**Background:** Detection of the factors that determine discontinuation of immunomodulatory drugs (IMD) treatment in multiple sclerosis (MS) may help to reach a better compliance among MS patients. The average rates of adherence in controlled clinical trials can be high, but in clinical daily practice the discontinuation rate sometimes is unknown. This analysis describes treatment discontinuation in relapsing remitting patients over 10 years of follow up. **Objective:** To determine the factors of drop-outs and proportion of patients that discontinue treatment in a RRMS group of patients treated with IMD. **Methods:** The follow up group included data from 1534 MS patients, from 7 MS centers in Argentina. Only patients with RRMS who initiated IMD treatment were included: We consider the following reasons of stopping treatment during visits: lack of efficacy (increase of relapse rate, progression of disease, changes in MRI); side effects; and pregnancy; other reasons (patient decision; medical decision; lost of social security; lost to follow up; death). **Statistical methods:** Epi info 2000. **Results:** The cohort included 784 patients with definitive RRMS treated with one of the four available IMD: intramuscular interferon beta 1a (AV); subcutaneous interferon beta 1 a (R22/R44); subcutaneous interferon beta 1b (BF) and glatiramer acetate (GA). **Observation period:** January 1996 to April 2006. 77.5% of patients (n=607) remained on the first treatment; and 22.5% of patients (n=177) discontinued any treatment (AV=38 p; R22=33 p; R44=31 p; BF=37 p; GA=38 p). Reasons for drop-out: Lack of efficacy: 44% (n=78); IFNs: 41% (n=73), GA: 3% (n=5). Side effects: 16% (n=29); IFNs: 1.6% (n=28), GA: 0.6% (n=1). Pregnancy: 3% (n=5); IFNs: 0.6% (n=1), GA: 2% (n=4). Other reasons: 36% (n=65); IFNs: 22% (n=40), GA: 14% (n=25).

**Conclusion:** Over 10 years of follow up 77.5% of treated patients in this cohort remain on the first IMD treatment demonstrating that adherence was relatively high. On the other hand, only 22.5% of drop-out patients stop treatment, in which lack of efficacy and side effects were the main reasons of discontinuation. Nevertheless, patients taking GA seem to have better compliance compared to IFNs.

**P324**

**Lipoatrophy in multiple sclerosis patients during interferon treatment**

C. Tilbery, I. Fernandes, M.F. Mendes, R. Thomaz; Santa Casa de Misericórdia de São Paulo (São Paulo, BR)

**Introduction:** Interferon beta (INF B) reduces disease activity in patients with Multiple Sclerosis. During the initial phase of treatment most patients experience influenza-like adverse effects and there are some rare reports of late reactions with the same medications, as lipoatrophy. **Objective and Conclusion:** Description of adverse effects in 308 MS patients with special reference of lipoatrophy in 4 of them. **Methods/Results:** We report headache in 90 patients (29%), generalize pain in 67 patients (21%), fever in 66 patients (21%), and others. In four
patients, females, 3 of then receiving interferon beta-1a, and 1 of then with interferon beta-1b, age between 20 to 35 years old, that developed lipatrophy after 1 year on subcutaneous injections. Conclusion: Lipatrophy has been described after intradermal and subcutaneous injection of numerous medications as cortisone, vaso-pressin, diphtheria-pertussis-tetanus vaccination and others, but rarely with interferon beta.

P325

BG00012, a novel oral fumarate, is effective in patients with relapsing-remitting multiple sclerosis

L. Koppus, D.H. Miller, D.G. MacManus, R. Gold, E. Havndorf, V. Limnroth, C.H. Polman, K. Schmieter, T.A. Youssy, M. Yang, M. Erksoy, E. Meluzinova, I. Rektor, G. O’Neill; University Hospital Basel (Basel, CH); Institute of Neurology (London, UK); University Clinic Göttingen (Göttingen, D); General Teaching Hospital (Prague, CZ); City Hospital of Cologne (Cologne, D); Vrije Universiteit Medical Centre (Amsterdam, NL); Biogen Idec Inc. (Cambridge, USA); University of Istanbul (Istanbul, TR); Faculty Hospital V Motole (Prague, CZ); Masaryk University (Brno, CZ)

Background: There is a need for more effective and more easily administered treatments in patients with multiple sclerosis (MS). Results of a phase 3 study showed that BG00012, a novel oral fumaric acid ester, is an effective treatment for chronic plaque psoriasis. Preliminary results from another study indicated that BG00012 may also be effective in patients with relapsing-remitting MS (RRMS). Here, we report the results of a randomised, double-blind, placebo-controlled, dose-ranging phase 2b study conducted to determine the efficacy of three dose levels of BG00012 on disease activity as reflected by the accumulation of new lesions on serial magnetic resonance imaging (MRI) in patients with RRMS.

Methods: Patients 18 to 55 years of age with a diagnosis of RRMS and an Expanded Disability Status Scale score between 0.0 and 5.0 were eligible. In addition, eligible patients were required to have either ≥1 relapse within 12 months prior to randomisation or ≥1 gadolinium-enhancing (Gd++) lesion on cranial MRI at the time of screening. During a 24-week treatment period patients received one of four treatments: BG00012 capsules 120 mg by mouth once daily (120 mg/day), 120 mg three times daily (tid) (360 mg/day), 240 mg tid (720 mg/day), or placebo. All patients then received BG00012 during a 24-week dose-blinded safety-extension period. The primary end point was the total number of Gd++ lesions, calculated as the sum of four MRI scans at Weeks 12, 16, 20, and 24. Additional MRI end points included the cumulative number of new Gd+ lesions from baseline to Week 24, the number of new and enlarging T2-hyperintense lesions at Week 24, and the number of new T1-hypointense lesions at Week 24. Relapses and disability progression were also assessed.

Results: A total of 257 patients were evenly randomised to the four treatment groups. Compared with placebo, treatment with BG00012 240 mg tid led to a 69% reduction in the total number of Gd+ lesions on scans acquired at Weeks 12 to 24 (4.5 ± 7.4 vs. 1.4 ± 3.8; p < 0.001), and a reduction in the number of new and enlarging T2-hyperintense (4.2 ± 5.4 vs. 2.2 ± 3.6; p = 0.001) and of new T1-hypointense lesions (1.7 ± 2.5 vs. 0.8 ± 2.0; p = 0.014). In addition, a 32% reduction in relapse rate was observed, however this effect was not significant. Conclusion: BG00012 significantly reduced MRI-detectable brain lesion activity in patients with RRMS over 24 weeks of treatment.

P326

Measurements of binding antibodies to interferon-beta

P.E.H. Jensen, F. Sellberg, P.S. Sørensen; Danish MS Research Center (Copenhagen, DK)

Treatment of MS-patients with interferon-beta (IFN-beta) may lead to the generation of IFN-beta binding antibodies (BAb). A fraction of the BAbS are neutralizing antibodies (NAbs) that inhibit IFN-beta from binding to its receptor causing significant decrease in treatment efficacy. Our goal was to develop a simple assay for BAb measurements that correlated with NAb measurements by use of capture ELISA. We have compared our BAb measurements to both measurements of NAbs with a cytopathic effects assay (CPE) in which we used a neutralizing capacity (NC) of 20% as cut-off limit for NAb positivity and with another BAb assay using protein-G affinity chromatography (AC) in which we defined BAb values above 16% as positive. In 36 negative control serum samples we obtained an average of 4 units ± 6 with range (0–22 units) in the BAb-ELISA. A minimum limit was set at 25 units for positive samples, based on three standard deviations. By screening of 247 samples of IFN-beta treated MS-patients we then found 73% of the samples to be negative, 14% in the range 25–100 units, and the rest were higher than 100 units, range (110–19.084). In the BAb-AC assay 56% of the samples were negative. All the BAb-AC negative samples were also negative for BAb-ELISA, except for one sample only. However, only two of 180 negative BAb-ELISA samples had positive NAb-values. Using 25 units as cut-off limit, we found 16% NAb-negative samples among the positive BAb-ELISA samples, suggesting that a higher cut-off limit for positivity could be preferable in the BAb-ELISA. Except for one, the samples with 25–100 BAb-ELISA units contained positive BAb-AC values. The NAb measures in these samples were negative with NC below 20% in all samples except 3 that were low positive with NC between 20 and 80%. Using a cut-off level of 100 units instead of 25 units in the BAb-ELISA gave only a minor increase from 1% to 2% positive NAb-values among the BAb-ELISA negative samples. In all, 12% of the samples were positive in BAb-ELISA, of which 7% turned out to be NAb-negative. Using logarithmic transformation, we found a better correlation between NAB NC and positive BAb-ELISA samples (r = 0.79) than with positive BAb-AC samples (r = 0.57). The correlation between the positive samples in BAb-AC and in BAb-ELISA was low (r = 0.53), demonstrating differences in the results of BAb analysis with the two methods. The results suggest that the ELISA provide a better correlation between BAbS and NAbs than the AC-method.

Clinical assessment tools

P327

The WHOQOL-BREF: a pivotal tool in the quest for suitably assessing the quality of life of persons with multiple sclerosis in continuing care

K. Turpin, S. Warren, D. Mike, K. Warren, J. Christopherson, T. Rust; University of Alberta (Edmonton, CAN); The Capital Care Group (Edmonton, CAN); MS Society of Canada (Edmonton, CAN)

Background: Difficulties inherent in studying a highly disabled group, including inapplicability of questions and floor/ceiling effects encountered, have made studying the quality of life (QOL) of MS patients in continuing care futile. The purpose of this study was to determine the ability of a relatively new satisfaction based QOL instrument, The World Health Organization QOL – Brief (WHOQOL-BREF), to counteract these difficulties. Methods: The WHOQOL-BREF was administered to thirty-nine MS continuing care residents, along with the MS-Related Symptoms Scale (MSRS), Hospital Anxiety and Depression Scale (HAD), and Emotional/Social Loneliness Inventory (ESLI), to evaluate the construct validity of the WHOQOL-BREF. Additionally, two general QOL items were taken from the MSQOL-54 survey: 1) rating of overall QOL and 2) rating of how one feels about their life as a whole. Results: The tool provides four summary QOL scores, ranging from 4 (worst QOL) to 20 (best QOL). The average domain scores ranged from 13.6 (physical) to 14.3 (social), with no floor or ceiling effects, and no missing data. Acceptable Cronbach alphas, demonstrating good

Downloaded from msj.sagepub.com by Shula Edelkind on October 1, 2010
internal consistency, were obtained for the physical (0.74), psychological (0.69), and environmental (0.76) domains, but not the social (0.57). We tested the following hypotheses (alpha = 0.10): the physical and environmental QOL domains would correlate with the MRS, HADS, and MSQOL-54 scores; the psychological with the MRS, HADS, ESLI, and the MSQOL-54 scores; and the social with the HADS, ESLI, and MSQOL-54 scores. The majority of our hypotheses were supported, except the psychological did not correlate with the MRS, and the environmental did not correlate with the HADS depression score. In addition, the only hypothesis supported in regards to the social domain was the correlation with the rating of how one feels about their life as whole. **Conclusions:** The WHOQOL-BREF appears to have potential for suitably evaluating the QOL of highly disabled MS patients. However, the Cronbach alphas are lower than what has been reported for this questionnaire in an international field trial. Also, the lack of complete agreement with all of our validity hypotheses suggests that perhaps the WHOQOL-BREF might better fit the MS population in general, and/or the disabled continuing care population (e.g.; MS, spinal cord injured), with some modifications, as has been done with other populations (e.g.; WHOQOL-OLD, WHOQOL-HIV).

**P328**

**Does the patient know best? Significant change in the multiple sclerosis impact scale (MSIS-29 physical) over four years**

L. Costello, K. O’Rourke, H. Kearney, C. McGuigan, L. Giblin, M. Duggan, L. Daly, N. Tubridy, M. Hutchinson; St. Vincent’s University Hospital (Dublin, IRL)

**Background:** The MSIS-29 is patient report measure responsive to change in short-term studies. The performance of the MSIS-29 physical over years has not been assessed nor has the minimally important change for the MSIS-29 been established. **Aims:** The aims of this study were 1) to examine the reliability and responsiveness to change of the MSIS-29 physical over four years in patients with EDSS scores 0–8.5 and 2) to quantify minimally important change in the MSIS-29 physical using the EDSS as an anchor measure. **Methods:** 214 MS patients, disability range EDSS 0–8.5, were followed for four years. Concurrent MSIS-29 physical and EDSS scores were collected prospectively. The stability of the MSIS-29 was assessed in 116 patients with unchanged EDSS. Responsiveness was assessed in 98 patients with EDSS change and effect sizes (ES) were calculated. ROC curves were used to compare the change in MSIS-29 physical scores in patients with and without EDSS worsening. **Results:** Stability. The stability of the MSIS-29 physical was better for lower grades of disability than higher. In 85 patients with EDSS 0 to 5.0 the mean MSIS-29 changed only by 0.1 points whereas in 31 patients with an EDSS in the range 5.5 to 8.0, the mean MSIS-29 physical fell by 8 points. This may represent a response shift phenomenon. **Responsiveness:** In the 44 patients with a baseline EDSS of 5.5 or more there were 76 changes in their EDSS. The correlation between change in EDSS and change in MSIS-29 was moderate (r = 0.56, p < 0.0001). In the 44 patients with a baseline EDSS of 0–3.0 there were 96 changes in EDSS scores. The correlation between change in EDSS and change in MSIS-29 was only mild (r = 0.33, p = 0.0002). For one point EDSS change the ES was 0.93, for two point EDSS change the ES was 1.4. **Minimally Important Change:** For EDSS 5.5–8.5, the ROC area under the curve was 0.85 (p < 0.0001); a changed MSIS-29 physical score of 8 had a sensitivity of 87% and a specificity of 67%. For EDSS 0–5.0 the ROC area under the curve was 0.73 (p < 0.0001); a changed MSIS-29 physical score of 14 had a sensitivity of 88% and a specificity of 36%. **Conclusions:** The MSIS-29 physical is a valuable patient report measure which should be used in phase III RCTs. Minimally important change scores have been determined which differ between patients with mild EDSS scores (impaired) and those with higher EDSS mobility disability. A response shift phenomenon was observed in stable patients with significant disability.

**P329**

**The responsiveness of the Multiple Sclerosis Impact Scale (MSIS-29) in relation to the Multiple Sclerosis Functional Composite (MSFC) over time**

L. Costello, K. O’Rourke, N. Tubridy, M. Hutchinson; St. Vincent’s University Hospital (Dublin, IRL)

**Background:** Patient’s assessment of their own level of impairment is important in chronic diseases such as MS. The responsiveness over time between the patient-rated MSIS-29 and the MSFC has not previously been investigated. **Aims:** To examine correlations between the MSIS-29 and the MSFC at a single time-point and longitudinally. To examine the responsiveness of the MSIS-29 physical in patients with change in MSFC over time. **Methods:** 204 MS patients with a disability range EDSS 0–8.0 were examined at baseline and 150 were examined 3 years later. The patient completed the MSIS-29 and the doctor scored the MSFC and EDSS. Change in MSFC Z score defined at 0.5, 0.32, and 0.15 SD from the baseline mean was calculated for 150 patients. Mean change in MSIS-29 physical was examined in patients who had improved and worsened for each level of change using unpaired Student T testing. Correlations between the scales at baseline and follow-up were assessed using Spearman’s rank correlation. **Results:** At baseline in 204 patients the MSIS-29 physical correlated moderately with the total MSFC score (r = −0.52), 25FTW (r = −0.48), and 9HPT (r = −0.54) but weakly with the PASAT (r = −0.22). At follow-up in 150 patients the MSIS-29 physical correlated moderately with the total MSFC (r = −0.58), the 25FTW (r = −0.71) and 9HPT (r = −0.61) but weakly with the PASAT (r = −0.26). No significant correlations were observed between the MSFC and the MSIS-29 psychological at either baseline or follow up. When MSFC change was defined at 0.32SD, 29 patients worsened and 12 improved. The MSIS-29 physical was responsive to change at this level (p = 0.05). At MSFC change of 0.15SD, 47 patients worsened and 29 improved. Again the mean change in MSIS-29 physical score was significantly different between those who had improved and worsened at this level of change (p = 0.03). When MSFC change was defined at 0.5SD, 19 patients worsened and 5 improved. At this level of MSFC change there was no difference in mean change in MSIS-29 physical between those who had improved or worsened (p = 0.39). **Conclusions:** The MSIS-29 physical correlates with the MSFC and its components at baseline and at follow-up. The MSIS-29 physical is responsive to change in MSFC when significant change is defined as 0.32 and 0.15 standard deviations from the baseline mean. MSFC change of 0.5SD is too stringent a definition in a heterogeneous population. The MSIS-29 is a valid and responsive instrument that is suitable for use in trials.

**P330**

**Longitudinal proxy measurements in multiple sclerosis: agreement between patients and their partners on the impact of MS on daily life over a period of two years**

F.A.H. van der Linden, J.J. Kragt, J.C. Hobart, M.A. van Bon, M. Klein, A.J. Thompson, H.M. van der Plouw, B.M.J. Uitdehaag, C.H. Polman; VU University Medical Centre (Amsterdam, NL); Neurological Outcome Measures Unit, Institute of Neurology (London, UK)

**Background:** The use of self-report measurements is increasing in clinical settings. This method of data collection might be problematic in patients with limitations that interfere with reliable self-assessment like cognitive impairment or serious mood disturbances, as may be the case in multiple sclerosis (MS). In these situations proxies (e.g. partners, relatives or close friends) may be considered, instead of the patient, to provide valuable information that otherwise would be lost. In order to use this information, one needs to be certain that proxies and patients give consistent ratings and that these rating remain stable over time. **Objective:** To examine patient-proxy agreement on the impact of MS on daily life at different points in time and to assess agreement between patients and proxies on possible changes in impact of MS. **Methods:** A group of 56 MS patients and their partners
completed the Multiple Sclerosis Impact Scale (MSIS-29) at baseline and follow-up, two years later. Patient and proxy agreement at both time points was assessed by calculating intraclass correlation coefficients (ICCs), exact and global agreement and the directional differences between groups to examine possible systematic bias. Agreement of change over time was assessed by calculating ICCs between changes scores. In parallel, global ratings of both patients and proxies of the extent to which the patient had improved or deteriorated over the past two years were collected to validate possible changes on the MSIS-29. Results: Preliminary analyses showed that, at both time points, agreement on the physical scale was higher than agreement on the psychological scale (ICCs at baseline were 0.81 for the physical scale and 0.72 for the psychological scale). At follow-up, the ICC values were 0.88 and 0.69 respectively. There was a tendency for proxies to report more physical and psychological problems than patients at follow-up compared to baseline. Conclusion: These preliminary results suggest that proxies might be useful sources for information. Stability of agreement over time needs to be further investigated.

P331

The Multiple Sclerosis Impact Scale (MSIS-29) is a reliable, valid and acceptable measure of quality of life: a population-based study

O.M. Gray, G.V. McDonnell, S.A. Hawkins; Royal Victoria Hospital (Belfast, UK)

Objective: To evaluate the psychometric properties of the MSIS-29 in a population-based study. Methods: A prevalence study included all MS cases residing in the north-east region of Northern Ireland with probable or definite MS as per the Poser criteria. Cases were assessed, with history, clinical examination and Kurtzke EDSS. Cases then completed the MSIS-29 questionnaire. The psychometric properties of data quality, scaling assumptions, acceptability, reliability and validity were assessed for both the physical and psychological scores of the MSIS-29. Results: Two hundred and forty-eight cases were included in this study – 166 females and 82 males (F: M 2:1). The mean age on entry to the study was 49.1 years (SD 12.4, range 22 - 87), with a mean time from onset of symptoms of 16.3 years (SD 12.1 range 0 – 64). Kurtzke EDSS ranged from 0 to 9.5 with a median of 6.0 (IQR 2.0 – 6.5). Data quality was excellent with responses to 99.8% of questions and computable scores for all individuals. Both physical and psychological impact scores spanned the entire scale range, with a mean physical impact score of 48.8 (SD 28.6) and a mean psychological impact score of 40.93 (SD 27.9). Floor and ceiling effects were small. Cronbach’s alpha was high for both the physical and psychological impact scores at 0.97 and 0.93 respectively, confirming the internal consistency of both scales. The convergent validity of the physical impact score of the MSIS-29 with the Kurtzke EDSS was confirmed with a high Spearman’s rank coefficient correlation of 0.63 (sig. at the 0.01 level). There was no such relationship between Kurtzke EDSS and psychological impact score (Spearman’s rank coefficient correlation of 0.12), confirming divergent validity. Conclusions: The MSIS-29 questionnaire is an acceptable, reliable and valid method of measuring quality of life in MS cases in the community.

P332

The multiple sclerosis impact scale is sensitive to factors predictive of increasing severity of multiple sclerosis

R. Nicholas, A. Vora, J.C. Hobart, O. Malik, R. Reynolds; West London Neurosciences Centre (London, UK); UK Multiple Sclerosis Tissue Bank (London, UK); Peninsula Medical School (Plymouth, UK)

Introduction: Quantifying disability in Multiple Sclerosis (MS) and factors that may influence disability is a key issue both economically and in measuring the potential benefits of treatment. The MS Impact Scale (MSIS-29), consisting of a motor and psychological sub-score, was developed to quantify the functional impairment and disability caused by MS. Method and Results: The MSIS-29 was sent to 1841 subjects registered with the UK Multiple Sclerosis Tissue Bank. Using analysis of covariance to model the MSIS-29 motor sub-score in the 973 subjects who responded (R squared 0.504) we found factors predicting a higher MSIS-29 motor score were: a higher MSIS-29 psychological sub-score; more severe disease as classified by the subjects themselves; wheelchair use (p <0.0005 for all of the above factors); increasing age at diagnosis (p = 0.004); and increasing length of disease (p <0.0005). We found each extra year of disease increased the MSIS-29 motor score by an average of 0.117 points and each extra year of age at diagnosis increased the MSIS-29 motor by an average of 0.162 points. The increase in the MSIS-29 motor score as a result of each extra year of disease was more evident in those who were non-wheelchair users (on average 0.227 extra points per year, p <0.012). Discussion: The MSIS-29 demonstrates sensitivity to previously identified factors predicting more severe MS. Furthermore, this study has identified and quantified an interaction between psychological and motor disability. This supports the utility of the MSIS-29 as a disability scale available at low cost that does not require expert examination making it an ideal tool for use in primary care.

P333

The timed tandem walk is a more informative measure of mild impairment in multiple sclerosis than the 25-foot walk

J. Herbert, K. Jakubowska-Sadowska, H.K. Russell, L.E. Kasten, N. Layer, A. Glueck, M. Frankel, J.T. Fromm, M. Del Bene; NVU Hospital for Joint Diseases (New York, USA); PROMETRIKA (Cambridge, USA)

Background: Simple, quantitative measures of impairment are essential for multiple sclerosis (MS) management and clinical trials. The 25-foot walk (T25W), a standard mobility measure for MS, is most informative in Expanded Disability Status Scale (EDSS) range 4.5 to 7.5 (overt gait impairment). However, the T25W is an unstructured measure and allows patients to compensate for ataxia. We therefore hypothesized that a stressed gait test would be more informative than T25W in mildly impaired patients (no overt gait limitation; EDSS range 0 to 4.0). Objective: To compare and correlate a stressed gait test (the 10-foot Timed Tandem Walk [TTW10]) with the unstressed T25W in EDSS range 0 – 5.0. Method: We measured the fastest time to tandem walk 10 feet along a straight line (TTW10), fastest unassisted T25W, and EDSS in 249 MS patients and 83 controls. TTW10 and T25W were calculated as the means of two consecutive measurements. Results: In normal controls, TTW10 correlated with gender whereas T25W correlated with age. For both normal controls and MS patients, TTW10 correlated moderately with T25W, and both TTW10 and T25W correlated with EDSS scores (p <0.0001 for all); however, correlation between EDSS and TTW10 (r = 0.49) was stronger than the correlation between EDSS and T25W (r = 0.36). TTW10 correlated significantly with cerebellar, pyramidal, sensory, cerebral and bowel/bladder Kurtzke functional systems, whereas T25W correlated only with cerebellar, pyramidal and sensory systems. Correlation of T25W with pyramidal and cerebellar functional systems was significantly weaker than the correlation seen with TTW10 (p <0.03 for both). MS patients were grouped into the following categories: EDSS 0 (n = 33), EDSS 1 – 1.5 (n = 62), EDSS 2 – 2.5 (n = 77), EDSS 3 – 3.5 (n = 47), EDSS 4 – 4.5 (n = 21). For all categories except EDSS 0, deviation from normal controls was greater for TTW10 than T25W. Thus, TTW10 appears to be a more informative measure of mild impairment than T25W. Seven of nine patients with EDSS 5.0 could not perform the T25W, establishing EDSS 4.5 as the upper limit of utility for TTW10. Conclusion: TTW10 is a simple, quantitative bedside measure of mild impairment in MS. TTW10 appears to be a more informative measure of impaired mobility than T25W in MS patients in the EDSS range 0 – 4.5. TTW10 should be investigated as a clinical measure for monitoring MS progression.
P334

Utilisation of multiple sclerosis functional composite in multiple sclerosis: 5-year follow-up
S. Ozakbas, E. Idiman; Dokuz Eylul University (Izmir, TR)

The Multiple Sclerosis Functional Composite (MSFC) includes quantitative tests of leg function (Timed 25-Foot Walk — [T25FW]), arm function (9-Hole Peg Test — [9-HPT]), and cognitive function (Paced Auditory Serial Additional Test — [PASAT-3 min version]). Previous observations suggest that the MSFC might be more sensitive to change in clinical aspects than the Expanded Disability Status Scale (EDSS). The aims of the present study were: 1. To investigate the difference in MSFC between MS patients who were treated with an immunomodulator and who were not, in a 5 year follow-up period, 2. to show the difference between patients with RRMS and secondary progressive MS (SPMS), in the same period and 3. to test the usefulness of MSFC instead of EDSS in years. 684 (541 female) patients with definite MS were enrolled in the present study. Patients were diagnosed having relapsing-remitting (RR) MS (n = 481), secondary progressive (n = 181, 28.4%), or primary progressive (n = 22, 3.3%) course. 79 patients who had at least two relapses in the last two years have received any of the immunomodulator agent, and followed-up 5 years. The MSFC and EDSS were performed at baseline and every year for 5 years to assess disability. Patients were divided into three subgroups: 1. RRMS patients who did not receive disease modifying therapy (DMT) — non-DMT group, 2. RRMS patients who received DMT — DMT group, 3. SPMS patients who did not receive DMT — SPMS group. The mean age was 39.2 ± 14.38 (18–53), mean disease duration was 28.45 ± 16.13. Multiple Sclerosis Quality of Life -54 (MSQoL-54) was also performed for all groups. EDSS was recorded 2.01 at baseline, 3.42 at the end of the second year, and 4.02 at the end of the fifth year for non-DMT group. For DMT group they were 2.49, 2.60 and 3.72, respectively. MSFC values were 1.47, 1.06, 0.98 for non-DMT group, and 1.33, 1.19 and 1.13 for DMT group respectively. Correlations between EDSS and MSQoL-54 were weak at baseline and at the end of fifth year (r = 0.19, 0.22 respectively). Correlation between MSFC and MSQoL-54 was very strong at baseline and at the end of the fifth year (r = 0.59 and even stronger at the end of the fifth year) (r = 0.69). Our study obtained the longest period data to conclude that the MSFC assesses aspects of neurological function not measured by the EDSS, suggesting that it is more sensitive to detect change over time and better able to demonstrate a therapeutic effect.

P335

The Faces-Symbol test and Symbol-Digit test are not reliable surrogates for the Paced Auditory Serial Addition Test in multiple sclerosis
K. O'Rourke, J. Williams, M. Hutchinson, N. Tubridy; St. Vincent's University Hospital (Dublin, IRL)

Objective: The paced auditory serial addition test (PASAT) is the established task for cognitive assessment in MS but is unpopular with patients. The faces-symbol test (FST) and symbol-digit tests (SDT) are alternative methods of cognitive testing in MS that are more acceptable; the purpose of this study was to evaluate the potential of the FST and SDT as surrogates for the PASAT. Methods: 50 unselected patients with MS (median EDSS 2.0; range 0–6.5) performed the three tests of cognitive function under standardised conditions. The raw PASAT, SDT, and FST data were transformed to Z-scores; Z-scores were compared using linear regression models. Agreement patterns were investigated using Bland-Altman graphs which plot the difference between a pair of test Z-scores against the average of the same pair of test Z-scores for each patient. Results: 1) Linear regression Z-FST-90 predicting Z-PASAT: R² 34.5%, 95% CI for slope 0.33, 0.81 (p < 0.0001), 95% CI for intercept −0.24, 0.22 (p = 0.90). Z-SDT-90 predicting Z-PASAT: R² 51.5%, 95% CI for slope 0.53, 0.95 (p < 0.0001), 95% CI for intercept −0.17, 0.23 (p = 0.76). Z-SDT-90 predicting Z-PASAT: R² 65.4%, 95% CI for slope 0.64, 0.99 (p < 0.0001), 95% CI for intercept −0.15, 0.20 (p = 0.76). The estimate of the intercept in each model is consistent with a zero intercept indicative of an unbiased assessment.

P336

Comparison of Kurtzke’s Expanded Disability Status Scale and the Multiple Sclerosis Functional Composite Index in a multiple sclerosis outpatient clinic
C. Michel, A. Dresse; Université Greifswald (Greifswald, D)

Background and Aim of the study: The Multiple Sclerosis Functional composite Index (MSFC) was developed to address shortcomings of the well established Kurtzke’s Expanded Disability Status Scale (EDSS) to allow assessment of disability in MS patients as an outcome parameter in clinical trials. The MSFC is a three dimensional quantitative composite score including an timed 25 foot walk, a nine hole peg test (9HPT) and the Paced Auditory Serial Addition Test (PASAT). Results are calculated as a z score, allowing quantitative comparison of the data. The test has been applied in several large phase III clinical trials, however, little information is available whether the MSFC can be useful in a clinical routine setting. The present study compared the MSFC to the EDSS in a MS outpatient clinic. Methods: In our MS clinic the MSFC is routinely applied to all MS patients by a trained nurse. We retrospectively analysed a three year period in which 714 paired MSFC and EDSS from 118 patients (age 17–70 yrs, median 43 yrs) were obtained. The correlation between the EDSS score and the MSFC and its subtests were calculated using the Spearman correlation. Results: The median EDSS score was 2.5 (range 0.0 to 7.5), the median MSFC (z score) was 0.1171 range (−6.949 to 1.422), the correlation r = −0.6250, p < 0.0001. The correlation for the 25 foot walk with the EDSS was best (r = −0.7144 p < 0.0001) followed by the 9HPT (r = −0.6446, p < 0.0001) while the PASAT had the weakest correlation (r = −0.3241 p = 0.0001). Conclusion: When used in a clinical routine setting the results of the MSFC and its subtests correlate with the EDSS. The correlation coefficients were similar to those reported in the initial validation study by Cutter et al. This suggests that the MSFC can be reliably applied in a MS clinic. However, to date, no cut offs have been established to identify clinical significant changes of the MSFC score in individual patients. Therefore, a more detailed analysis of changes that define relapses or clinical disease progression is warranted.

P337

Evaluation of the Expanded Disability Status score during post-relapse follow-up of multiple sclerosis patients treated with Avonex®; SENSE study
P. Vermersch, T. De Broucker, P. Hinau, C. Bazetquet; University of Lille II (Lille, F); Service de Neurologie (Saint Denis, F); Cours Raphael Binet (Rennes, F); Biogen Idec (Nanterre, F)

Background: Several authors have underlined the risk of classification errors when the EDSS is measured too early after Multiple Sclerosis 2006; 12: 51–5228 www.sagepub.co.uk

Downloaded from msj.sagepub.com by Shula Edelkind on October 1, 2010
Sclerosis (MS) relapses or when different thresholds for changes in disability are adopted, wrongly conducting to the conclusion of therapeutic failure. The goal of the SENSE study is to better assess these disability variations by distinguishing transient changes from those that persist for up to 12 months after a relapse. 

Methods: This is a French multicenter, prospective, longitudinal, observational study of patients treated with Avonex® in the course of their ongoing clinical management. Primary objective: Evaluate the 9% of patients experiencing disability improvement as defined as at least 1 point decrease in the Expanded Disability Status Score (EDSS) compared to EDSS reference value collected 1–2 months following the onset of the relapse. To be confirmed, this improvement will have to be observed at 2 visits apart. Secondary objectives: Evaluate the correlation between disability improvement and several parameters: time since diagnosis, relapse rate in the 2 previous years, clinical symptomatology, use of corticoid therapy for the current relapse, reference EDSS score and treatment initiation date. The mean relapse to disability improvement will also be determined. Evaluations: Four evaluations may be fulfilled, according to the physician's discretion, between 1 and 11 months, after the relapse. Inclusion criteria: patients already treated with Avonex presenting with a recent relapse (between 1 and 2 months). Sample size: the % of patients whose EDSS improved within the 12 month-period after a worsening episode with sustained disability is close to 30% (Based on Rio J, publication, Ann Neurol 2002; 52: 400–406). We assumed that the rate of improvement over 12 months after a relapse will be similar to this figure. For a measurement precision of 4.5%, i.e. CI = [25.5–34.5%] and alpha = 5%, it will be necessary to evaluate 399 patients. In order to take into account patients with missing data, 520 patients will be recruited. Results: 260 French neurologists have currently recruited 499 patients. Baseline data of these patients will be presented.

P338

The EDSS software program for handheld devices: a study of reliability and validity

C. Markowitz, D. Mikol, L. Shi, D. Denney on behalf of the EDSS Calculator Study Group

Background: The Expanded Disability Status Scale (EDSS) measuring multiple sclerosis disability is derived from seven Functional System scores determined through standard neurological examination and tabulated manually. Objectives: To test the reliability of the EDSS scores obtained through the EDSS software program for handheld devices (EDSS Calculator) versus those obtained through the manual method; to evaluate test-retest reliability of the calculator-based scores; and to examine their validity through correlation with the Ambulation Index. Design/Methods: A sample of 62 MS patients from six study centers had their EDSS evaluated two times during a single office visit, using separate raters for each of the tabulation methods: EDSS Calculator and manual method. Method order was randomly assigned. Patients returned for a second office visit 7–10 days later when their EDSS was re-evaluated with the Calculator by the same rater who had used the EDSS Calculator at visit 1. The Ambulation Index was also measured at this visit. Results: The mean EDSS of the sample was 3.5±2.2 when obtained with the EDSS Calculator and 3.4±1.95 when obtained by the manual method; scores ranged from 0 to 8 with both methods. There was a high degree of inter-rater/method reliability between the EDSS scores obtained with the EDSS Calculator and those obtained through the manual method (Kappa: 0.84; CI: 0.74 to 0.94); differences between the scores were far more likely the result of differences in symptom presentation during the two evaluations than differences in the methods for tabulating the scores. A high degree of test-retest reliability was observed with the EDSS Calculator (Kappa: 0.93; CI: 0.86 to 0.996). The correlation between the EDSS Calculator scores and the Ambulation Index was 0.73 (p < 0.001). Conclusions: Relative to the manual method, the EDSS Calculator is highly reliable and provides valid results when measuring disability in MS patients.

P339

Short-term prognosis in relapsing-remitting multiple sclerosis


Objective: Prognosis of Multiple Sclerosis (MS) is highly variable. Several prognostic factors based on demographic, clinical and magnetic resonance imaging (MRI) data have been described. Moreover, we have recently demonstrated that intrathecal IgM synthesis (ITMS) against myelin lipids predicts an unfavourable disease course in MS. The aim of this study was to analyze the impact of these factors independently or in combination on short-term prognosis in MS. Methods: We prospectively studied 78 patients with relapsing-remitting MS according to the Poser's criteria since the initial stages of the disease. Patients were followed during 56.99 ± 2.85 months. Seven prognostic factors were recorded: age at onset (≥ 40 years of age versus < 40 years of age); gender; symptoms at onset (motor versus non-motor); MRI findings at onset; presence of ITMS against myelin lipids; interval between the first 2 attacks (< 2.5 years versus > 2.5 years); and attack frequency in the first 2 years (> 2 versus < 2). Disability was measured by the Expanded Disability Status Scale (EDSS). Disease evolution was evaluated by monitoring final EDSS scores and changes in EDSS during follow-up. Results: Final EDSS score was not influenced by gender, motor symptoms at onset, burden of lesions on initial MRI, interval between the first and second attack and number of relapses during the first 2 years. It was significantly higher in patients with older age at onset and with ITMS against myelin lipids. When the progression index (defined as EDSS score increase per year) was evaluated, we only found it related with the presence of ITMS against myelin lipids (p = 0.0063) and with suffering a second attack before 2.5 years (p = 0.025). We observed a strong correlation between these two variables. Although no significant, we found a trend between age at onset and the progression index (p = 0.07). We used Kaplan-Meier analyses and Cox regression models to determine the influence of these variables on the time to disability progression. Median times to increase one point in EDSS score were exclusively influenced by the presence of ITMS against myelin lipids (p = 0.0002). Conclusions: ITMS against myelin lipids predicts an aggressive disease evolution from the first stages of MS. This study also shows a worse outcome in patients with an older age at onset. Conversely, we did not find an association between the burden of T2 lesions on initial MRI and disability progression in the early phase of the disease.

P340

Serum uric acid levels in multiple sclerosis patients correlate with disability

A.L. Guerrero, E. Laherré, F. Gutiérrez, I. Martin-Polo, A. Garrachaga, M. Rodríguez-Gallego, C. Alcázar, J. Peralta; Hospital Rio Carrion (Palencia, E)

Introduction: Uric acid (UA), the end product of purine metabolism in humans, is an endogenous antioxidant, acting as a natural peroxynitrite scavenger. Peroxynitrite, the product of the free radicals nitric oxide and superoxide, exerts a toxic effect on neurons, axons, and glia cells and contributes to demyelination, oligodendrocyte destruction, and axonal damage in Multiple Sclerosis (MS). Since in 1998 it was found that gout patients rarely, if ever, develop MS, some studies have described that MS patients have lower serum UA levels than controls, although it has not been established whether UA is primarily deficient or secondarily reduced due to its scavenging activity. UA has also been proposed as a indicator of
Abstracts

disease activity, as some authors have shown that UA levels are lower during clinical or magnetic resonance imaging (MBI) activity than in remission. Up to date, previous studies have only found a trend towards an inverse correlation of serum UA concentration with disability. Patients and Methods: We retrospectively reviewed 478 serum UA levels obtained in 94 MS patients followed in our Neurology Unit. 90 samples were collected during a relapse, before steroids treatment beginning. We collected data concerning demographic and clinical variables. We evaluated the Expanded Disability Status Scale (EDSS) when outside a relapse. Correlation between UA levels obtained during a relapse, or a relapse free period and comparison between UA and EDSS score was tested using a t test. A p value less than 0.05 was considered statistically significant. Results: UA levels were significantly lower when measured during a relapse (n: 90) than in a remission period (n: 388) (t: −0.16, p: 0.003). UA levels measured outside a relapse, inversely correlated with EDSS score (r: −0.015, p: 0.001). Conclusion: Our results indicate that lower uric acid levels in Multiple Sclerosis patients are associated with clinical relapse; serum UA might serve as a possible marker of disease activity in MS. According to our knowledge, this is the first description of an inverse correlation of serum UA levels with disability as assessed by EDSS score.

P341
OSE: A simple tool for the evaluation and follow-up of patients with relapsing-remitting multiple sclerosis.
Results on 183 patients
D. Vernay, G. Edan, T. Moreau, J.M. Visy, C. Gury on behalf of the OSE study group

The heterogeneity in the progression and symptomatology of the relapsing-remitting type of multiple sclerosis fueled the development of clinical scales for its evaluation and follow-up. Among them, the UKNDS (United Kingdom Neurometric Disability Scale – ex GNDS, Guy’s Neurological Disability Scale) is simple and well accepted, and was first translated and introduced in France by Gilles Edan (CHU of Rennes). We further simplified the UKNDS into a version named OSE (Outil Simple d’Evaluation). OSE is based on: (i) 13 sets of pictograms for the rapid and systematic evaluation of symptoms, therapeutic and evolution aspects and (ii) a corporal scheme, allowing disability localization. In March 2004, we started a well-designed, multicentric study in France (69 participating neurologists), aimed to evaluate OSE in patients with the relapsing-remitting type of multiple sclerosis. Here we present the results obtained in 183 patients (44 males, 139 females), aged 41.3 ± 9.6 years. Multiple sclerosis was diagnosed 7.0 ± 6.1 years before the inclusion. 98 patients were treated with Interferon and 81 with Copaxone. At V1, residual EDSS was 2.6 ± 1.75. Patients were evaluated with OSE during two visits (V1 and V2) after the inclusion. The mean period between V1 and V2 was 4.5 ± 2.6 months. Index of satisfaction, as evaluated by the physician and the patient, were scored on a scale from 1 to 5 (none, modest, important, very important, full satisfaction). At V2, satisfaction index was 2.25 ± 0.78, as evaluated by the physician and 3.0 ± 1.11, as evaluated by the patient. Important factors influencing the satisfaction index were: previous use of OSE and hospital practice for the physician and age, duration of the disease and physician’s help for the patient. Similar results were obtained at V1. In conclusion, the present results show a good acceptance by both, clinicians and patients, and suggest that OSE is simple and rapid to fulfill.

P342
Impact of fatigue in multiple sclerosis: the Fatigue Impact Scale for Daily Use
J. Benito-León, J.E. Méndez-Martín, B. Frades, C. de Andrés, M.L. Martínez-Ginés, I.E. Meca-Lallana, A.R. Antigué, B. Hueté-Antón, E. Rodríguez-García, J. Ruiz-Martínez; Móstoles General Hospital (Madrid, E); National Center for Epidemiology (Madrid, E); University Hospital “Gregorio Marañón” (Madrid, E); University Hospital “Virgen de la Arrixaca” (Murcia, E); Basurto Hospital (Bilbao, E); Hospital “Severo Ochoa” (Madrid, E); Mendiko Hospital (Guipúzcoa, E)

Objective: To determine the metric properties of the daily fatigue impact scale (D-FIS) in multiple sclerosis (MS) patients. The D-FIS is an 8 item-instrument designed to measure the subjective daily experience of fatigue. Methods: 124 consecutive patients with operationally defined MS underwent the D-FIS. Usual clinical measures for MS, the Montgomery-Asberg Depression Rating Scale and the Functional Assessment of Multiple Sclerosis (FAMS) to assess health-related quality of life (HRQoL) were also applied. In addition, patients with fatigue (68 patients, 54.8%) completed the Fatigue Descriptive Scale, the Multidimensional Fatigue Inventory (MFI), a visual analogue scale for fatigue (VAS-F) and a Global Perception of Fatigue scale (GPF). Results: Relevant metric D-FIS results were: floor effect = 1.54%; ceiling effect = 1.54%; skewness = −0.28; item homogeneity = 0.58; Cronbach’s alpha = 0.89; item-total correlation = 0.62 (item 1) - 0.84 (item 6); standard error of measurement = 2.19; convergent validity with other fatigue measures = −0.57 [VAS-F] (p < 0.001); 0.28 [GPF] (p < 0.001); 0.46 [MFI-General fatigue] (p < 0.001). In a multiple linear regression model, fatigue independently influenced HRQoL. Conclusions: The D-FIS is a valid, short, and easy-to-administer instrument for measuring MS-related fatigue, a frequent symptom, which impairs patients’ HRQoL.

P343
Derivation of a unidimensional scale from the Fatigue Impact Scale
L.C. Doward, D.M. Meads, J.D. Fisk, S.P. McKenna, B.J. Eckert; Galen Research (Manchester, UK); Dalhousie University (Halifax, CAN); Novartis Pharmaceuticals Corp. (New Jersey, UK)

Objectives: The Fatigue Impact Scale (FIS) is a 40-item questionnaire designed to assess fatigue. The FIS has 3 subscales; cognitive, physical and psychosocial functioning. The purpose of the study was to develop a unidimensional scale from the FIS with improved responsiveness that would be particularly suitable for assessing fatigue in multiple sclerosis (MS). Methods: Rasch analysis (one-parameter logistic item response theory) was applied to existing FIS data available from 188 patients with MS. Misfitting items and those with differential item functioning (DIF) related to gender or age were removed. Qualitative interviews conducted with MS patients in the UK identified potential gaps in instrument coverage. Face and content validity were assessed via cognitive debriefing interviews with a new MS patient sample in the UK. Results: MS-patient samples: FIS data sample: n = 188, 25% male; mean age 50.9, SD 10.5; 39.6% relapsing remitting, 36.9% primary progressive, 23.5% secondary progressive. Qualitative interview sample: n = 35, 43% male; mean age 50.0, SD 13.2; mean MS-duration 17.7 years, SD 14.2; 31.4% relapsing-remitting, 28.6% secondary-progressive, 5.7 primary-progressive, 34.3% other or missing. Cognitive debriefing sample: n = 15, 33% male; mean age 48.4, SD 14.5; mean MS-duration 14.5, SD 14.0 years; 53.3% relapsing-remitting 33.3% primary-progressive, 13.3% secondary-progressive. The 40-item FIS did not fit the Rasch model. Five misfitting items and 4 exhibiting DIF by age or gender were removed to produce a unidimensional scale that fitted the Rasch model (Chi² p < 0.005). Data from qualitative interviews were used to generate 9 new items to improve sensitivity with patients with milder fatigue. The recall period was reduced from 4 to 1 week. Cognitive debriefing interviews revealed problems with only 3 items; 1 was removed and 2 modified. Patients found the new 39-item scale clear and relevant. Conclusions: A single unidimensional scale of fatigue was produced from the FIS that was acceptable to UK MS patients and with good coverage of fatigue severity. The scale is currently being adapted for use in France, Germany, Italy, Spain, Sweden, USA and Canada (French and English). Psychometric and scaling properties for all language versions will be assessed.

Multiple Sclerosis 2006; 12: 51 – 5228 www.sagepub.co.uk
The development of a work instability scale for multiple sclerosis

E. McFadden, H.L. Ford, A. Tennant; University of Leeds (Leeds, UK)

Background: The capacity to work is often altered in those with chronic illness such as multiple sclerosis. More than 50% of persons with multiple sclerosis (MS) are unemployed within 10 years from diagnosis unrelated to level of disability. Currently the “Gold Standard” way to identify those at risk of work disability is for an experienced therapist to carry out a Vocational Assessment.

Objectives: To devise a valid, reliable Work Instability Scale for multiple sclerosis (MS WIS) in order to identify those at risk of premature work loss. Methods: The approach comprised a number of discrete stages and combined the theoretical and developmental strengths of established methodologies in qualitative research with the statistical strength of the Rasch model. The Rasch models are built on three fundamental concepts: Unidimensionality, order and additivity. They ensure that the items in a scale map onto a single underlying construct, for example, work instability, that there is a hierarchical structure to these items such that they each represent different levels of risk of work loss and that the items are equally spaced along the construct so providing an interval level scale.

Study design: 1. Qualitative interviews to generate the item pool for the patient completed Multiple Sclerosis Work Instability Scale (MS-WIS). 2. Item selection to produce a draft questionnaire. 3. Cognitive debriefing to assess the face and content validity of the draft MS WIS. 4. Internal Construct Validity (Rasch analysis) to test the properties of and reduce the number of items in the scale. 5. Criterion Validity to compare the scale against the “Gold Standard” assessment to assess the reliability and sensitivity of the draft measurement. 6. Reliability (Rasch analysis) to assess the test-reliability and internal consistency of the scale confirming the validity of the questionnaire. Results: Qualitative analysis of the data obtained from the interviews highlighted the main factors contributing to work instability and identified TRUE/ NOT TRUE statements for the scale. Rasch analysis on the answers provided by the study population yielded a twenty one item Work Instability Scale. Conclusions: The MS WIS is brief, easy to complete and interpret in the hospital setting and provides the clinician with a validated tool to identify those at risk of unemployment due to MS and refer appropriately for job retention measures.

P345

A final United Kingdom scale for measurement of self efficacy in multiple sclerosis

R.J. Mills, J.A. Woolmore, C.P. Hawkins, C.A. Young; Walton Centre for Neurology and Neurosurgery (Liverpool, UK); University Hospital of North Staffordshire (Stoke on Trent, UK)

Background: Self efficacy (SE) is the individual’s belief that they have the ability to overcome challenges presented to them. Two self efficacy in MS.

Methods: The approach comprised a number of discrete stages and combined the theoretical and developmental strengths of established methodologies in qualitative research with the statistical strength of the Rasch model. The Rasch models are built on three fundamental concepts: Unidimensionality, order and additivity. They ensure that the items in a scale map onto a single underlying construct, for example, work instability, that there is a hierarchical structure to these items such that they each represent different levels of risk of work loss and that the items are equally spaced along the construct so providing an interval level scale.

Study design: 1. Qualitative interviews to generate the item pool for the patient completed Multiple Sclerosis Work Instability Scale (MS-WIS). 2. Item selection to produce a draft questionnaire. 3. Cognitive debriefing to assess the face and content validity of the draft MS WIS. 4. Internal Construct Validity (Rasch analysis) to test the properties of and reduce the number of items in the scale. 5. Criterion Validity to compare the scale against the “Gold Standard” assessment to assess the reliability and sensitivity of the draft measurement. 6. Reliability (Rasch analysis) to assess the test-reliability and internal consistency of the scale confirming the validity of the questionnaire. Results: Qualitative analysis of the data obtained from the interviews highlighted the main factors contributing to work instability and identified TRUE/ NOT TRUE statements for the scale. Rasch analysis on the answers provided by the study population yielded a twenty one item Work Instability Scale. Conclusions: The MS WIS is brief, easy to complete and interpret in the hospital setting and provides the clinician with a validated tool to identify those at risk of unemployment due to MS and refer appropriately for job retention measures.

P345

P344

The development of a work instability scale for multiple sclerosis

E. McFadden, H.L. Ford, A. Tennant; University of Leeds (Leeds, UK)

Background: The capacity to work is often altered in those with chronic illness such as multiple sclerosis. More than 50% of persons with multiple sclerosis (MS) are unemployed within 10 years from diagnosis unrelated to level of disability. Currently the “Gold Standard” way to identify those at risk of work disability is for an experienced therapist to carry out a Vocational Assessment.

Objectives: To devise a valid, reliable Work Instability Scale for multiple sclerosis (MS WIS) in order to identify those at risk of premature work loss. Methods: The approach comprised a number of discrete stages and combined the theoretical and developmental strengths of established methodologies in qualitative research with the statistical strength of the Rasch model. The Rasch models are built on three fundamental concepts: Unidimensionality, order and additivity. They ensure that the items in a scale map onto a single underlying construct, for example, work instability, that there is a hierarchical structure to these items such that they each represent different levels of risk of work loss and that the items are equally spaced along the construct so providing an interval level scale.

Study design: 1. Qualitative interviews to generate the item pool for the patient completed Multiple Sclerosis Work Instability Scale (MS-WIS). 2. Item selection to produce a draft questionnaire. 3. Cognitive debriefing to assess the face and content validity of the draft MS WIS. 4. Internal Construct Validity (Rasch analysis) to test the properties of and reduce the number of items in the scale. 5. Criterion Validity to compare the scale against the “Gold Standard” assessment to assess the reliability and sensitivity of the draft measurement. 6. Reliability (Rasch analysis) to assess the test-reliability and internal consistency of the scale confirming the validity of the questionnaire. Results: Qualitative analysis of the data obtained from the interviews highlighted the main factors contributing to work instability and identified TRUE/ NOT TRUE statements for the scale. Rasch analysis on the answers provided by the study population yielded a twenty one item Work Instability Scale. Conclusions: The MS WIS is brief, easy to complete and interpret in the hospital setting and provides the clinician with a validated tool to identify those at risk of unemployment due to MS and refer appropriately for job retention measures.

P346

Medium-term longitudinal follow-up in kinematic analysis surveyed patients

P. Nisipeanu, R. Carasso, A. Dagan; Hadera Medical Center (Hadera, IL)

Background: Kinematic analysis (KA) of hands movements was shown to be sensitive for the quantitative evaluation of movement. We previously reported that KA was able to detect subtle deterioration of arm function in mild-moderate relapsing-remitting and secondary progressive multiple sclerosis (RRMS, SPMS) patients who did not significantly worsen on other measures and who were followed for a period of 18 months. Objective: To verify if this finding will be confirmed by clinical evolution. Methods: 14 patients (10 women), who participated in the first study were entered in a 18-month follow-up, being examined at 3-month interval. In addition to neurological examination at each session Expanded Disability Status Scale (EDSS), Functional System Scales (FS), Timed 25-Foot Walk (T25) and Nine-Hole Peg Test (9-HPT) were assessed. Results: All patients completed at least 5 3-month evaluations. Patients whose KA of hands showed deterioration at the end of the previous 18 month study were significantly worse at the end of the actual study, especially at the FS and 9-HPT measures. Conclusions: KA of hands may be useful in the medium term prognostic of clinical changes less well assessed by EDSS.

P347

Computerised analysis of cerebellar symptoms in multiple sclerosis - “TremorAn”, a new bedside test

I. Koehler, G. Schäfer, M. Dietrich, A. Fuldung; Johannes Gutenberg-Universität (Mainz, D)

Objectives: The aim of this study was to develop a new bedside test for computerized quantitative analysis of cerebellar symptoms of the upper extremity in patients with MS. The test battery consists out of 3 different drawing exercises starting with “drawing the line” over “zick-zack-course” to “star” characterized by an increasing difficulty and one target exercise “target-target” to proof the accuracy of movements. Methods: Based on a tablet pc (Compaq Tablet PC TC1000) a individual software was developed to analyse quantitatively following parameters: 1) Curve under the line, 2) number of maxima, 3) number of intercept points, 4) overshoot distance, 5) number of interruptions and 6) needed time of the drawing tests and 7) sum of distance from target and 8) length of distortion of the target test. Normative data were evaluated by testing 50 healthy subjects (20 women, mean age 25 years). Sensitivity of these data were proved in 8 patients with secondary progressive MS, EDSS 4.5 – 7.5 but clinical different severity of cerebellar symptoms of the upper extremities (2 patients without, 3 with mild, 2 with moderate and 1 with severe signs and symptoms). In addition the nine-hole peg-test was documented and the results were compared to the new bedside test. For statistical analysis based on SPSS 11.0 special curve analysis was used with respect to the highest stability index to calculate the normative data of the single tests. Results: All healthy subjects could perform the test battery accurate without any problems. Based on the normative data with this new bedside test movement

www.sagepub.co.uk

Multiple Sclerosis 2006; 12: 51 – 2228

Abstracts 591
Abstracts

P348
Assessing blinding methodology in multiple sclerosis clinical trials
A. Scalfari; Oxford University (Oxford, UK)

Empirical studies show that the poor quality of trials may distort results. The internal validity of a study might be threatened by bias which could occur at any stage of the trial, producing results that differ from true values. The blinding methodology is widely used to reduce as much as possible such bias, and it has therefore become a principle of randomised controlled trials. In order to determine the quality of a trial many assessment scale have been created, but none of these scales consider specifically the problem of blinding. Considering the extreme importance of blinding, we have focused on the need to have a reliable tool in order to assess the quality of blinding methodology in MS clinical trials. We have created a specific checklist which will allow us to evaluate all the situations in which participants should be kept blinded. The checklist focuses on several different aspects: blinding of treating and assessing neurologist, blinding of patients, blinding of neuroradiologists, blinding of statisticians and blinding of randomization (treatment allocation concealment). Using the checklist readers will be able to assess whether all the efforts in order to make blinding successful were done or not. The checklist is based on a score system in order to rate the trials analyzed in terms of “quality of blinding methodology”. A score is awarded to each item when the item is applicable. The total score is then divided by the total possible score, and non applicable item scores are not counted in the denominator. Finally an overall blinding quality index of each trial is obtained. Each applicable item will be scored and confirmed by the written explanation taken from the paper. For each item readers will score a full point when clear explanations of blinding procedures have been provided, a half point when unclear (insufficient) explanations have been provided and no point when no explanations have been given. Since each single item has not got the same relevance we decided to consider them differently. Thus blinding of assessing neurologist will be scored as 4, blinding of patient as 3, blinding of neuroradiologist as 2 and all the other items as 1. Therefore, when all the items are applicable, a maximum score of 12/12 can be obtained. In order to assess the reliability of the check list, two different reviewers have tested it independently on 5 MS clinical trials. The results have been compared.

P349
Comparison of retinal nerve fibre layer measurements and visual function among patients with multiple sclerosis
F. Costello, E. Eggengerber, C. Demeulemeester, S. Coupland, H. MacLean, H. Rubinovitch, M. Freedman, I. Pan, W. Hodge; The University of Ottawa (Ottawa, CAN); Michigan State University (East Lansing, USA); OHRI (Ottawa, CAN)

Background: Retinal optic neuritis (ON) is a common cause of vision loss among patients with Multiple Sclerosis (MS). MS patients have a predilection for sub-clinical involvement of the afferent visual pathway, and commonly manifest visible defects of the retinal nerve fiber layer (RNFL). Optical coherence tomography (OCT) measured RNFL values are reduced among MS patients, and correlate with diminished visual performance. The objectives of this study were to compare RNFL values among patients with progressive MS, relapsing remitting MS (RRMS), ON as a clinically isolated syndrome (CIS), recurrent ON and normal control subjects. We also aimed to compare tests of visual function with RNFL measurements among MS and CIS patients to determine whether axon loss in the afferent visual pathway is the substrate for persistent visual impairment.

Methods: A cross sectional study was conducted on MS and CIS patients seen at the University of Ottawa. Patients underwent standard ophthalmic evaluations, including best-corrected Snellen visual acuity and visual field analysis (Humphrey perimeter 30–2 Threshold testing.). OCT (Stratus version 3, Zeiss Meditec, Dublin, California) testing was used to determine the average and quadrant sector RNFL values for MS, CIS and normal eyes. RNFL values were compared to tests of visual function among patients. Results: 148 patients underwent OCT and ophthalmic testing (71 RRMS; 54 CIS; 9 progressive MS and 14 recurrent ON). The mean overall and quadrant RNFL values were reduced in all MS sub-types (p < 0.0001) as compared to disease free control patients. Patients with prior ON (recurrent and as a CIS) and progressive MS had significantly lower RNFL values and reduced visual function scores (log MAR visual acuity/visual field sensitivity) as compared to control subjects.

Conclusions: In this study, MS and CIS patients demonstrated reduced RNFL values as compared to disease control patients. Patients with recurrent ON; prior ON as a CIS; and progressive MS had significantly lower RNFL values and reduced scores on tests of visual function as compared to normal control patients and RRMS patients. Reduced RNFL values correlated with diminished visual function in all subsets of MS and CIS patients. OCT testing detected sub-clinical optic nerve damage among patients, and RNFL values correlated with tests of visual function. OCT may be a reliable biomarker of axonal integrity in the afferent visual pathway.

P350
Cognitive demand affects functional mobility in multiple sclerosis
S. Khurana, M. Cline, A. DiGiacomo, J.D. Bowen, R. Wadhani, G. Kraft; University of Washington (Seattle, USA)

Background: Multiple sclerosis (MS) is an unpredictable and disabling condition that has been associated with decreased mobility, balance problems, and cognitive dysfunction. It has been speculated that persons with MS also have more difficulty multi-tasking compared to age-matched adults. The objective of this study is to analyze the affects of cognitive distractions on ambulation in people with multiple sclerosis (MS). Research Design: A prospective case-control study conducted at The University of Washington, Seattle, Washington. Methods: Ninety ambulatory (EDSS ≤ 6.0) community-dwelling adults (50 with MS, 40 age- and sex-matched controls) are being enrolled. Two ambulation measures are being used: the Timed Up and Go Test (TUG) and a 100-foot walking test. These measures are performed under three conditions: no cognitive task; a simple cognitive task: reciting the alphabet; a more difficult cognitive task: counting backwards by three. In addition, subjects will count backwards by three while seated and complete the Paced Auditory Serial Addition Test. Results: In MS subjects (n = 28) and controls (n = 10) studied to date, both simple and complex cognitive tasks adversely affect motor performance. MS subjects showed trends towards greater differences in speed between the TUG alone and the TUG with a cognitive task. The control group was 5.1% slower when performing the TUG with a cognitive task; MS subjects were 15.7% slower. Similar results were seen in the 100-foot walking tests while performing the easier cognitive task. Conclusions: This study is relevant to multiple sclerosis as it addresses the interplay between two areas that are commonly affected by MS: cognition and motor function. The results allow us to better understand the impact of distraction on common motor functions. By better understanding the conditions under which multi-tasking occur, the treating physician will be able to employ rehabilitation strategies more effectively, thus minimizing morbidity in this population.
Multidimensional assessment of upper limb impairment in patients with multiple sclerosis: a multicentre study

L. Padua, V. Nociti, S. Bartalini, F. Patti, A. Quattrone, P.A. Tonali, M. Ulivelli, P. Valentino, M. Zappa, A.P. Batocchi; Catholic University, Fondazione Don Gnocchi (Rome, I); University of Siena (Siena, I); University of Catania (Catania, I); Magna Grecia University (Catanzaro, I)

Background: Although there is increasing interest in measuring the quality of life (QoL) and disability in Multiple Sclerosis (MS) patients, no studies were focused on the upper limb impairment in a multiperspective way. The aim of this multicentric study is to multidimensionally evaluate the impairment of upper limb in MS patients using validated patient-oriented, clinical and disability measurements.

Methods: We enrolled 80 patients (22 men, 58 women; mean age 37.6 years; 68 with relapsing-remitting MS, 12 with secondary-progressive MS) fulfilling McDonald diagnostic criteria. To evaluate the upper limb impairment was used a patient-oriented questionnaire as the disability arm shoulder hand questionnaire (DASH) and an objective test of dexterity as the 9 Hole Peg Test (9HPT). DASH provides 2 scores: on symptoms and functional status (DASH-S/F) and on the use of upper limb at work (DASH-W). We also used SF-36. Statistical analysis was performed. Non-parametric analysis of the correlation was assessed by Spearman’s R test. Multivariate analysis was also used. Results: We observed high significant relationships between DASH scores and 9HPT (p = 0.0005, r = 0.4 and p = 0.01, r = 0.3 respectively). Fatigue was related to the timing of the upper limb task (p = 0.0007, r = 0.4) but even more to the patient’s perspective of upper limb impairment (DASH-S/F p ≤ 0.0000001, r = 0.7; DASH-W p ≤ 0.000000, r = 0.5). Some aspects of QoL (SF36 scores) are influenced by the upper limb task duration: 9HPT was highly related with physical function (p ≤ 0.0000001, r = −0.5), mildly with the social function (p = 0.04, r = −0.2) and highly related with the main physical score (p < 0.0008, r = −0.4).

The duration of the disease was mildly related to the upper limb performance as perceived by the patient: it was related to the symptoms/function (DASH-F/S p = 0.03, r = 0.2) but not to the performance at work (DASH-W: NS). To evaluate which are the clinical features that influence the upper limb performance we performed a preliminary multivariate regression analysis: the objective measurement of upper limb impairment (9HPT) was highly related only to the EDSS while the perception of upper limb function (DASH-F/S) was highly related only to the fatigue. Conclusions: By these preliminary data we can assess that upper limb impairment strongly involves daily life in MS patients and that many clinical features seem to influence the manual task, probably we should consider it as an outcome measure in clinical trial.

Immunomodulation - Part I

P353

Long-term therapy with IV-immunoglobulins in multiple sclerosis: Evaluation of a prospective documentation of 668 patients

E. Maida; Evangelic Hospital (Vienna, I)

Multiple sclerosis (MS) can neither be cured nor stopped completely, but by immunomodulating therapies the occurrence of severe incapacies can be delayed in the majority of patients, especially, when introducing the treatment during the first years after the onset of the disease. The immunomodulating properties of i.v.-immunoglobulins (ivIG) refer to both T-helper- and B-cells and and they serve for treatment in several autoimmune diseases. Trials in MS already have been performed since more than 20 years. Although from many studies encouraging results had been obtained, the use of i.v.-immunoglobulins in MS is still controversial. The actual presentation is based on data obtained from an open prospective documentation, which started in 1993, of relapse rates, EDSS-scores and MRI observations of relapsing-remitting MS-patients, who had received 200 mg/kg body weight of 75-immunoglobulines every 21-28 days for at least 2 years (earlier withdrawals included). 668 patients were evaluated. In 10% therapy was stopped earlier (mostly because of problems with veins and allergy). Before starting ivIG the mean duration of MS was 7.6 years, the mean relapse rate was 2.8 per 24 or less months, the mean EDSS was 3.5. The mean duration of ivIG-treatment until the actual evaluation was 35 months.

Results: 51% of the patients remained relapse-free, a mean reduction of relapse rates of −2.2 was observed, the mean EDSS-score showed a reduction of −0.3. No further progression and/or Gd+ activity was seen in the majority of MRI scans of both brain and cervical spine. An additional evaluation of subgroups of the patients showed a positive correlation of ivIG effects on relapse rates, EDSS-scores and stability in MRI respectively and the duration of MS at the time of starting the treatment. Conclusion: The evaluation of a large number of patients points out the high effectiveness on reducing the progression of MS both clinically and in MRI by ivIG-therapy. Furthermore it was excellently tolerated. The rather low dosage, which was used for the present documentation showed similar good effects like studies with higher dosages of ivIG, beta-interferons and glatiramer-acetate, but was more economic. The actual data revealed the high therapeutic value of ivIG in relapsing-remitting MS.
Predictors of intravenous immunoglobulins effectiveness in multiple sclerosis
G.N. Bisaga; Military Medical Academy (St.Petersburg, RUS)

Introduction: Among immunomodulators for multiple sclerosis (MS) treatment intravenous immunoglobulins (IVIg) are marked out not only low frequency of side effects and ability to reinforce remyelination in CNS, but also high clinical efficiency, which depends on a number of factors, which we had analyzed. Methods: 45 clinically defined MS patients (F/M = 29/16, aged 4 – 54 (35.4 ± 11.2) years, EDSS 1.5 – 9.5 (4.4 ± 2.2)) were studied. RRMS 26 pts, SDMS 13, PPM-S and RPPM-S. The duration of IVIgS treatment was 3 – 72 (11.1/– / 10.8) months. The patients received IVIg enriched lgM (Pentaglobin “Bioteo”; n = 24) and IVIg, on 99% consisting from IgG (Intraglobin “Bioteo”; n = 21). Dosages 0.4 g/kg within 3 days and then monthly 0.15 – 0.20 g/kg within 3 – 72 months. A positive clinical effect was fixed in case of reduction of relapse number at least on 50% and/or decrease of EDSS on 0.5 or more points. Results: Side effects (only slight and intermittent) were seen in 11% of the patients. The positive effect of IVIgS treatment is established in 54% of cases: at RRMS in 74%, SPMS-39% (p < 0.05), PPM-S0%, RRMS-0%. Among the patients with RRMS with a positive effect at 59% was revealed strong positive effect, at 41% – moderate; among the patients with SPMS only moderate positive effect (100%) was revealed. The relapse rate before treatment was 1.4 ± 1.2 per year, during treatment – 0.64 ± 1.0, during a half a year after treatment – 0.65 ± 0.95. Average reduction of EDSS during IVIgS treatment was 0.41 ± 0.40. Higher clinical efficiency of IVIg enriched lgM is established in comparison with containing only IgG: 60% and 48% accordingly. The efficiency of IVIgS did not depend on age of the patients and duration of disease. The weak positive correlations were revealed between efficiency of treatment and relapse rate before treatment (r = 0.30; p < 0.05), moderate-between effect from treatment and its duration (r = 0.53; p < 0.01) and weak negative correlations established between result from treatment and initial EDSS (r = −0.31; p < 0.05).

Concentrations of cytokines and adhesion molecules in commercial immunoglobulin preparations: relevance for the treatment effect in MS patients
K. Retzlaff, C. Roth-Langer, R. Reuß, S. Vogel, C.V. Burger, M. Kaps, P. Oschmann; University Giessen (Giessen, D)

Introduction: Many studies have shown that intravenous immuno- globulin preparations (IVIg) are effective in the treatment of relapsing remitting multiple sclerosis (RRMS). Up to know the definite mode of action is still unknown. A wide spectrum of action is observed not only low frequency of side effects and ability to reinforce remyelination in CNS, but also high clinical efficiency, which depends on a number of factors, which we had analyzed. Methods: 45 clinically defined MS patients (F/M = 29/16, aged 4 – 54 (35.4 ± 11.2) years, EDSS 1.5 – 9.5 (4.4 ± 2.2)) were studied. RRMS 26 pts, SDMS 13, PPM-S and RPPM-S. The duration of IVIgS treatment was 3 – 72 (11.1/– / 10.8) months. The patients received IVIg enriched lgM (Pentaglobin “Bioteo”; n = 24) and IVIg, on 99% consisting from IgG (Intraglobin “Bioteo”; n = 21). Dosages 0.4 g/kg within 3 days and then monthly 0.15 – 0.20 g/kg within 3 – 72 months. A positive clinical effect was fixed in case of reduction of relapse number at least on 50% and/or decrease of EDSS on 0.5 or more points. Results: Side effects (only slight and intermittent) were seen in 11% of the patients. The positive effect of IVIgS treatment is established in 54% of cases: at RRMS in 74%, SPMS-39% (p < 0.05), PPM-S0%, RRMS-0%. Among the patients with RRMS with a positive effect at 59% was revealed strong positive effect, at 41% – moderate; among the patients with SPMS only moderate positive effect (100%) was revealed. The relapse rate before treatment was 1.4 ± 1.2 per year, during treatment – 0.64 ± 1.0, during a half a year after treatment – 0.65 ± 0.95. Average reduction of EDSS during IVIgS treatment was 0.41 ± 0.40. Higher clinical efficiency of IVIg enriched lgM is established in comparison with containing only IgG: 60% and 48% accordingly. The efficiency of IVIgS did not depend on age of the patients and duration of disease. The weak positive correlations were revealed between efficiency of treatment and relapse rate before treatment (r = 0.30; p < 0.05), moderate-between effect from treatment and its duration (r = 0.53; p < 0.01) and weak negative correlations established between result from treatment and initial EDSS (r = −0.31; p < 0.05).

Concentrations of cytokines and adhesion molecules in commercial immunoglobulin preparations: relevance for the treatment effect in MS patients
K. Retzlaff, C. Roth-Langer, R. Reuß, S. Vogel, C.V. Burger, M. Kaps, P. Oschmann; University Giessen (Giessen, D)

Introduction: Many studies have shown that intravenous immuno- globulin preparations (IVIg) are effective in the treatment of relapsing remitting multiple sclerosis (RRMS). Up to know the definite mode of action is still unknown. A wide spectrum of action is observed not only low frequency of side effects and ability to reinforce remyelination in CNS, but also high clinical efficiency, which depends on a number of factors, which we had analyzed. Methods: 45 clinically defined MS patients (F/M = 29/16, aged 4 – 54 (35.4 ± 11.2) years, EDSS 1.5 – 9.5 (4.4 ± 2.2)) were studied. RRMS 26 pts, SDMS 13, PPM-S and RPPM-S. The duration of IVIgS treatment was 3 – 72 (11.1/– / 10.8) months. The patients received IVIg enriched lgM (Pentaglobin “Bioteo”; n = 24) and IVIg, on 99% consisting from IgG (Intraglobin “Bioteo”; n = 21). Dosages 0.4 g/kg within 3 days and then monthly 0.15 – 0.20 g/kg within 3 – 72 months. A positive clinical effect was fixed in case of reduction of relapse number at least on 50% and/or decrease of EDSS on 0.5 or more points. Results: Side effects (only slight and intermittent) were seen in 11% of the patients. The positive effect of IVIgS treatment is established in 54% of cases: at RRMS in 74%, SPMS-39% (p < 0.05), PPM-S0%, RRMS-0%. Among the patients with RRMS with a positive effect at 59% was revealed strong positive effect, at 41% – moderate; among the patients with SPMS only moderate positive effect (100%) was revealed. The relapse rate before treatment was 1.4 ± 1.2 per year, during treatment – 0.64 ± 1.0, during a half a year after treatment – 0.65 ± 0.95. Average reduction of EDSS during IVIgS treatment was 0.41 ± 0.40. Higher clinical efficiency of IVIg enriched lgM is established in comparison with containing only IgG: 60% and 48% accordingly. The efficiency of IVIgS did not depend on age of the patients and duration of disease. The weak positive correlations were revealed between efficiency of treatment and relapse rate before treatment (r = 0.30; p < 0.05), moderate-between effect from treatment and its duration (r = 0.53; p < 0.01) and weak negative correlations established between result from treatment and initial EDSS (r = −0.31; p < 0.05).

Concentrations of cytokines and adhesion molecules in commercial immunoglobulin preparations: relevance for the treatment effect in MS patients
K. Retzlaff, C. Roth-Langer, R. Reuß, S. Vogel, C.V. Burger, M. Kaps, P. Oschmann; University Giessen (Giessen, D)

Introduction: Many studies have shown that intravenous immuno- globulin preparations (IVIg) are effective in the treatment of relapsing remitting multiple sclerosis (RRMS). Up to know the definite mode of action is still unknown. A wide spectrum of action is observed not only low frequency of side effects and ability to reinforce remyelination in CNS, but also high clinical efficiency, which depends on a number of factors, which we had analyzed. Methods: 45 clinically defined MS patients (F/M = 29/16, aged 4 – 54 (35.4 ± 11.2) years, EDSS 1.5 – 9.5 (4.4 ± 2.2)) were studied. RRMS 26 pts, SDMS 13, PPM-S and RPPM-S. The duration of IVIgS treatment was 3 – 72 (11.1/– / 10.8) months. The patients received IVIg enriched lgM (Pentaglobin “Bioteo”; n = 24) and IVIg, on 99% consisting from IgG (Intraglobin “Bioteo”; n = 21). Dosages 0.4 g/kg within 3 days and then monthly 0.15 – 0.20 g/kg within 3 – 72 months. A positive clinical effect was fixed in case of reduction of relapse number at least on 50% and/or decrease of EDSS on 0.5 or more points. Results: Side effects (only slight and intermittent) were seen in 11% of the patients. The positive effect of IVIgS treatment is established in 54% of cases: at RRMS in 74%, SPMS-39% (p < 0.05), PPM-S0%, RRMS-0%. Among the patients with RRMS with a positive effect at 59% was revealed strong positive effect, at 41% – moderate; among the patients with SPMS only moderate positive effect (100%) was revealed. The relapse rate before treatment was 1.4 ± 1.2 per year, during treatment – 0.64 ± 1.0, during a half a year after treatment – 0.65 ± 0.95. Average reduction of EDSS during IVIgS treatment was 0.41 ± 0.40. Higher clinical efficiency of IVIg enriched lgM is established in comparison with containing only IgG: 60% and 48% accordingly. The efficiency of IVIgS did not depend on age of the patients and duration of disease. The weak positive correlations were revealed between efficiency of treatment and relapse rate before treatment (r = 0.30; p < 0.05), moderate-between effect from treatment and its duration (r = 0.53; p < 0.01) and weak negative correlations established between result from treatment and initial EDSS (r = −0.31; p < 0.05).
Background: Immunomodulatory treatment (IMT) for MS has been evaluated in large, randomised, placebo controlled trials. All treatments produce a significant reduction in relapse rate (RR) compared to placebo and the relative reduction is similar between treatments. Although not enough information about switching IMT is available, movement from one IMT to another should be considered when lack of efficacy is observed. Objective: The efficacy on relapses and accumulated disability parameters, were compared by consideration between 2 groups: treatment 1 (before switch) vs. treatment 2 (after switch). On both groups the primary endpoints were relapse rate and annual relapse rate and the secondary endpoints were proportion of relapse-free patients, time to first relapse and mean EDSS change.

Methods: 109 patients with definitive RRMS treated with IMT were evaluated:. 30 patients were switched from low dose to high dose of interferon (INF) (IFN/INF); 52 from IFNs to glatiramer acetate (GA) (IFN/GA); 13 from IFNs to immunosuppression (IMS) (INF/IMS); 14 from GA to IFNs (GA/INF). Neurological examinations and Kurtzke EDSS scores were evaluated every 3 months. Follow up: 2 years

Statistical methods: Epi info 2000; differences between groups in continuous variables were assessed using non-parametric tests (Kruskall Wallis for multiple groups). Results: Pre-treatment data: age, gender, disease duration, number of relapses in the previous two years, annual relapse rate, and EDSS of both groups were evaluated. 2 years follow-up data: RR: IFN/INF: 0.44; RR IFN/INF: 0.66; RR GA/INF: 0.46; RR INF/IMS 0.46. Change in EDSS: +0.17 INF/GA; +1.36 GA/INF (p = 0.0035)

Conclusions: After 2 years of follow up, our results support that switching to GA from INF results in a significant improvement on relapse rate and accumulative disability compared to switching between low dose to high dose of INF, and from GA to IFN. Unfortunately there is not Class I data supporting the underlying assumption that switching therapy improves clinical outcome, nevertheless this could be evaluated in the setting of clinical practice of real-life situation.

P358

Treatment of relapsing neuromyelitis optica. A review


School of Medicine (Cienfuegos, CUB); School of Medicine (Santa Clara, CUB)

Introduction: There is still not a specific treatment for Relapsing-neuromyelitis optica (R-NMO). Objective: To review the present situation of R-NMO treatment. Methods: All studies with series of cases and clinical trials related to R-NMO. Results: Acute relapses: Although there is no scientifically proved treatment for acute relapses steroids are administered parenterally in the same way as multiple sclerosis relapses. Patients who do not respond to this therapy could benefit from plasmapheresis with a good response in 60% of cases in a series of cases and clinical trials related to R-NMO. Prevention of relapses: a) Interferon Beta-1b (IFNB-1b): A Japanese randomized clinical trial evaluated the efficacy and safety of two doses of IFNB-1b in Optic-Spinal MS (OS-MS)/R-NMO. The primary end point was the annual relapse rate. Patients received either 18 (50 μg) or 25 (250 μg) INF-1b subcutaneously for 2 years. The ratio of the annual relapse rate in the 250 μg group to that in 50 μg was 0.608 (−39.2%) (p = 0.09). The annual relapse rate with spinal cord lesions from disease onset to study entry was 0.571 (−46%) with a p = 0.07. The proportion of relapse-free patients was 28% (5/18) in the 50 μg and 27% (6/22) in the 250 μg group (p = 0.653). The dose-dependent reductions in relapse rate were observed in the OS-MS/NMO group with 39% in comparison with 25% in classical MS. This study raises questions about the presumption, which is unsupported by any clinical trial evidence, that IFN is ineffective in R-NMO. Comments on this study suggest a nonsignificant trend to reduction of relapse frequency, but no effect on proportion of relapse free patients. The discrepancy could be due to inadequate power, and the trend regarding the attack frequency outcome may mean that IFNB-1b is effective in OS-MS/NMO. b) Other therapies in series of cases of R-NMO an improvement was observed: Mitoxantrone (4–5 cases), intravenous immunoglobulin (2–2); Glatiramer acetate (1–1), Rituximab (6–8). c) Combined therapies with an improvement in 7 with Azathioprine/glycocorticoids and methotrexate/prednisone, in 8. Conclusions: IFNB-1b produced a nonsignificant trend to reduction of relapse frequency, but no effect on the proportion of relapse free patients. This discrepancy could be due to inadequate power, and the trend regarding the attack frequency outcome may mean that IFNB-1b is effective in OS-MS/NMO. Other isolated or combined drugs have shown an improvement in series of cases treated exclusively.

P359

Survey on the use of immunomodulating, complementary and alternative medicine amongst individuals with multiple sclerosis in the Netherlands

L.H. Visser, A van der Zande for the Dutch National MS foundation

Background: Disease modifying therapy (DMT) for patients with relapsing-remitting multiple sclerosis (MS) is available since 1996 in the Netherlands. Some patients with MS are on treatment at an early stage of the disease, while others with the same disease pattern are not on DMT. The reason for this difference is not known. Also switching of DMT seems to increase, but the prevalence and reasons for switching are not well-known. Furthermore, complementary and alternative medications are frequently used, but the prevalence and patterns of use is not known. Objectives: To get more insight:

1. in the use of medications in patients with MS by a large survey
2. in the way a choice is made to start or not to start with DMT
3. how many patients have switched from DMT and the reasons for this switch.
4. in the prevalence and patterns of use of complementary and alternative medicine amongst individuals with MS in the Netherlands.

Methods: With the help of TNS-NIPO, the Dutch custom research group, a questionnaire has been developed. The first questions are about age, gender, self-reported disease activity, MS duration and education level to examine the relationship between patient characteristics and the use of DMT. The inquiry deals with four categories: never been on DMT, still on the first DMT, switchers and patients who stopped with DMT. Questions will assess the reasons for being in one of the four categories. Lastly, the questionnaire will examine the prevalence, patterns of use and effect of complementary and alternative medicine. Surveys are mailed to the entire mailing list of the Dutch MS foundation, instituting of 5000 individuals with MS and via the website patients will be asked to participate in this survey. Results: The questionnaire is now finalised. The content of this survey and questionnaire will be presented. Already at this moment more than 1400 patients with MS have responded. Conclusions: This study will give insight in the prevalence and use of DMT, complementary and alternative medicine in a large group of MS patients in the Dutch population.

P360

Pregnancy and disease-modifying therapies in patients with relapsing-remitting multiple sclerosis

N.S. Oztekin, M.F. Oztekin; SB Diskapi Hospital (Ankara, TR)

Objective: Multiple Sclerosis (MS) is a common neurological disorder which affects young people especially women during their child bearing age. Previous studies have shown that MS has no apparent affect on birth outcomes compared to general population. But there is lack of information about the disease modifying therapies, especially interferons (IFN) and Copaxone during pregnancy, birth and breast feeding. Introduction: The aim of the study is to evaluate whether women with R-R MS receiving disease modifying therapies and get pregnant during therapy are more likely to have poor outcomes
Factors influencing adherence to immunomodulatory treatment in a sample of 324 Portuguese patients with multiple sclerosis

M. J. Sá, J. Guimarães, S. Castro, P. Carinha, J. Reis, M.E. Rio; Hospital S. João (Porto, P)

Introduction: Adherence of multiple sclerosis (MS) patients to immunomodulatory drugs (IMD) is a major concern in the medical practice. This chronic therapy benefits the disease course modestly, only if drugs are taken. In Portugal IMD are provided free to patients and prescription is restricted to MS neurologists working in public Hospitals. Despite the close management of MS patients it can be hard to assess the adherence to IMD, due to individual and social factors. We decided to address this issue in our MS Unit. Objective: Evaluation of adherence to IMD in MS patients during 2004.

Material and Methods: From the 324 MS patients (224 women; 100 men; median age: 40.5 yr) under IMD in 2004 (Avonex®-97; Betaseron®-93; Copaxone®-85; Rebif®-22; Rebif®-6) with complete anamnesis are included, to reduce biases as regression paper updates results to dec.2005.

Results: Age and gender data of patients from Groups A and B was similar and fit the normal. Non of the patients in both groups did not breast feed their children. Comment: Considering the therotical abortive potential of these drugs there were no spontaneous abortions and other pregnancy complications among both groups. There were no differences in terms of birth outcomes compared to normal population and these results are even better. Due to the small number of patients a realistic statistical evaluation was not possible. A longitudinal study with a larger population will be more informative to establish the effects of MS therapies on pregnancy and breast feeding.
Kruskal-Wallis and post hoc tests for relapse-rates (r-rates) and EDSS; Odds-Ratios for RM data; multiple regression for longitudinal data. 

**Results and Discussion:** 129 pts are included (31 M, 98 F; age at onset 11–50, m29.12), with a N of 1313 years (no therapy = 106, years before therapy = 682; IFN1b = 90, IFN1a 1/w = 154, IFN1a 3/w = 136, AZA = 96, COP = 28; other = 21). Before therapy r-rate is similar (m 0.69, SD 0.726), so we made a single control group. r-rate for IFN1b is 0.489 (SD 0.753), for IFN1a 1/w 0.610 (0.838); for IFN 1a 3/W 0.492 (0.788); for AZA 0.5 (0.799) for COP 0.643 (0.989); r-rate is lower vs. pre-therapy years (p = 0.0002); without post hoc differences among the drugs. The only important side effect is fever. IFN 1b and IFN 1a 3/w show significantly lower switching rate. All drugs reduce RM lesions. New lesions: IFN1b: OR = 0.20 (0.10–0.38); IFN1a 1/w: OR = 0.20 (0.12–0.34); IFN1a 3/w OR = 0.21 (0.12–0.37); AZA OR = 0.21 (0.09–0.47); COP OR = 0.25 (0.09–0.70). Enhanced lesions: IFN1b OR = 0.20 (0.09–0.46); IFN1a 1/w OR = 0.32 (0.18–0.57); IFN1a 3/w OR = 0.36 (0.20–0.65); AZA OR = 0.17 (0.06–0.51); COP OR = 0.31 (0.09–0.98). Comparing the drugs till the fifth year, r-rate decreases in the years before therapy (regression to mean, p = 0.000), and, with lower scores, with IFN 1b (p = 0.0043) IFN1a 3/w (p = 0.0040) and COP (p = 0.0017). EDSS increases before therapy (p = 0.015), in contrast with non significant decrease during treatment with any drug. 

**Conclusion:** Longitudinal follow-up is the main contribution of postmarketing studies on SM therapy, literature on which is rather poor, the most prolonged studies being extensions of the original trials. Our data can be comparable to others in literature, for “year-patient” method, though some limits, is simple, reliable, cheaper, and allows more powerful data with smaller casistics.

**P364**

**Long-term follow-up of immunomodulatory therapies: beta interferons and glatiramer acetate in early relapsing-remitting multiple sclerosis**

**J. Haas**; Jüdisches Krankenhaus (Berlin, D)

**Background:** Early and sustained treatment with immunomodulating drugs is recommended as first-line RRMS therapy, but longterm comparative data of available immunomodulatory agents are rare. The objective of this study is to evaluate the long-term efficacy and patient adherence of immunomodulatory therapies in daily clinical practice over a period of 6 years. 

**Design/Methods:** This 6 year follow up analysis is based on a prospectively organized clinical database (MUSIS) including patients being on immunomodulating therapies (IMTs) and clinically followed-up since a period of at least 6 years at the time of analysis. In order to compare the efficacy of and adherence to beta-interferons (INFbeta-1a im (Avonex), INFbeta-1b sc (Betaferon), INFbeta-1a 22µg sc (Rebbi)), and glatiramer acetate in patients with RRMS (EDSS ≤ 3.5), 285 patients starting IMT were included for statistical analysis, whereas only “completees” are looked at in this current study. The primary target parameter for efficacy was relapse rate, the secondary parameter was the EDSS score. A “Completer” analysis was done in order to compare the treatment groups over the course of time and in order to focus on comparability of adherence at 72 months of continuous therapy. 

**Results:** Concerning the relapse rate and the EDSS the three Beta interferons were not statistically significant different. Only the relapse rate between Copaxone and Avonex reaches a statistically significant difference after 72 months. The dropout rate within the entire study so far ranges from 75% for Avonex (60/80), 72% for Betaferon (56/77), 71% for Rebbi (22/35) to 48% for Copaxone (38/79). This difference was statistically significant. 

**Conclusions:** During 6 years of ongoing IMT, it can be assumed, that the number of patients remaining in the ongoing study is selected by treatment response. Therefore, the clinical parameters are well comparable among all of these responders in the long term. However, since switch or escalation of therapy is the main reason for dropping out, the safety and dropout rates over time are an excellent surrogate indicator for the efficacy of long-term IMTs. In the long-term side effect profiles play a minor role concerning discontinued therapy. Whereas in the current study >70% of the patients dropped out under IFNs, only 48% dropped out under Copaxone. This difference, together with a stable treatment response, is statistically significant and may be clinically meaningful.

**P365**

**Outcome of T1 hypodensities in patients with early forms of multiple sclerosis randomised to Betaseron® or Copaxone® and followed prospectively by monthly 3T MRI for up to 2 years: preliminary analysis of the BECOME study**

**D. Cadavid, M. Gómez-Choco, M. Alemany, L. Segal, S.D. Cook, J. Skurnick, L. Wolansky; UMDNJ-New Jersey Medical School (Newark, USA)**

**Introduction:** BECOME is the first rater- blinded, randomized, prospective study comparing the efficacy by MRI of standard doses of Betaseron (Interferon beta 1b) vs. Glatiramer acetate (Copaxone) in patients with relapsing forms of multiple sclerosis (MS). An important radiological outcome of treatment for MS is the burden of T1 hypointensities, referred to as black holes (BH). There is evidence suggesting that treatment with Copaxone decreases the evolution of new lesions to BH, but whether a similar effect occurs with Interferon Beta remains to be determined. Until the BECOME study there has never been a head-to-head comparison of these treatments. 

**Objectives:** One of the secondary outcome measures of the BECOME study is a comparison of the evolution of T1 hypointensities after randomization to either Betaseron or Copaxone in an intention to treat analysis. 

**Methods:** All MRI for the BECOME study were performed at a single 3T dedicated head unit at New Jersey Medical School. Patients were recruited from the MS Centers at New Jersey Medical School and Holy Name Hospital, both in New Jersey. For this preliminary analysis we compared the evolution of T1 hypointensities after randomization to either Betaseron or Copaxone in an intention to treat analysis. 

**Results:** For the percentage of new lesions evolving into BH, masked examiners analyze new enhancing lesions (NEL) on follow up T1W MRIs without fat suppression 6 and 12 months after onset to determine whether they persist as hypointense. Of 661 lesions that have been analyzed, 42 (6.4%) and 21 (3.2%) remain as BH after 6 and 12 months of follow up, respectively. The volume of lesions that evolved to BH was significantly larger than in the lesions that did not (mean volumes 510 and 84 mm³, respectively; p < 0.001 for both). Volumetric analysis of change in T1 hypointensity after 1 year is in progress. 

**Conclusions:** In this preliminary analysis less than 4% of NEL evolved into BH. Larger new lesions have a higher likelihood of becoming BH. The results of the volumetric analysis and the percentage of NEL evolving to BH after 6 and 12 months of randomization to either Betaseron or Copaxone will be presented at the meeting. Funding. Supported by an investigator-initiated grant from Bence/Shering AG to UMDNJ.

**P366**

**Optimisation of immunomodulating therapy with Copaxone® daily 20 mg sc in combination with a patient self-monitoring programme in the Netherlands (OPTIVIT): baseline results**

**P.I.H. Jongen, E.A.C.M. Sanders, L.H. Visser, C.P. Zwanikken for the OPTIVIT Study Group**

**Background:** Benefit of immunomodulating therapy in relapsing-remitting multiple sclerosis depends on the individual clinical effect as well as patient compliance. Compliance to Copaxone® (glatiramer acetate; GA) seems lower in patients who have been using immunomodulating therapy than in those who did not. It is well known that perception of self-control and self-efficacy are important factors determining the adherence to chronic treatment, therefore an online self-monitoring programme for patients starting GA therapy was set up in The Netherlands. 

**Objectives:** To evaluate the relapse rate (RR)
Abstracts

P367
Betaseron® vs. Copaxone® in multiple sclerosis with triple-dose gadolinium and 3-T MRI Endpoints (BECOME): efficacy of the optimised MRI protocol and announcement of primary study outcome

L.J. Wolansky, S.D. Cook, V. Shemyzn, J. Skarnick, W.C. Liu, Y. Vidgap, N. Bhagat, K. Talishe, D. Cadavid; New Jersey Medical School (Newark, USA)

Background/Purpose: The BECOME trial (Betaseron vs. Copaxone in MS with triple-dose gadolinium and 3-T MRI Endpoints) is the first head-to-head trial focusing on the MRI outcomes of subjects treated with these two medications. The primary outcome measure is for each patient the mean # of combined active lesions per scan (CAL) with the unit of statistical analysis being the patient. CAL is defined as # of enhancing lesions plus # of new T2/FLAIR (new-T2) lesions unassociated with enhancement. The protocol is optimized to detect enhancement by using triple-dose gadolinium, post-injection delay, 3-T MRI, and an off-resonance saturation pulse for a magnetization transfer contrast (MTC) effect. This communication's purposes are to provide: 1) a report on the efficacy of the MRI protocol and 2) an announcement of the primary outcome measures of the study.

Methods: 75 subjects with MS were randomized to one of two treatment arms: 20 mg glatiramer acetate injected subcutaneously (sc) on a daily basis vs. 250 mcg of interferon beta-1a injected sc every other day. Drug efficacy was monitored with monthly MRI up to 24 months (greater than 1200 MRI's total), as well as other measures. The MRI protocol consisted of precontrast scans followed by injection of 0.1 mmol/kg of Gadopenetate Dimeglumin (Gd), a 15–20 min. post-injection delay, an injection of an additional 0.2 mmol/kg of Gd followed by an 2nd 15–20 min. post-injection delay. T1 images were then obtained with MTC using 5 mm sections. The MRI scans were read in a blinded fashion by a single experienced neuroradiologist with expertise in MS. The number of CAL was tabulated. Results: At the time of this analysis 35 of the 75 subjects had their MRI scans read. Among these, there were 460 available MRI scans. On these, there were 536 enhancing and 21 new-T2 lesions for a total CAL of 557. Of the 35 subjects, 9 demonstrated no CAL at any time point. Most of the 26 patients with lesions, the median number per scan was 1.5. 22 subjects never demonstrated a new-T2 lesion. 376 scans had a previous MRI within 6 weeks. In this group, there were 414 enhancing lesions but only 4 new-T2 lesions. Conclusions: (1) When an MRI protocol is used that is optimized for the detection of enhancement, the frequency of new-T2 lesions in the absence of gadolinium enhancement may be as low as 1%. (2) The MRI efficacy results comparing interferon beta-1b to glatiramer acetate will be presented at the meeting.

P368
Treatment of relapsing-remitting multiple sclerosis with glatiramer-acetate: our 7-year clinical experience

P. Hradilek, O. Zapletalová, I. Wizniová; University Hospital (Ostrava, CZ)

Background: Glatiramer-acetate (GA) is an approved effective treatment for relapsing-remitting multiple sclerosis (RRMS). It acts as both immunomodulatory and neuroprotective agent. Objective: To evaluate clinical parameters (relapse rate and EDSS change) and side effects in RRMS patients treated with glatiramer-acetate. Material and Methods: Our group consisted from 108 RRMS patients (96 women and 12 men). Treatment with GA started in 1999. In 95 patients GA was their first disease-modifying drug, 13 were previously treated with interferons. GA was administered to RRMS patients presenting with high disease activity (at least 2 relapses per year or 3 relapses/2 years) and MRI finding consistent with the diagnosis of MS. The average period since the diagnosis of MS to the beginning of the treatment with GA was 6.75 years (1-30). Relapse rate and EDSS change was evaluated in the subgroups treated at least 1, 3 or 5 years. Results: The average relapse rate (R-R) before the treatment was 1.5. After one year of the treatment R-R decreased to 0.68, after three years to 0.51 and in the subgroup treated at least 5 years the R-R was 0.31. Among the patients with stable EDSS score the average change of EDSS after five years of the treatment was 0.1 point. Only 5 patients discontinued the treatment. Except two skin allergic reactions leading to discontuation of the treatment we did not observe any major side effects. 11 patients became pregnant during GA therapy, which was interrupted the day the subjects knew about their pregnancy. There were 3 spontaneous abortions and 8 normal deliveries. We did not observe any foetus abnormalities. Conclusion: We consider treatment of RRMS patients with GA safe and very effective with positive impact on both R-R and disease progression (EDSS score).

P369
Long-term treatment of multiple sclerosis patients with glatiramer acetate: clinical efficacy and anti-glatiramer acetate antibodies profile

D. Teteletham, T. Bremer, O. Abramys, C. Sicirc, R. Amon, D. Karussis; Weizmann Institute of Science (Rehovot, IL); Hadassah University Hospital (Jerusalem, IL)

We have previously demonstrated that glatiramer acetate (GA) treated multiple sclerosis (MS) patients developed GA antibodies (Abs). These Abs did not interfere with the biological activity of GA. In the contrary, relapse-free patients tended to develop higher Abs titers. The latter study followed Abs response during the two first years of treatment. A retrospective study was designed to determine and characterize the anti-GA responses after GA-treatment of >2 years, to test the in vitro neutralizing activity and correlate these parameters with the clinical course. Serum samples from 126 MS patients treated with GA for 2 to 15 years were collected and measured using ELISA. Neutralizing activity was determined by the capacity of the serum to inhibit the proliferation of GA specific T cells. Anti-GA abs could be detected I almost all of the patients. The IgG2 isotype (Th1-related isotype) significantly decreased after 2 years of treatment and remained low up to 15 years. In contrast IgG1 and IgG4 isotypes (Th2-related isotypes) were not affected and the efficacy of GA treatment seems to be related to an up-regulation of IgG4. Only 6/126 patients demonstrated a minimal neutralizing activity (inhibition...
of 10–25% of GA-specific T-cell proliferation). Clinically, patients treated with Copaxone during a mean 6.65 years period showed only a minimal mean increase in of 0.65 degrees in the EDSS scale (mean annual increase per patient = 0.10 degrees). GA treatment favorably affected the natural history of MS: after a mean disease duration period of 10.75 years, 86% of the patients had not reached an EDSS score of 5.0 and 77% had a score of less than 4.0. These results confirm the long-term efficacy of GA and indicate that the immunological effects of GA are maintained upon long-term treatment.

### P370

A longitudinal observational study of a cohort of patients with relapsing-remitting multiple sclerosis treated with glatiramer acetate

T. Moreau, F. Brudon, M. Debouverie, O. Heinzlef, C. Lebrun-Frenay, C. A

Glatiramer acetate (GA) has been demonstrated to reduce relapse rate and slow accumulation of disability in first-line therapy of patients with relapsing remitting multiple sclerosis (RRMS). Switching to GA is also an appropriate treatment strategy in patients who cannot be prescribed, or fail treatment with, b-interferons. Before GA became available in France, treatment with this drug could be initiated as part of a compassionate use programme in those patients with RRMS for whom b-interferons could not be prescribed. This programme was initiated in December 1997 and continued until registration of GA in June 2002. Subjects were then eligible to continue treatment as part of a three-year observational study. The objective of this observational study was to evaluate the middle- and long-term effectiveness and acceptability of GA. The study included adult patients with RRMS in whom b-interferons had been discontinued. Recruitment into the observational study started in March 2003. Patients who had not been evaluated during the preceding year were ineligible. Patients were evaluated at inclusion into the compassionate use programme, at entry into the observational study and thereafter annually. Data were collected on relapses, disability (EDSS scores), conversion to a secondary progressive form and adverse events. 655 patients were included in the compassionate use programme and 198 continued into the observational study. The mean total treatment duration was 733±716 days, including 362±716 days in the compassionate use programme. 84.6% of patients experienced three or more relapses. One year after initiation of GA, the mean relapse rate had fallen to 0.46, and thereafter remained stable. The EDSS score at inclusion was 3.4. In 74.6% of subjects, the EDSS did not evolve over the study. 26 patients (4%) converted to a progressive form of the disease. The most frequently reported adverse events were local injection site reactions. Nine patients discontinued GA treatment for adverse events. In conclusion, treatment with GA in a naturalistic treatment setting is well-tolerated and associated with a beneficial clinical outcome in subjects with RRMS to whom b-interferons cannot be prescribed.

### P371

Effect of glatiramer acetate on diffusion imaging in patients with multiple sclerosis

R. Zivadinov, S. Hussein, N. Abdelrahman, D.L. Cookfair, M. Meyer, N. Garg, J.L. Cox, M.G. Dwyer, B. Weinstock-Guttman; University of Buffalo (Buffalo, USA)

Background: In multiple sclerosis (MS), the two main pathological processes affecting the brain are demyelination and neurodegeneration; they can alter the geometry of brain tissue orientation, resulting in an increase of water diffusivity measurable with different diffusion-weighted imaging (DWI) indices. No studies have looked at the effect of disease-modifying treatment on DWI measures over the short- and long-term. **Objective:** To evaluate the effect of glatiramer acetate (GA) on the 1-year changes in DWI measures in patients with either relapsing-remitting (RR) or secondary-progressive (SP) MS. **Methods:** Twenty-four (24) consecutive MS patients (RR = 19, SP = 5), with mean disease duration 10.7 yrs, mean age 43.8 yrs and mean EDSS 5 were included in the study and followed for a mean follow-up of 12.6 months. Inclusion criteria were: age 18–65, RR or SP disease course, EDSS score ≤5.5, disease duration 1–20 years and presence of GA monotherapy for a minimum of 6 months prior to study entry (mean exposure 3.5 years). Patients received 1.5T MRI scan and clinical examination at study entry and at the end of follow-up. 1-year changes were determined in DWI and other non-conventional and conventional MRI measures. **Results:** All patients completed clinical and MRI follow-up. Treatment with GA promoted recovery in DWI mean parenchymal diffusivity (MFD) (−7.2%, p = 0.003). In patients who were clinical responders (no relapses and no disability deterioration during the study, N = 11), there was a significant improvement in DWI-MFD (p = 0.04). No significant within-patient deterioration in magnetization transfer ratio (MTR) measures of whole brain (−0.3%), normal appearing (NA) brain tissue (−0.3%), NA white matter (−0.1%) and NA gray matter (−0.4) was determined in total population. Over the follow-up, there was a significant decrease in GA-enhancement (−83%, p = 0.007) activity, Measures of central brain atrophy showed improvement (−3.5% for the third ventricular width) or stabilization (whole brain) over 1 year. Only two patients progressed 1.0 point in their EDSS and 4 presented ≥1 relapse during the follow-up period. **Conclusions:** The data from the present study suggests that GA can prevent macroscopic and microscopic tissue damage in the brain, as measured by the DWI. Treatment with GA stabilized/prevented progression on a number of other non-conventional and conventional MRI measures. This study was funded by a grant from Teva Neuroscience.

### P372

Randomised, double-blind, parallel-group, dose-comparison study of glatiramer acetate in relapsing-remitting multiple sclerosis

J. Cohen, M. Rovaris, A. Goodman, D.R. Wynn, D. Ladkani, M. Filippi for the 9006 Study Group

**Background:** Three pivotal trials support the safety, tolerability, and efficacy of Copaxone (glatiramer acetate; GA) 20 mg/day by subcutaneous injection in relapsing-remitting multiple sclerosis (MS). There are few data concerning other doses. **Objective:** This nine-month multi-center, randomized, double-blind, parallel-group, Phase II study evaluated the safety, tolerability, and efficacy of GA 30 mg compared to the currently-approved 20 mg dose. **Methods:** Eligibility criteria included clinically-definite MS with a relapse in the prior year, Expanded Disability Status Scale score 0–5.0, no prior use of GA, and 1–15 gadolinium-enhancing (GdE) lesions on a screening MRI (which also served as the pre-treatment baseline MRI). Subjects then underwent MRI at months 3, 7, 8 and 9, and neurological assessments at baseline then months 3, 6, and 9. **Results:** Of 229 subjects screened, 90 were randomized to GA 20 mg (n = 44) or 40 mg (n = 46). The groups were well-matched at baseline on demographic, clinical, and MRI characteristics. The primary efficacy endpoint, total GdE lesion number at months 7, 8, and 9, showed a 38% reduction from baseline for GA 40 mg (relative risk = 0.62; 95% CI = 0.36–1.0; p = 0.0898) with mean lesions per scan (SD) equal to 0.79 (1.36) for the 40 mg group versus 1.32 (1.51) for the 20 mg group. A difference emerged as early as month 3, 1.33 (1.8) lesions for the 40 mg group versus 2.61 (4.22) lesions for the 20 mg group (52% reduction; p = 0.0051). There was a trend favoring 40 mg for relapse rate with significant benefit on proportion of relapse-free subjects (p = 0.0183) and time to first relapse (p = 0.0367). For the 20th percentile, time to the first relapse was delayed from 80 days in the 20 mg group to 213 days in the 40 mg group.
group. The proportion of patients being both relapse-free, and free of GdE lesions at months 7, 8, and 9 (or with the mean number of GdE lesions reduced by 50% or more versus baseline), was higher for the 20 mg dose (69% versus 38.5%, Odds Ratio = 3.52 [1.39–8.88], p = 0.0078). GA 40 mg was well-tolerated with a safety profile similar to the 20 mg dose. Injection site reactions and immediate post-injection reactions were somewhat more common and severe with the higher dose. Conclusions: This study demonstrated that GA 40 mg is safe and well-tolerated. The overall efficacy results provided evidence that the 40 mg dose of GA may be more effective than the currently approved 20 mg dose in reducing MRI activity and clinical relapses.

P373
Prevention of demyelination and possible remyelination by glatiramer acetate in EAE mice as visualised by Novel Wet SEM technology
R. Aharoni, A. Vassilheine, V. Behar, W. Bräck, R. Arnon; The Weizmann Institute of Science (Rehovot, IL); Quantumix (Rehovot, IL); Georg August University (Gottingen, D)

In both Multiple Sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE), the detrimental process provoked by autoimmune inflammatory mechanisms leads to dissemi- nated demyelination, axonal damage and neuronal loss. Although degenerative processes contribute to the permanent neurological disability, demyelination is the primary pathological mechanism that characterizes these diseases. It is therefore important to assess current MS treatments for their ability to prevent demyelination and/or enhance remyelination. Glatiramer acetate (GA, Copaxone®), an approved drug for the treatment of MS, has been shown to induce specific Th2/3 cells that penetrate the CNS, expressing in situ anti- inflammatory cytokines as well as neurotrophic factors (BDNF, NT-3, NT-4). GA affects also the actual target of the pathological process—the resident CNS population. Hence, IL-10, TGf-b and BDNF expression was augmented, not only by regulatory T-cells, but also by astrocytes and neurons. Moreover, as recently demonstrated, GA treatment induces neurogenesis resulting in generation of new neurons that migrate into injury sites and differentiate to mature neuronal phenotype. This study relates to our investigation of the effect of GA on the process of demyelination and/or remyelination. For that purpose we use scanning electron microscopy (SEM) for imaging of wet biological specimens, a novel methodology WETSEM™ that allows rapid and high resolution imaging of myelin in mouse spinal cord, using EAE model induced by MOG 35–55 peptide. Evaluation of spinal cords from EAE infected mice, 22 and 32 days after disease induction, with grade 3 disease, revealed inflammation, myelin vacuolation, edema and parenchymal damage. In contrast, spinal cords of GA-treated mice, in which treatment (10 daily injections, 2 mg/mouse) started together with disease induction (prevention), were similar to those of naive mice, indicating that GA blocked demyelination as well as its clinical manifestation. Moreover, even when GA treatment was applied after the appearance of clinical symptoms, in the chronic phase, 22 or 56 days after induction (suppression and delayed suppression), no pathology was observed. These results suggest that GA arrested the detrimental process after demyelination had already occurred and/or induced remyelination.

P374
The mode of action of Fingolimod (FTY720), an oral sphingosine 1-phosphate receptor modulator that is highly effective in human multiple sclerosis (MS) (Phase II)
V. Brinkmann, B. Metzler, M. Mattoubian, H. Mittwepper; Novartis Institutes for Biomedical Research (Basel, CH); UCSF (San Francisco, USA); Max Planck Institute for Infection Biology (Berlin, D)

Fingolimod (FTY720), an oral immunomodulator with a novel mechanism of action, has shown excellent efficacy in human multiple sclerosis (MS) in a Phase II study. Six months data showed a highly significant reduction in the relapse rate and in the number of brain lesions detected by MRI scan, as well as a longer time to first relapse. Mechanistic studies show that Fingolimod does not impair T- and B-cell activation, proliferation and effector function, but interferes with cell traffic between lymphoid organs and blood. Fingolimod, after phosphorylation, acts as high affinity agonist at the G protein-coupled sphingosine 1-phosphate receptor-1 (S1P1) on lymphocytes, thereby down-modulating the receptor. This renders the cells unresponsive to the serum lipid sphingosine 1-phosphate (S1P), depriving them of an obligatory signal required to transmigrate through the sinuses-lined endothelium in the lymph nodes (LN) and to egress into efferent lymph and blood. This process reduces the recirculation of disease-relevant auto-reactive T-cells from their site of activation in the LN to the central nervous system (CNS) and, as a consequence, abrogates the inflammatory process. Since blood lymphocytes comprise only about 2% of the total lymphocyte pool of the body, the trapping of T-cell subsets does not result in detectable cell accumulation in LN. Fingolimod differentially affects T-cell subsets depending on their recirculation characteristics. The drug effectively traps naive T-cells (Tn) and central memory T-cells (Tcm) in the LN, but spares peripheral effector memory T-cells (Tem). This relates to the fact that Tem do not express the LN homing receptors CDX2 and CCR7 and, thus, do not traffic through LN. These data suggest that pathogenic T-cells may primarily reside within the Tcm subset which could cross-react on autoantigen (AA) in draining LNs and, in the absence of Fingolimod, would recirculate to the CNS. In contrast, peripheral Tem (i.e. in gut epithelial surfaces, small intestine lamina propria, lung, liver, kidney, peritoneum, bone marrow, blood) would not reach the CNS but could provide local defense against infection. Accordingly, Fingolimod did not affect clearance of Listeria monocytogenes infection in mice. The available data establish S1P1 as the key target of Fingolimod, and further identify the novel class of G-protein-coupled sphingosine 1-phosphate receptors as therapeuti- cally relevant drug targets in autoimmune indications, including MS.

P375
Pleiotropic actions of FTY720-phosphate in cells of oligodendroglial lineage
C.G. Jung, V.E. Miron, S. Cook, C.A. Foster, J.P. Antel, B. Soliven; The University of Chicago (Chicago, USA); McGill University (Montreal, CAN); Novartis Institutes for Biomedical Research (Vienna, A)

Background: Fingolimod (FTY720) is an oral sphingosine-1-phosphate (S1P) receptor modulator in late stage development for relapsing multiple sclerosis (MS). Its efficacy in patients and in MS animal models has been attributed to the retention of myelin-autoactive T cells in peripheral lymphoid organs as a consequence of S1P1 ligation. It is not known what effect direct modulation of S1P1 receptors on glial cells in the central nervous system may have on oligodendrogial regeneration and remyelination. Objectives: Delineate the effect of FTY720 and its phosphorylated form (FTY720-P) on the functional properties of cultured oligodendrocytes (OLG) and OLG progenitor cells (OPC). Methods: The effect of S1P receptor modulators on the survival, differentiation and cell cycle progression in cultured neonatal rat OPC and OLG was examined using MTT microelisa assay, immunofluorescence method, flow cytometry and Western blot analysis. Human OPC and OLG were used in a subset of experiments. Results: S1P1 and S1P5 are expressed by cells of OLG lineage from neonatal rat brains; similar expression was confirmed on human oligodendrogial progenitor cells and mature oligodendrocytes. We found that treatment for 48 hr with FTY720-P but not FTY720 at low concentrations (≤0.1 μM) during serum withdrawal led to improved survival of rat OLG, yet had no effect on OPC survival. Correlating with the effect on survival, we found that FTY720-P stimulated phosphorylation of extracellular signal regulated kinases (ERK1/2) in OLG but not in OPC. Treatment of OPC with FTY720-P (1 μM) led to a decrease in MBP+ cells with myelin sheet structures (mature OLG) when compared to untreated or vehicle-treated cul-

Multiple Sclerosis 2006; 12: 51–5228
www.sagepub.co.uk
Oral fingolimod (FTY720) in relapsing multiple sclerosis: 24-month results of the Phase II study
L. Kappos, J. Antel, G. Comi, X. Montalban, A. Radue, A. de Vera, H. Pohlmann, P. O'Connor on behalf of the FTY720D2201 Study Group

Background: In a 6-month (M), placebo-controlled (PL-C) study including 281 patients with relapsing MS (89% RRMS, 11% SPMS patients), the oral sphingosine-1-phosphate receptor modulator fingolimod significantly reduced inflammatory disease activity on MRI by up to 80% and relapses by more than 50% at both doses. The annualized relapse rate (ARR) was 0.77 with PL versus 0.35 with 1.25 mg (P = 0.009) and 0.36 with 5 mg fingolimod (P = 0.014). Objective: To report the M24 safety and efficacy results of this Phase II study. Methods: 250 of 255 patients completing the 6-M PL-C phase entered the extension. Patients on fingolimod continued their originally-assigned treatment; those on PL were re-randomized to fingolimod (PL-fingolimod) groups. Evaluations were scheduled monthly during M0-6 and 3-monthly during M7-24. Between the M15 and M24 visit all patients receiving 5 mg were switched to 1.25 mg fingolimod. The original treatment allocation was not disclosed to the sites until all patients completed M24 visit. Results: The M0-24 ARR was 0.20 in the continuous fingolimod and 0.33 in the PL–fingolimod groups (6 M on PL, 18 M on fingolimod). At M24, 84% of patients in the continuous fingolimod and 85% in PL-fingolimod groups were free of Gd+ lesions. 79% of patients in the continuous fingolimod and 78% in the PL-fingolimod groups were free of 3-M confirmed disability progression. Nasopharyngitis and influenza were the most frequent adverse events reported. Clinically asymptomatic increases in ALT and increase in blood pressure (average of 5 mmHg within first the 6 months remaining stable thereafter) were also observed. Conclusions: At 6 months, fingolimod reduced disease activity on MRI up to 80% and relapse rate by more than 50% versus PL. Over 2 years of treatment, disease activity (MRI and clinical) remained low in the continuous fingolimod groups. In patients switching from placebo to fingolimod, disease activity consistently decreased after the switch and remained low thereafter. No unexpected side-effects emerged in patients treated for up to 24 months compared with the 6-month placebo-controlled phase. These positive results support further evaluation of fingolimod as an oral treatment option in the ongoing Phase III program in RRMS. Study supported by Novartis Pharma AG

Pharmacodynamic effects of oral fingolimod (FTY720) R. Schnieder, S. Aradhya, P. O’Connor, L. Kappos; Novartis Pharmaceuticals Corporation (East Hanover, USA); St. Michaels Hospital (Toronto, CAN); University Hospital Basel (Basel, CH)

Background: Fingolimod is an oral sphingosine-1-phosphate (S1P) receptor modulator that reduced relapse rate by more than 50% and MRI inflammatory lesion activity by up to 80% compared to placebo at 6 months in a Phase II study in relapsing multiple sclerosis (MS) patients. Low disease activity on fingolimod was maintained with continued treatment for up to 18 months. Interaction of fingolimod with S1P receptors induces a transient reduction in heart rate upon treatment initiation and inhibits the egress of lymphocytes from the lymph nodes. Objectives: To describe the effects of fingolimod on peripheral lymphocyte counts and on heart rate, with special focus on doses currently used in the Phase III MS studies (0.5 and 1.25 mg/day).

Methods: We reviewed data from approximately 150 healthy volunteers (HV) and 134 MS patients treated with 0.5 or 1.25 mg fingolimod. Results: In HVs receiving single-dose fingolimod, blood lymphocyte counts were reduced in a dose-dependent manner. In HVs receiving fingolimod 1.25 mg/day for 7 days, mean lymphocyte counts decreased from 1900 cells/mm³ at baseline to 400 cells/mm³ at day 7. After cessation of dosing, lymphocyte counts began to increase within 2–3 days and had recovered to 74% of baseline (mean 1400 cells/mm³) 4 weeks after the last dose (end of study). In the Phase II MS study, the reduction in circulating lymphocytes with 1.25 mg fingolimod was similar to that seen in HVs. Lymphocyte counts remained stable over 12 months of continuous therapy (mean 570 cells/mm³ at month 12). There were no correlations between low lymphocyte counts and severe or serious infections under fingolimod treatment. In both HV and MS patients, decrease in heart rate after the first dose of fingolimod was maximal between 4–5 h post-dose (a reduction of 10–13 bpm on average for 1.25 mg and 5 bpm for 0.5 mg), returning towards baseline with subsequent dosing. In HVs there was no effect on circadian autonomic rhythm and the heart’s responses to intrinsic/extrinsic stimuli were intact. Of the 134 MS patients entered in the Phase II study, symptomatic bradycardia was reported in only 1 patient and resolved without treatment. Conclusion: Transient reduction in heart rate is a

Oral fingolimod (FTY720) in relapsing multiple sclerosis: MRI results of a placebo-controlled Phase II study and active drug extension E-W. Radue, P. O’Connor, J. Antel, G. Comi, X. Montalban, A. de Vera, D. Tang, L. Kappos on behalf of the FTY720D2201 Study Group

Background: The oral sphingosine-1-phosphate receptor modulator fingolimod has shown efficacy in the 6-month (M), placebo-controlled (PL-C) phase of a study in patients with relapsing MS. Objectives: To present detailed information on the effects of fingolimod on MRI parameters in this PL-C Phase II study and its extension up to M12 and M24. Methods: Of the 281 patients randomized (1:1:1 to 1.25 mg fingolimod and PL), 255 completed the PL-C phase of the study (M0-6) and 250 entered the extension. T1-weighted images pre- and post-Gadolinium (Gd) 0.2 mmol/kg injection as well as PD/T2-weighted images were performed monthly during M0-6 and every 6 months during M7-24 according to a standardized protocol. The MRIs were evaluated (blinded) by the MS MRI Evaluation Center in Basel. Results: At 6 M, the median (mean) cumulative number of new and persistent Gd-enhanced lesions was 5 (14.8) for PL, 1 (8.4) for fingolimod 1.25 mg (p < 0.001 vs. PL) and 3 (5.7) for 5 mg (p = 0.006 vs. PL). The reduction in inflammatory activity occurred early as evidenced by the significantly lower number of new T2 lesions developing between M1 and M2 compared to PL (1.0 for PL, 0.32 for 1.25 mg and 0.26 for 5 mg, p < 0.003 for both doses vs. PL). All other analyses of Gd-enhanced and new T2 lesions showed consistent and statistically significant positive results vs. PL over 6 months with no differences between the two doses. At M12, the number of Gd-enhanced lesions remained low in the continuous fingolimod groups, and were significantly reduced in the PL-fingolimod groups (M12 vs. M6: p < 0.005 for both doses). 73% of the patients on continuous fingolimod treatment did not develop any new T2 lesion between M6 and M12. Other variables showed consistently low MRI lesion activity in the continuous fingolimod groups and marked reduction of Gd-enhanced and new T2 lesion activity at M12 compared to M6 in the PL-fingolimod groups. Conclusions: Oral fingolimod induces a marked and consistent therapeutic effect on MRI inflammatory activity. The effect of fingolimod on MRI lesions was seen early, becoming significant after 1 month of treatment. The significant effect seen on all MRI lesions was sustained for up to 12 months. The two doses of fingolimod (1.25 mg and 5 mg) were equally effective. Results of the M24 MRI will be presented.

Oral fingolimod (FTY720) in relapsing multiple sclerosis: 24-month results of the Phase II study and active drug extension L. Kappos, J. Antel, G. Comi, X. Montalban, A. de Vera, H. Pohlmann, P. O’Connor on behalf of the FTY720D2201 Study Group

Background: In a 6-month (M), placebo-controlled (PL-C) study including 281 patients with relapsing MS (89% RRMS, 11% SPMS patients), the oral sphingosine-1-phosphate receptor modulator fingolimod significantly reduced inflammatory disease activity on MRI including 281 patients with relapsing MS (89% RRMS, 11% SPMS patients). Process retraction in OPC proliferation, OLG survival and remodeling of processes indicates that S1P receptors may play a role during oligodendroglial regeneration and remyelination.

Abstracts S101
well-characterized biological effect of fingolimod and is clinically benign in HIV and MS patients. Fingolimod induces a dose-dependent reduction in peripheral lymphocyte counts, an effect that is thought to contribute to its therapeutic effects in MS.

**P379**

*Oral teriflunomide is effective and well tolerated in multiple sclerosis with relapses: results of an open-label 144-week extension study*

P. O'Connor, D. Li, M. Freedman, A. Bar-Or, G. Rice, C. Confavreux on behalf of the Teriflunomide Multiple Sclerosis Trial Group

**Background:** Teriflunomide is an oral immunomodulator that reduced the number of combined unique active (CUA) lesions on MRI by more than 61% compared to placebo over 36 weeks of treatment in a phase II randomized, double-blind placebo-controlled trial (Neurology 2006; 66: 894–900). This is a report of the 144-week open-label extension phase of that trial. **Methods:** Patients originally enrolled in the phase II trial had clinically definite MS with age 18–65, baseline EDSS score less than 7, and two documented relapses in the previous 3 years. 147 patients who consented to participate entered the extension study. Placebo patients were randomized to either teriflunomide 7 or 14 mg per day and followed along with the original teriflunomide-treated patients. Adverse event (AE) and clinical efficacy assessments were performed every 12 weeks with brain MRI every 48 weeks. Safety and efficacy data after 144 weeks of extension study treatment were analyzed. **Results:** At baseline the 4 groups consisted of placebo-7 mg switch (N=29), placebo-14 mg switch (N=26), continuous 7 mg (N=52) and continuous 14 mg (N=40). The incidence of AEs, serious AEs and AEs leading to treatment withdrawal were similar in the 4 groups. Elevations in liver function tests beyond 3 times the upper limit of normal were uncommon and not dose related. The overall drop-out rate was less than 10% per year. The annual relapse rate was similar between groups (approximately 0.4/year) as was the proportion of relapse-free patients up to week 144 (approximately 54%). Efficacy data in patients completing 144-weeks of treatment (n=114) were compared to the extension-study baseline. Placebo patients switched to 7 mg/day experienced a 65% decrease in MRI CUA lesions at week 144 (P=0.02) versus an 85% decrease for placebo-14 mg patients (P=0.02). The patients continuously on therapy throughout the study experienced no change in the number of CUA MRI lesions during the extension phase. There were no significant differences in clinical parameters between groups. **Conclusion:** Teriflunomide was well tolerated with few drop-outs during this 144-week extension study. Patients switched from placebo to active treatment experienced a significant drop in the number of CUA MRI lesions, further supporting the results of the initial double-blind placebo-controlled phase of the study. There were no statistically significant changes in relapse rates or EDSS between groups during the extension study.

**P381**

*T cell vaccination in multiple sclerosis with CSF-derived activated cd4+ T cells: results of a placebo-controlled trial*

P. Stiinissen, R. Meder, N. Hellings, E. Vanroose, F. Barkhof, J. Raus; Hasselt University (Diepenbeek, B); Image Analysis Center (Amsterdam, NL)

Activated anti-myelin T-cells accumulate in the cerebrospinal fluid (CSF) of MS patients, indicating that these T cells may represent a source of disease-related T cells. A previous pilot trial of T cell vaccination (TCV) with activated CD4+ T cells derived from CSF demonstrated safety, feasibility and immune effects in MS patients. A double-blind placebo-controlled trial was performed with early relapsing-remitting MS patients to study the effects of TCV on disease activity as measured by magnetic resonance imaging (MRI). Twenty-nine MS patients that demonstrated MRI activity in a pre-entry period of 6 months were randomized into active (TCV) (n=20) and placebo (n=9) groups. Three immunisations with irradiated CSF vaccines (5–10 million cells) or placebo were administered subcutaneously with an interval of 2 months. Patients were monitored for 12 months after the final immunisation for MRI activity (every 2 months), clinical scores and immune responses. The vaccinations were well tolerated and no toxicity or adverse effects were reported. Anti-myelin T cell responses were reduced after TCV in 16 out of 20 MS patients. The mean number of active MRI lesions and the volume of active MRI lesions and T2 lesions was reduced in the treated group, but not in the placebo group. These differences were however not statistically significant. The mean EDSS scores remained stable in both groups. Patients with a high immune response to the vaccine cells showed a trend for improved MRI and clinical responses to TCV. Despite the lack of statistically significant differences due to the low patient number, this study further demonstrates safety and feasibility of TCV in MS patients, and suggests that possible therapeutic effects of TCV may be more prominent in patients that show high immune responses to the vaccine.

**P380**

*A responder group-targeted phase II/III double blind placebo controlled multi-centre study of MBP8298 in subjects with secondary progressive multiple sclerosis*

T. Verco, L.Z. Ferenczi, M.J. Krantz; BioMS Medical Corp (Edmonton, CAN)

**Objective:** To design and carry out a large confirmatory clinical trial of MBP8298 which is powered to achieve a clinical endpoint (Expanded Disability Status Scale (EDSS)) in a Human Leukocyte Antigen (HLA)-defined subset of multiple sclerosis (MS) patients. **Background:** A responder cohort of patients with HLA haplotypes DR2 and/or DR4 was identified in a phase II study of MBP8298 in chronic progressive multiple sclerosis (MS) patients. **Methods:** The primary endpoint is the clinical efficacy of MBP8298, as measured by EDSS, compared to placebo in subjects with HLA-DR2 and/or DR4 haplotypes; assessment of clinical efficacy in subjects with other HLA-DR haplotypes is a secondary objective. A total of 553 subjects will be enrolled, with 453 expected to be in the primary study group where 408 evaluable subjects are required. Male or female subjects, 18–65 years of age, with a documented history of secondary progressive MS (SPMS) will be accepted. An absence of relapse in the 3 months prior to the first study-specific procedure and an EDSS score of 3.5 to 6.5 are required. MBP8298 (500 mg) or placebo in 10 mL will be given intravenously every 6 months for 2 years. The primary endpoint is a clinically and statistically significant increase in the time to confirmed worsening of disability in the MBP8298 treatment group, as measured by EDSS. For statistical analysis, subjects will be stratified by baseline score (3.5–5.0 or 5.5–6.5) and presence or absence of HLA haplotype DR2 and/or DR4. Criteria for safety include adverse events, physical examinations, haematology, serum chemistry, 12-lead electrocardiograms and brain magnetic resonance imaging. **Results:** Enrolment is underway in Canada and Europe, and is expected to be complete in 2006. To date, no safety concerns have been identified by an independent data and safety monitoring board. **Conclusions/Relevance:** This large confirmatory clinical trial of MBP8298 targets an identified responder subgroup, but the potential for clinical responses in less common HLA-DR haplotypes is being evaluated in the same protocol.

**P382**

*Natalizumab alters transcripational expression profiles in peripheral blood of multiple sclerosis patients*

R.L.P. Lindberg, M. Achtnichts, J. Kuhle, F. Hoffmann, L. Kappos; University Hospital Basel (Basel, CH)

**Background:** In two large scale Phase III trials, natalizumab reduced the number of re-lapses and the risk of sustained progression in...
Methods: We performed large-scale transcriptional analysis of peripheral blood of 11 RR-MS patients (6 females, 5 males), participating in the AFFIRM study [1]. PAXgene blood samples were drawn at baseline, week 4 (W4), W8, W12, W24 and W52 and at Month 1, 2 and 3 of the open label extension study. We used human genome U133A microarrays (Affymetrix), which cover approximately 22'000 sequences. Remote analy-sis computation for gene expression data (RACE, http://race.unil.ch) was used for ex-perimental quality assessment, and robust multi array analysis (RMA) was applied for normalization of expression signals. NetAffx (Affymetrix) tools, GeneMAPP/MAPPFinder and EASE (Expression Analysis Systemic Explorer) were employed for pathway and gene ontology investigations. Results: By using filtering criteria of a fold change of “1.5” and p<0.01, we found 64 genes, which were differentially expressed throughout time points W8, W12, W24 and W52. Based on the known biological functions (47 genes), the altered transcripts were classified into gene ontology-based biological processes. Genes related to “response to stimulus” (p<0.0017), “cell communication” (p<0.0003) and “immune response” (p<0.0031) were among the major significant biological groups altered during natalizumab treatment. The immune response genes included interleukin-6 and 8 receptors, leukocyte immunoglobulin-like recep-tor, FYN binding protein and complement compo-nent 5 receptor 1. Interestingly, also a neutrophil specific gene, elastase 2, was altered. In addition, several regulated genes had a chemotaxis function. Extra-cellular matrix (ECM) related genes, basigin (metallopeptidase in-ducer), TIMP2 (metallopeptidase in-hibitor 2) and a disintegrin and metalloproteinase domain 8 (ADAM8), were also regu-lated. Conclusions: Transcriptional regulation of T, B-lymphocytes, monocytes and neutro-philos, is altered during natalizumab treat-ment. The continuous down regulation of im-mune related genes suggest the association to persistent clinical efficacy. Further stud-ies on expression profiles in various cell subpopulations are needed for better under-standing of cell type specificity of gene expression changes. Moreover, functional inves-tigations of regulated genes, will increase current knowledge of the mode of action of natalizu-mab. Supported by Biogenidc Germany and the Swiss MS Society.

Reference
1. NEJM 2006; 354: 899 - 910

P384
Influence of repeat intrathecal triamcinolone acetonid application on cerebrospinal fluid and serum biomarkers in multiple sclerosis patients
M. Aba-Muhgeisib, A. Petzold, A. Grossmann, F. Kamin, W. Kohler, F. Hoffmann, J.H. Faiss, I. Ringel, R. Benecke, U.K. Zettl; University of Rostock (Rostock, D); University College London (London, UK); Sachsisches Krankenhaus Hubertusburg (Wernsdorf, D); Staedt. Krankenhaus Martha- Maria Halle-Doelau (Halle, D); Landesklinik Teupitz (Rostock, D)

Background: Neurofilaments (NF) are a biomarker for axonal degeneration and glial fibrillary acidic protein (GFAP) is a biomarker for glial activation and astroglisis. Objective: This prospective pilot study aimed to investigate the effects of repeated intrathecal triami-cinolone acetonid (TCA) application on cerebrospinal fluid (CSF) and serum NF heavy chain (NIH SMI 35) and CSF GFAP in multiple sclerosis patients. Methods: 10 Patient suffering from MS (4 relaps remitting [RR], 6 secondary or primary progressive [SP/PP]) were included in this pilot study that is still continuing: mean age 51.2 ± 9.1 years, disease duration 13,75± 9 years, mean Expanded Disability Status Scale (EDSS) 5,1 ± 1,96 at baseline. 3 TCA applications were performed as part of an ongoing trial every second day to reduce spasticity. NIH and GFAP levels were measured using standard ELISA techniques at baseline and at each TCA application. A change of more than 16% (NFH) or 6% (GFAP), representing the intraassay coefficient of variation (CV), from baseline values was interpreted as an increase/ decrease. Additionally MRI was used for determination of the T2-hyperintense lesion load and the number of Gd-enhancing leasions.

Results: The median EDSS was 5,5 (range 1– 8) at baseline and 4,5 (1 – 6) at follow up. The median CSF NIH levels were 0.06 ng/mL at baseline and correlated those at time-point 2 (0.07 ng/mL, R = 0.75, p = 0.012) and 3 (0.09 ng/mL, R = 0.72, p = 0.017). Correcting for repeated measurements there was no change of the CSF NIH levels over the observation period. There was a tendency towards increasing CSF NIH levels in patients with SP/PP- compared to RRMS. The median serum NIH levels were 0.18 ng/mL at all three time-points. The respective values for the CSF GFAP levels were 0,01 ng/mL and not measureable thereafter. The median CV for individual NIH concentrations was 26% in the CSF and 3% in the serum. No changes at the MRI examination were measured. Conclusion: This pilot study demonstrated a small, but measurable short term variability of the individual CSF but not serum NIH levels of relevance for the interpretation of this biomarker as a potential secondary outcome measure. This treatment trial is ongoing and future lumbar punctures are planned.

References

www.sagepub.co.uk
P385
Expansion of CD8+ regulatory T-lymphocytes and fall of activated CD8+ T-lymphocytes after IV methyl-prednisolone for multiple sclerosis relapse
C. Arístimuño, J. Navarro, M. Martínez-Gines, S. Giménez-Rohdán, E. Fernández-Cruz, S. Sánchez-Ramón, C. de Andréis; General Universitary Hospital (Madrid, E)

Introduction: Multiple sclerosis (MS) is a multifocal chronic inflammatory demyelinating disease of the central nervous system. Axonal damage correlates with the presence of macrophages and CD8+ T-lymphocytes at brain lesions. The gold standard of therapy at MS relapse are IV glucocorticoids (GC). The aim of the study was to assess the changes on the different subsets of circulating CD8+ T-lymphocytes at relapse and after IV GC therapy. Patients and Methods: We consecutively studied 20 patients at MS relapse and 5-days after initiation of IV methyl-prednisolone (MP) therapy (1 g for 3 to 5 days). CD4+ and CD8+ T-lymphocytes subsets were studied by multiparametric flow-cytometry. As control group, 18 healthy subjects were studied. Results: Treatment with IV MP suppressed activated (CD8+CD27−CD45RO+) T-lymphocytes (p<0.05) and effector memory (CD8+CD27−CD45RO+) T-lymphocytes (p=0.07). Furthermore, an increase of CD8+ naive (CD8+CD27+CD45RO−) T-lymphocytes was observed (p<0.002). At MS relapse, there was an inverse correlation between regulatory CD6+CD25+CD28− T-lymphocytes and activated CD4+ (r=−0.66; p=0.012) and CD8+ (r=−0.66; p=0.004) T-lymphocytes. After IV MP treatment, positive correlation between regulatory CD6+CD25+ high T-lymphocytes and CD8+CD25+ T-lymphocytes was observed (r=0.74; p<0.0001). Conclusions: Our data suggest that IV GC may contribute to changes observed on the differentiation of CD8+ T-lymphocytes, namely blocking their complete maturation, and expansion of regulatory CD8+ T-lymphocytes. We hypothesize that IV GC may control axonal damage by this way, and additionally may play a neuroprotective effect on MS relapse.

P386
Long-term corticosteroid administration prevents relapse in neuromyelitis optica
S. Watanabe, T. Misu, K. Fujihara, I. Nakashima, I. Miyazawa, Y. Shiga, Y. Itoyama; Tohoku University School of Medicine (Sendai, JP)

Background: Neuromyelitis optica (NMO) is an inflammatory demyelinating disease characterized by severe, often bilateral optic neurtis and transverse myelitis. Disease-modifying therapy for NMO has not been established, although a combination therapy of azathioprine and corticosteroid, and anti-CD20 monoclonal antibody have been reported to be effective to prevent relapse in the devastating disease. There have been some cases of NMO in which tapering dosage or discontinuation of corticosteroid resulted in relapse. Moreover, NMO can be associated with collagen vascular diseases like systemic lupus erythematosus and Sjogren syndrome. Thus, long-term corticosteroid administration may be effective to lessen relapse in NMO, but such studies are lacking. Patients and Methods: We retrospectively reviewed the medical records 25 cases of NMO who were admitted in Tohoku University Hospital due to relapses from January 1, 2000 to December 31, 2005. Twenty-four of them were women, and median age at onset was 38 years old (range; 22–60). Median follow-up was 58 months (range; 12–165). We compared the annual relapse rate (ARR) during the period of corticosteroid administration (CSP) with the ARR during the period of no corticosteroid administration (NCSP). CSP was defined as the period with oral administration of prednisolone (PSL) for over 60 days (maintenance dose; 5–10 mg/day) after high dose intravenous methylprednisolone (HIMP) at relapse, and NCSP was defined as the period without PSL. (PSL was discontinued within 60 days of HIMP.) Autoantibody status, such as antinuclear antibody (ANA), anti-SS-A antibody (SS-A), and neuromyelitis optica IgG (NMO-IgG), was also compared between CSP and NCSP. Results: The ARR during NCSP (mean ±SD) was 2.0 ± 2.8, while that during CSP was 0.29 ± 0.45 (p<0.05). On the other hand, antinuclear antibody (ANA), anti-SS-A antibody (SS-A), and neuromyelitis optica IgG (NMO-IgG) were examined in 24, 19, and 22 patients and the number of seropositive patients for each antibody was 18, 7, and 12, respectively. As for ANA, a significant difference was seen between the ARR during NCSP and that during CSP (p<0.05), but there was no significant difference for SS-A (p=0.82) or NMO-IgG (p=0.78) between CSP and NCSP. Conclusions: Daily low-dose PSL may be effective to prevent relapse in NMO patients, especially in those who are seropositive for ANA.

P387
A prospective evaluation of oral high-dose methylprednisolone for acute exacerbations in multiple sclerosis
I. Bosca, A. Pascual-Lazano, F. Coret, R. Casanueva; Hospital la Fe (Valencia, E); Hospital Clínico (Valencia, E)

Background and Objective: There is only a limited evidence from clinical trials that support high-dose methylprednisolone (MP) therapy for relapses of MS and practically none supporting oral administration. We assessed the feasibility of treating relapses of MS at home with high dose of oral MP. Method: Twenty-one MS patients who were consecutively presented at our unit with a relapse of less than 2 weeks duration were treated in an open label trial with oral MP 1 gr (in one morning dose), supplements of potassium and 80 mg of omeprazol per day, for 3 consecutive days. All included patients were previously visited every six months and we had previous EDSS and functional scales available. Patients were scored with the expanded disability status scale (EDSS) and the MS functional composite scale (MSFC) before the treatment and after 1 and 4 weeks of treatment. Adverse effects (AE) were also registered. Results: Oral, high dose of MP failed in three patients. After 4 weeks of oral MP treatment, the rest eighteen patients recovered the previous EDSS. In the other hand, after 4 weeks of oral MP treatment the Z-score got worse respect to previous value. No serious AE were seen. The most frequent were digestive and skin symptoms and light headache. Conclusions: 1. Oral high dose of MP is a safe and effective treatment to relapses of MS. A randomized study is still needed to compare this treatment regimen with IV therapy, before the standardization. 2. MSFC was the most sensitive scale to detect a small but permanent injury after relapses, it could help us to asses better the responses to new treatments.

P388
Immunomodulation of experimental autoimmune encephalomyelitis by the novel copolymer PI-2301

Years of clinical experience have shown that daily subcutaneous administration of the peptide copolymer Copaxone™ (Cop-1), a mixture of millions of peptides composed of the four amino acids YEAK in random order, is a safe and efficacious treatment for relapsing-remitting multiple sclerosis (RR-MS). Cop-1 was modeled to mimic myelin basic protein (MBP) and turned out to ameliorate experimental autoimmune encephalomyelitis (EAE) in mice, most likely by shifting the immune response away from an effector T4+1 to an immunoregulatory T4/2/T11 immune response through presentation by MHC class II molecules. Recently, a series of new peptide copolymers have been synthesized with the goal to improve the in vivo efficacy of Cop-1. In the present study, we show that Copolymer PI-2301 produced by substituting E with F has improved beneficial effects in a relapsing-remitting murine model of EAE. Unlike Cop-1,
PI-2301 shows long-term therapeutic efficacy when administered either daily or weekly at disease onset. In an adoptive EAE setting, efficacy of PI-2301 administered daily at the time of autoreactive lymph node cell transfer correlates with decreased serum level of Metalloproteinase-9 and increased level of Metalloproteinase Inhibitor-1. Antibody production against PI-2301 and T-cell recall proliferation assay using splenocytes from PI-2301-treated mice underscores the induction of a Tc2/T3,5 immunity. Evidences of the expansion of T-cells with regulatory properties expressing Forkhead box protein P3 (FoxP3) and producing Interleukin box protein P3 (FoxP3) and producing Interleukin-10 following PI-2301 treatment are also apparent. We propose that PI-2301 is a potential novel immunomodulatory compound for the treatment of RR-MS. Clinical trials will soon be started to test this hypothesis.

P389
Preclinical studies with methylthioadenosine for the treatment of multiple sclerosis
B. Moreno, J. Sepulcre, C. Berasain, F. Corrales, M. Avila, P. Villoslada; University of Navarra (Pamplona, E)

Background: Methylthioadenosine (MTA) is able to prevent acute Experimental Autoimmune Encephalomyelitis (EAE) and ameliorate chronic-relapsing EAE (RR-EAE) by modulating T cell activation, decreasing inflammation and demyelination in the central nervous system (Moreno et al. Ann Neurol, in press). Objective: To conduct the preclinical studies (dose comparison, efficacy comparative study against approved immunomodulatory drugs and toxicity) in order to move it to the clinical phase. Methods: We study the effect of different doses of intraperitoneal MTA in the acute (prevention trial) and chronic EAE (treatment trial) models by quantifying clinical and histological scores and by performing immunohistochemistry stains of the brain. Toxicity was assessed by measuring transaminases levels and by scoring clinical adverse effects and by in cell death vitro assays.

Results: We compared 3 different doses of MTA: 45, 96 and 192 45 uM/kg/day. We found that the lower dose did not suppress acute or RR-EAE. The other higher doses were able to prevent acute EAE and ameliorate RR-MS. We found no clinical signs of side effects and serum transaminases levels were normal in the different groups of animals at the end of the experiment. In vitro assays did not detect signs of toxicity. Conclusions: MTA is efficacious and safe for preventing and ameliorate rodent EAE in a doses-response way. Next preclinical studies will be to compare the efficacy of MTA treatment against standard interferon B and Copaxone and to test oral formulations.

P390
Short-term blockade of CD154 potentiates long term clinical and MRI remission of RRMS: a five-year follow up
L. Kasper, C. Fadul, C. Ryan, J. Smith, H. Wishart, I. Kasper, R. Noelle; Dartmouth Medical School (Lebanon, New Hampshire, USA)

Objective: To assess clinical and MRI progression in subjects with relapsing MS following blockade of CD154, a critical co-stimulatory ligand required for CD4+ T cell effector activation. Background: MS is associated with a Th1 skewed immune response that requires costimulation via CD40/CD154 ligation. Pre-clinical studies have suggested the importance of this co-stimulatory interaction in the pathogenesis of MS. A Phase I clinical trial (Kasper LH et al. ECTRIMS 2003) was performed to assess safety and tolerability in response to anti-CD154 blockade. Design/Methods: For the Phase I trial, four cohorts (n=3 subjects/group) with relapsing MS each received doses of 1, 5, 10, or 15 mg/kg of fully humanized anti-CD154 every other week (n=4 doses/patient). Clinical, laboratory and MRI evaluations were performed at serial time points thru week 18 to assess safety and toxicity. All patients completed treatment without serious adverse events. Thereafter, all patients were followed clinically every 6 months and by MRI with Gd enhancement every year. Patients with acute flares were treated with steroids if indicated. Nine patients elected to be on approved immune modulating drug (3-high dose IFNb, 6- GA) during the first year post anti-CD154 treatment. No immediate effect of therapy on either EDSS or MRI was observed at 18 weeks post treatment.

Results: No statistically significant change in EDSS for the 4 groups of subjects was observed during the five year follow-up (Baseline EDSS 2.3 ± 0.5 and 5 years 2.5 ± 1.6 p = 0.622). A correlation between dose and EDSS was significant (r = −0.51 p < 0.05). Higher dose of anti-CD154 is associated with lower EDSS at 5 years. The average annual relapse rate during the five years was 0.125 (compared to 1.0 without immunotherapy). At 5 years post treatment, MRI lesion volume in subjects treated with anti-CD154 is 1.4 times that of baseline study. 3/12 patients have elected to remain on current approved therapies at 5 years. Conclusion: The 5 year follow-up data for the Phase I trial using anti-CD154 demonstrates a profound reduction in clinical relapse rate that compares favourably to all currently approved drugs. These findings suggest that blockade of CD154 is a potentially important therapy for the treatment of RRMS. These promising long-term clinical outcomes warrant further studies in a closely monitored Phase II trial. Funding for this trial is in place and awaits availability of product. Supported by NIH AI061938.

P391
Daclizumab phase I/II trial in relapsing-remitting multiple sclerosis: MRI and clinical results
J. Rose, J. Burns, J. Bjerklund, J. Klein, H. Watt, N. Carlton; Neurovirology Research Lab VASLCHCS (Salt Lake City, USA)

Objective: A phase I/II trial of Daclizumab in patients with relapsing and remitting multiple sclerosis (RRMS) was performed to determine effects on MRI and clinical parameters. Background: Daclizumab is a humanized monoclonal antibody specific for the Interleukin 2 receptor alpha chain which has shown promising effects either in combination with interferon or as a monotherapy. Methods: MS patients on interferon therapy but with continuing relapses and contrast enhancing lesions (CEL) were selected for participation. Patients were evaluated with monthly MRI scans and clinical rating scales including Timed Ambulation, EDSS and NRS from 3 months prior to Treatment (baseline) and then at 0.5 months to 27.5 months during treatment. Daclizumab (1 mg/Kg) was initiated at baseline and administered again in 2 weeks followed by every 4 week treatments according to a protocol developed at NIH and approved by the University of Utah IRB. Interferon was continued until 5.5 months after Daclizumab was initiated. Patients without continuing CEL were placed on Daclizumab monotherapy and patients with recurrent CEL were continued on interferon with Daclizumab therapy at (1.5 mg/Kg) every 28 days per protocol. The primary outcome measure was the number of total and new CEL with clinical scores as secondary measures. Results: Eight patients qualified for treatment with Daclizumab. One patient developed a severe relapse prior to Daclizumab initiation and therapy was discontinued after two treatments. Seven patients continued on treatment. Two patients were observed to have recurrent new CEL and restarted interferon in addition to an increase in Daclizumab dose. Total CEL and new CEL were compared between pretreatment scans & subsequent scans in 3-month intervals. Significant four fold reductions in total CEL (p < 0.05 to 0.001) and new CEL (p < 0.001) were observed at all seven intervals after treatment initiation. Significant reductions in Timed Ambulation, EDSS & NRS were observed. Treatment resulted in a significant decrease in the number of relapses (p < 0.001). Discussion: Daclizumab appears to be effective in reducing CEL and improving clinical scores in RRMS patients with active disease not controlled by interferon alone. Optimal therapy for some patients may require combination treatment with interferon. These results provide evidence for long-term efficacy and support further clinical development of daclizumab in patients with active relapsing-remitting MS.
P392
Pharmacokinetic and pharmacodynamic properties of the VLA-4 inhibitor CDP323
M. Baker, A. Shock, T. Parton, P. Hales, G. Parker; UCB Celltech Ltd (Slough, UK)

Introduction: CDP323 is a small molecule inhibitor of vascular cell adhesion molecule 1 (VCAM) binding to alpha-4 integrin (VLA-4) in development for the treatment of immune system disorders including multiple sclerosis (MS). Aims and Methods: Three Phase I clinical studies were conducted in 75 healthy volunteers to investigate the pharmacokinetics (PK), safety and tolerability of CDP323 administered for up to 7 consecutive days. Ex-vivo inhibition of a-4/VCAM binding was utilized as a pharmacodynamic (PD) biomarker. An in vitro whole blood VCAM binding assay showed that the IC50 in human blood for CT7758, the active moiety of the prodrug CDP323, was 60 nM (95%CI 33–108). Study 1 evaluated 6 single ascending-dose levels, study 2 was a 7-day, multiple dose study of 3 dose levels, while study 3 was a male/female PK/PD comparison. Results: CDP323 was well tolerated by human volunteers at oral doses up to 1000 mg bid for 7 consecutive days with an adverse event profile comparable to that observed with placebo. A PK model was developed to describe the disposition of CT7758 and an active metabolite, CT533652, which displayed linear kinetics over the doses investigated without gender effect. The PD effect closely followed the plasma concentration time course of the two active compounds and enabled the development of a PK/PD model that described the plasma concentration disposition of both compounds and directly linked these concentrations into an effect model without hysteresis. The IC50 value for the inhibition of VCAM binding derived for CT7758 (incorporating the pharmacodynamic contribution from the metabolite CT533652) from the PK/PD model in healthy volunteers was 63 nM (95%CI 48–78), similar to that derived in vitro. Conclusion: Oral administration of CDP323 resulted in the inhibition of VCAM binding in human volunteers. The development of a PK/PD model has assisted in the selection of dose regimen for evaluation in therapeutic clinical trials, providing evidence that the inhibition can be maintained throughout a 12 or 24 hour dose interval at dose levels well tolerated by human subjects.

Quality of life
P393
Effects of aquatic exercise on fatigue and the quality of life in women with multiple sclerosis
H. Kooshian, M. Moshtagh, M. Sandar, M. Fouroghipour, M. Shakeri; Mashhad University Medical Sciences (Mashhad, IR); Farabi Hospital (Mashhad, IR)

Background: Fatigue is the most common and disabling symptom of multiple sclerosis (MS). It can disrupt the occupational and social function of patients. The participation of the patients is one of the most important factors in developing the health behavior and the life quality. Also exercise is another factor, so the aim of this paper was to detect: Effects of Aquatic exercise on fatigue and the quality of life in women with MS. Methods: This clinical trial was carried out from 2005 to 2006 in Ghaem MS clinic in mashhad, IRAN. So that 40 affected with MS outpatients that their illnesses were approved clinically were selected by Convenience and purposeful sampling. Then samples were divided randomly in two case and control groups. In case group Aquatic exercise were accomplished for 8 weeks and 3 sessions weekly, for 45 minutes each session then levels and severity of fatigue and quality of life were evaluated with modified fatigue impact scale (MFI), fatigue severity scale (FSS) and Mezzich quality of life questioner in two groups before and after study. Results: The mean age of the patients was 30.63 years (ranging from 19 to 45) and the most frequency belonged to houseswives and then university students. in two groups, the rate of relapsing-remitting was (75.7%) and the average of fatigue in case group was (43.81) before and (32.56) after exercise, that the difference was statistically significant (p = 0.0). and the mean of fatigue was also significant between two groups after trial (p = 0.04), the mean of fatigue in case group before and after study at physical and psychosocial level was significantly different as well (p = 0.001) but there were no differences at cognitive level (p = 0.06). and the average difference in fatigue severity in Case before and after exercise were (4.63) and (3.89) respectively, that was significant (p = 0.003) also The mean of the score of life quality in case group were before exercise (63.13) and after it (80.06) respectively, that was significant too (p = 0) and there was a significant difference (p = 0) between the mean of life quality score in control (66.52) and case (80.06) after research. Conclusion: Aquatic exercise could decrease fatigue in MS patients and develop physical-physiological health and quality of life but it wasn't effective in complications related to disease (especially cognitive), therefore exercise can be used as supplement treatment to cope with everyday patients problems.

P394
An evaluation of cognitive analytic therapy for psychological problem in patients with multiple sclerosis
A. Adamopoulou, G. Ganyfallos, M. Paschalidou, K. Katsigiamopoulos, P. Pazarlis, I. Dronos, I. Milonas; Community Mental Health Center-NW District (Thessaloniki, GR); Aristotle University (Thessaloniki, GR)

Objectives: The objective was to investigate the evaluation of Cognitive Analytic Therapy (CAT) in patients with multiple sclerosis (MS) who manifested psychological problem. Material and methods: Twenty-seven (27) patients with MS who visited a Community Mental Health Center (CMHC) in Thessaloniki (Greece) followed an eight (8) session psychotherapeutic program in CAT once per week. All patients completed - before and after the psychotherapy-the Minnesota Multiphasic Personality Inventory (MMPI) and the Eysenck Personality Questionnaire (EPQ). Results: Three (3) patients did not start the psychotherapy and six (6) patients stopped it. All the eighteen (18) patients -who have completed the CAT- were examined in a two (2) month follow-up study with the same questionnaires, and have manifested statistical significant improvement in clinical scales As, D, Hy, Pt, Sc, Sf of MMPI as well as in sum clinical scales. Also they manifested statistical improvement in N and E scales of EPQ. Conclusions: CAT is an effective psychotherapy for psychological problems in MS patients.

Reference

P395
Predicting depression and anxiety in a multiple sclerosis clinic population: the contributions of illness severity, illness management, and perceived cognitive impairment
L.M. Stepleman, K. Lester, M.D. Hughes; Medical College of Georgia (Augusta, USA)

Background: Within the multiple sclerosis (MS) population, high prevalence of psychiatric concerns, such as depression and anxiety, has been well documented in the literature. However, there is less clarity regarding the specific factors that contribute to an MS patient's experience of these psychiatric symptoms at clinically significant levels, especially for anxiety. The purpose of this study was to examine physical, psychosocial, and cognitive variables that predict higher depression and anxiety levels in an MS clinic sample. Method: Eight-two patients utilizing MS clinical services at a regional MS center in the southeastern United States were included in the study. Participants completed survey packets measuring the predictor variables,
including MS physical severity (disability status, duration of illness, and physical impact of MS), perceived illness management (self-efficacy and illness intrusiveness), and perceived cognitive impairment as well as the outcome variables (depression and anxiety).

Results: Results from the hierarchical regression analyses indicated that each set of predictor variables uniquely contributed to the prediction of depression and anxiety (total variance accounted for was 63% for depression and 45% for anxiety). Additionally, cognitive impairment predicted a significant amount of the variance for depression and anxiety over and above MS physical severity and perceived illness management. In particular, increased depression and anxiety were related to shorter duration of illness, higher physical impact of MS, and greater perceived cognitive impairment. Higher illness intrusiveness was related to increased depressive symptoms; however, it did not contribute to a significant amount of variance in anxiety. Self-efficacy was not related to either depression or anxiety in the regression models, but there was a trend toward significance.

Conclusions: The results of this study support past research findings and expand on them, particularly regarding predictors of anxiety in individuals with MS. Overall, the findings of this study support the clinical consideration and further investigation of physical severity, illness management, and cognitive impairment in the treatment of anxiety and depression in the MS population.

P396

Overcoming mental health care barriers for individuals with multiple sclerosis: innovations in psychological consultation

L.M. Stepleman, S.F. Shelton, M.D. Hughes; Medical College of Georgia (Augusta, USA)

Background: The diagnosis and progression of MS can have a profound impact on patient quality of life, especially when compounded by issues of depression, anxiety, and cognition dysfunction. Despite the need for psychological providers, dedicated MS mental health programs are scarce and traditional mental health settings are underutilized due to obstacles like expense, fatigue, and mobility. Stigma and misinformation about psychology also contribute to treatment reluctance. To overcome these barriers, we developed a psychology consultation service concurrent to medical clinics at a regional Multiple Sclerosis Center in the US. Method: In 2003, a needs assessment was conducted by the Department of Psychiatry for the Augusta MS Center (both part of the Medical College of Georgia) to determine how a psychology consult service could best benefit MS patients. As the Center is the largest in the region and serves over 1,000 patients, we anticipated the need for a variety of services. Thus, it was determined that the onsite service would include emotional and cognitive screening, brief evaluation, crisis intervention, limited psychotherapy (1–3 sessions), and referral services. Psychology residents and fellows would staff the psychology service and work along side the MS team. Consultants would be trained in the physical, cognitive, and emotional aspects of MS. Consultations could be alongside the MS team. Consultants would be trained in the physical, residents and fellows would staff the psychology service and work.

Results: Since 2004, the psychology consultation service has played an integral role in the MS Center. During this time period, 418 consultations and psychotherapy interventions have been performed. We have increased the availability of an onsite psychology consultant from 4 hours in 2004 to 16 hours in 2006. Further, funding from foundations and drug companies have made services available regardless of ability to pay.

Conclusions: This model has been successful and has benefits of 1) early detection of distress, 2) a comprehensive multidisciplinary treatment approach, 3) decreased stigma associated with mental health leading to greater utilization, 4) increased well-being resulting in greater compliance with MS medical regimen, 5) decreased transportation and mobility demands by having psychology services onsite and concurrent with medical appointments, and 6) increased quality of life. This psychology consultation model would be applicable to many national and international MS programs and further evaluation is warranted.

P397

Goal disengagement and goal re-engagement among multiple sclerosis patients: relationship to well-being and illness perceptions

E. Neter, A. Litvak, A. Miller; Rappin Academic Center (Emeg Hefer, IL); Tel-Aviv Yafe College (Tel-Aviv, IL); Carmel Medical Center (Haifa, IL)

Background: Research on adaptive human behavior often emphasizes the role of goal attainment, alongside related variables such as optimism, self-efficacy, and persistence. Equally important is giving up personal goals when the latter are unattainable. People’s capacity to withdraw effort and commitment from an unattainable goal is a constant challenge. The goal re-engagement model can be adopted by freeing personal resources to other areas in life and by affecting people’s feelings of mastery and well-being (Wrosch, Scheier, Miller, Schultz & Carver, 2002). The present study extends previous findings to a clinical population of multiple sclerosis patients. Patients with multiple sclerosis are often individuals in the prime of their life, i.e., most are in their 20–40’s; the illness sometimes blocks the attainment of previously set goals, and confronts patients with the need to disengage from these goals and set up new ones.

Methods: 100 MS patients, filled out questionnaires pertaining to goal disengagement and goal reengagement, illness perception, anxiety, depression, purpose in life, self esteem and illness intrusion. Background variables such as demographic characteristics, years since diagnosis and disease stage (i.e., expanded disability status (EDSS) scale) were recorded. Results: Goal disengagement and goal reengagement had good reliability also in this population and were moderately correlated. Goal disengagement and goal reengagement were unrelated to illness perception beside the cure/control scale, so that the more control people felt they had over their disease, the more they were inclined to both disengage and re-engage in new goals. Goal re-engagement was also positively related to purpose in life and self esteem. Regression analyses on depression and anxiety uncovered an interaction between goal disengagement and re-engagement, so that those who were high on disengagement and low on re-engagement were more prone to depression and anxiety. Regression analyses indicated that illness perception variables predicted self-esteem, depression and anxiety and illness intrusion, with identity the most consistent predictor. There were no consistent gender and social support differences in the illness perception variables. Discussion: The difference between purpose in life, on the one hand, and disengagement and goal reengagement is discussed. The centrality of Identity in illness perception is highlighted.

P398

The specialist liaison nurse: Improving care and management of people with MS

D. McArdle, J. Roche, O. Hardiman; Beaumont Hospital (Dublin, IRL)

Multiple Sclerosis (MS) an autoimmune disease is one of the most common neurological conditions and is the commonest cause of disability amongst adults (Paty et al 1997). Introduction: To date much of the nursing care has been provided within the hospital leaving the patients to depend on their own external supports while in the community. The provision of a liaison nursing service contributes to improve quality of care for patients in the community but can help to fast track people who require early intervention and hence reduce waiting time and hospital admissions (Campion, 1996; Lesaux et al., 1999; Winters et al., 1989; Freeman et al 1997). Aim: To improve the quality of life for patients by providing an individualised home visitation by the liaison nurse specialist. Objective: To assess the physical, psychological and emotional needs of the person along with their carers’ needs and to provide an appropriate pathway of care. Method: From 2003 to end 2005 the nurse provided 279 visits within the hospital catchment area. The care pathway used to assess patients was the Roper, Logan, and Tierney model of nursing. Results: Firstly, the results showed: total number of visits in the 3-year period was 279. In 2003–91 visits, 2004–90 visits, 2005–98 visits. The average was 93
showing a 9% increase. Secondly, the result indicates the need for a multidisciplinary approach along with comprehensive planning of home-based intervention implemented by the nurse specialist in conjunction with the interdisciplinary team may provide a cost-effective approach to management of MS and improve quality of life (Pozzilli et al. 2003).

P399
The family caregiver pilot projects and a strategy for supporting caregivers in the MS Society of Canada
M. Temme; MS Society of Canada (Canada, CAN)

From 2000 - 2005, the National Client Services department of the MS Society of Canada developed and evaluated an innovative caregiver program through a two-phase pilot project. Central to this was a Caregiver Wellness Funding Pilot Project which provided funding to a maximum of $300/caregiver. This afforded many opportunities to hear directly from caregivers of people with MS regarding their needs and valued supports.

Three foundational principles guided the projects:

- Caregiver Voice-Caregivers should be involved in the assessment of their needs, have opportunities to express their needs and identify how they feel their needs can best be met.
- Caregiver Choice-Caregivers should have an opportunity to identify and choose individualized service options instead of having to fit into a narrowly or rigidly defined service.
- Respite as an outcome-A caregiver’s relief or renewal, whether emotional, psychological, spiritual, physical and/or social, which results from services and strategies intended to help them maintain their own health and achieve greater balance in their lives.

This in turn enables caregivers to secure better quality of life for themselves and the family members or friends for whom they provide care and support.

To conclude the project, stakeholders from across Canada reviewed the project findings and related implications for the MS Society’s role in supporting caregivers. This resulted in a draft Strategy for Supporting Caregivers of People with MS, that includes a series of fundamental beliefs regarding caregivers arising out of project findings, and recommended actions in support of caregivers. Broader caregiver engagement on this strategy was then sought to ensure that it accurately reflected caregiver beliefs, values and needs. Over 500 additional caregivers responded to a survey on the Strategy. Of these:

- 96% reported that the Fundamental Beliefs capture the important aspects of caregiving
- 97% felt that the Society’s Recommended Actions in support of caregivers will benefit them

The Strategy was approved by the Society’s National Board of Directors in November, 2005 and additional multi-year funding was secured to facilitate its implementation at all levels of the Society from coast-to-coast.

P400
Therapeutic camp intervention for teens with multiple sclerosis
M. Milazzo, P. Block, W. MacAllister, N. Slota, A. Belman, L. Krupp; SUNY Stony Brook (Stony Brook, USA)

Background and Goals: Multiple Sclerosis is an autoimmune disorder which targets the central nervous system and most frequently affects young adults. Although nearly 5% of patients are diagnosed prior to age 18, this fact is unknown to many health care providers. When speaking to friends or relatives, affected children often hear statements such as, “MS doesn’t happen in kids.” They often struggle in school and have difficulty engaging with peers. Parents of children with MS often report feeling “lost.” They rarely have the opportunity to meet other children/parents sharing a similar experience. Available literature of pediatric MS is scant. Factors that predict better coping with chronic illness include external support from friends and health care providers. A Teen Adventure Weekend for adolescents living with MS was developed to bring teens with MS together, to share common experience, and develop a support network for teen and family. Therapeutic camp interventions have been utilized in the treatment of other pediatric conditions such as asthma, diabetes, and HIV infection. Studies have indicated that the benefits of camp-style interventions are far reaching, and improve mood, self-perception, interpersonal functioning, and adherence to treatments. Methods: Weekend retreat interventions were undertaken in the summers of 2004 and 2005. Participants were recruited from across the US. Therapeutic activities included kayaking, sailing, and a ropes course designed to encourage group work. These activities promoted team building and the development of support networks. Post-intervention ethnographic data was collected by a cultural anthropologist via telephone. Results: To date, twenty-eight children and adolescents with MS, ranging in age from 11 to 19 years participated in the program. Post-event interviews have shown a feeling of cohesion and belonging among attendees. Team building was fostered during the program, and post -camp, communication continues among the attendees. Medication compliance, by self-report, has improved since the camp program. Parents relate feeling more connected and less isolated. Conclusion: A therapeutic camp intervention for teens with MS is a positive experience for both affected teen and family. Parental perceived stress and coping is improved and a support network develops among the families.

P401
Longitudinal study of health-related quality of life and disability at early stages of MS
A. Andriaharinony, E. Salort-Campana, M. Deloire, M. Bonnet, K.G. Petry, B. Brochet; University V. Segalen (Bordeaux, F)

Background: Health-related quality of life (QOL) scales have been proposed to measure the broad impact of Multiple Sclerosis (MS) on daily life. Few longitudinal studies examine so far the relationship between disability and QOL at early stage of disease. Objective: To assess if QOL assessment in patients with MS a few weeks after the diagnosis is correlated with disability 3 year later. Methods: A population based sample of 69 consecutively patients were recruited by the AQUISEP network less than six months after a diagnosis of MS. 68 participated to this study, 57 with relapsing-remitting MS (RRMS) and 11 with progressive MS. Each patient were evaluated using the 15 domains of the French validated SEP-59 scale (French adaptation of MSQOL-54), EDSS, MS Functional Composite (MSFC), depression scale (MADRS) and fatigue (fatigue item of UKNDS). Patients were reassessed after one, two and three years. Results: At baseline overall QOL (general well-being domain) was 67.5% (SD 18.2) (normal value = 100%). The mean mental composite score of QOL (MCS, including distress, general well-being, emotional well-being, role-limitation-mental and cognitive domains) was 62.3% (20.8) and the mean physical composite QOL score (PCS, including physical activity, general health, energy, role-limitation-physical, pain, sexual function, social-well-being and distress domains) was 59.0% (18.2) (normal values = 100%). During the 3 years of follow-up only role-limitation physical domain significantly improved in the whole group (p = 0.004) (ANOVA). In RRMS patients social support domain deteriorated significantly during follow-up (p = 0.007). Multivariate analysis showed that overall QOL is significantly correlated with disability and depression at baseline and follow-up. EDSS after
3 years was significantly correlated with overall QOL (r = 0.48; p = 0.0002), MCS (r = 0.44; p = 0.0008) and PCS (r = 0.50; p = 0.0005) at baseline. EDSS deterioration is significantly associated with PCS change during follow-up (r = 0.53; p = 0.0006). **Conclusion:** Overall QOL and QoL composite scores correlate with disability and depression during the 3 years following the diagnosis. This work is supported by grants of ARSEP and Schering France SA.

**P402**

**Health-related quality of life predicts change in disability in multiple sclerosis**

J. Draulovic, T. Risse, M. Nortvedt, T. Pekmezovic, M. Maniguda, S. Mesaros; Institute of Neurology, CCS (Belgrade, CS); University of Bergen (Bergen, N).

The natural history of multiple sclerosis (MS) shows a marked variation when it comes to the gradual development of disability. The aim of our study was to analyze whether the 8 scales of the SF-36 could predict change in the Expanded disability status scale (EDSS) score over a follow-up period of 3 years. A group of 156 clinically definite MS patients, diagnosed according to Posner criteria (1983), were included in this 3-year follow-up study. None of the patients have been evaluated during current MS exacerbation. Additionally, none of these patients has ever been treated with evidence-based disease-modifying therapies neither during the 3-year follow-up period, nor previously. We measured disability and HRQOL at baseline and at 3-years of follow-up. To assess disability status, the EDSS was used, rated by the same neurologists (MM, SM). The patients reported their quality of life using a self-administered SF-36 questionnaire. At a 3-year follow-up retesting, 32 out of 156 patients (20.5%) had dropped out of the study. Out of these patients, 3 could not have been reached due to displacement, 19 were not willing to participate in retesting, and 10 patients died. Eight patients had died from MS-related causes, one patient died from myocardial infarction, and in one case cause of death was unknown. A total of 123 patients had scores of EDSS at baseline and follow-up. The mean score increased from 3.7 at baseline to 4.5 at follow-up three years later. The level of EDSS itself at baseline but who rated their physical functioning better. Those who rated their own physical functioning as low had a higher increase in EDSS compared with patients with the same level of EDSS at baseline but who rated their physical functioning better. This implicates that the patients understanding of their situation gives information important for development of the disease that is not contained in the more objective measure of disease status (EDSS).

**P403**

**Health-related quality of life assessed by MSQoL-54 in Serbian patients with multiple sclerosis: 3-year follow-up study**

D. Kasic, J. Kostic, N. Stojsavljevic, S. Mesaros, T. Pekmezovic, J. Draulovic; Institute of Epidemiology, School of Medicine (Belgrade, CS); Institute of Neurology, CCS (Belgrade, CS).

The aim of our study was assessment of quality of life in patients with multiple sclerosis (MS) over a follow-up period of 3 years by using disease-specific questionnaire MSQoL-54 (Serbian version). This panel study comprised 109 patients with MS diagnosed according to McDonald Criteria (2001). Exclusion criteria were exacerbation of MS in the last month, severe chronic diseases, preexisting psychiatric and psychological disorders, antidepressive and/or corticosteroid therapy in the last month. In order to assess quality of life in relation to physical disability, cognitive function, depression and anxiety, fatigue, and sexual and sphincter function, following instruments and scales were used: MSQoL-54, EDSS, MMS, Hamilton depression and anxiety rating scales, Fatigue severity scale, Sasz's scale for sexual functioning and questionnaire for sphincter functions examination. We measured HRQOL and aforementioned clinical parameters at baseline and at 3-years of follow-up. In data analysis, correlation and regression analyses were used. Between baseline and at 3 years of follow-up, statistically significant differences in scales of MSQoL-54 were registered in domains of physical health (p = 0.001), energy (p = 0.001), cognitive functioning (p = 0.001), health distress (p = 0.001) and sexual functioning (p = 0.001). In the multiple regression analysis, significant predictive value for depression was registered for the following domains: physical health (beta coefficient = -0.529, p = 0.006), emotional functioning (beta coefficient = -0.397, p = 0.001), energy (beta coefficient = -0.344, p = 0.003), health perception (beta coefficient = -0.381, p = 0.001), and social (beta coefficient = -0.306, p = 0.001) and sexual functioning (beta coefficient = -0.396, p = 0.001). Our findings suggest that certain MSQoL-54 domains could be predictive factors for worsening of the depression in MS patients.

**P404**

**Culture-specific differences in the linguistic expression of functional disability in multiple sclerosis: implications of a pilot study on German and Polish translations of the English FAMS Version 4**

A. Pluta-Fuerst for the Austrian-German-Polish Study Team

**Background:** The Functional Assessment of Multiple Sclerosis (FAMS) and its translations are validated instruments measuring health-related quality of life (QOL) in MS. Some of the items deal with culture-specific (CS) concepts which may result in response shifts despite appropriate translation. This study looked for differences between the patients’ perception of CS and unspecific items in the German and Polish FAMS. Methods: Patients in Austria (A), Germany (D), and Poland (PL) completed the FAMS. They were selected from 3 EDSS groups (EDSS < 3.5; EDSS 3.5–6.0; EDSS > 6.0), and stratified according to sex (ratio 1:1) and age (20–60 years). Patients with no FAMS completion within the previous 12 months, with the target language (TL) as mother tongue and means of communication with both parents, and school education in TL were included. Results: The data are consistent (n = 389, Cronbach Alpha = 962) showing uniformly a decreasing QOL with increasing EDSS in all centres. Patient groups in A, D, and PL were comparable as to mean age, disease duration, income, professional activity, and marital status. Mean EDSS was slightly lower in patients from A (4.23; p < 0.005) than in those from D and PL (4.3 and 4.45, respectively). Polish patients reported living in bigger families (3.28 members; p < 0.001) than did patients in A (2.45) and D (2.39). The total FAMS score was highest for patients from A followed by those from D and PL (p < 0.05) which reflects a gradual decrease in QOL between the 3 countries. For CS items, there was a significant relationship between the patients’ country and their answers (p < 0.001) with a difference between the data from Austrian patients versus those from D and PL, the former being lower (p = 0.017), the latter higher (p < 0.001) than expected in view of the total FAMS score. Conclusions: The FAMS is reliable showing a strong association with patient disability. There are differences in the way patients in A, D, and PL interpret CS items. These differences become most evident when comparing Austrian patients with those from D or P. German patients seem more pessimistic, Poles more optimistic in their QOL assessment. In this context, a potentially relevant aspect is the fact that family structure analyses show Polish MS families to be bigger (with a higher number of both adults and children) than those in A or D. Supported by an unrestricted educational grant from Schering AG Berlin/Germany, in co-operation with Schering Austria GmbH and Schering Polan.
Multiple sclerosis quality of life inventory in patients at risk for clinically definite multiple sclerosis: results from the CHAMPIONS trial

D.M. Miller, C. Kolbman, R.P. Kinkel for the CHAMPIONS STUDY GROUP

The negative impact of multiple sclerosis (MS) on quality of life (QoL) is well documented in persons with moderate to severe clinically definite MS (CDMS). QoL consequences for those at risk for or recently diagnosed with MS are not understood. These individuals have persistent symptoms not found on clinician assessment. We evaluated Multiple Sclerosis Quality of Life Inventory (MSQOL) scores, which includes the SF-36 and 9 disease-specific scales in a group of 5 patients 5 years after they were determined to be at risk for CDMS. CHAMPIONS is an ongoing open-label study for participants from the earlier, 2-year, CHAMPS study of the benefit of intramuscular interferon beta-1a treatment for individuals at risk for CDMS. The MSQOL was administered to CHAMPIONS subjects 5 years after enrolling in CHAMPS. 196 of 203 patients enrolled in CHAMPIONS (97%) completed the MSQOL at their 5-year visit. Of the 196 patients, 82 had developed CDMS by the 5-year visit. There were significant differences (p < 0.001) in 7 of 11 MSQOL subscales in patients who had developed CDMS compared with patients who had not. Patients who had developed CDMS reported more fatigue (Modified Fatigue Impact Scale score 33.5 ± 18.0 vs. 21.5 ± 17.5) and pain (Pain Effects Scale score 12.9 ± 6.2 vs. 9.2 ± 4.8), more problems with bladder (Bladder Control Scale 3.2 ± 4.9 vs. 1.0 ± 2.8) and bowel control (Bowel Control Scale 2.8 ± 4.2 vs. 1.0 ± 1.9), and more cognitive impairment (Perceived Cognitive Deficits Questionnaire score 24.8 ± 17.2 vs. 18.0 ± 14.5) than patients without CDMS. Patients with CDMS also had lower scores for the SF-36 Physical Component (43.1 ± 11.8 vs. 50.5 ± 8.5) and the Mental Health Inventory (61.3 ± 18.4 vs. 68.2 ± 17.8). There were no differences between patients with CDMS and those without in SF-36 Mental Summary, Sexual Satisfaction Scale, Impact of Visual Functioning Scale, or Modified Social Support Scale. Based on the results of the MSQOL, patients with CDMS have decreased quality of life compared with patients who are at risk for, but have not yet developed, CDMS.

Multiple sclerosis quality of life inventory in patients at risk for clinically definite multiple sclerosis: results from the CHAMPIONS trial

D.M. Miller, C. Kolbman, R.P. Kinkel for the CHAMPIONS STUDY GROUP

The negative impact of multiple sclerosis (MS) on quality of life (QoL) is well documented in persons with moderate to severe clinically definite MS (CDMS). QoL consequences for those at risk for or recently diagnosed with MS are not understood. These individuals have persistent symptoms not found on clinician assessment. We evaluated Multiple Sclerosis Quality of Life Inventory (MSQOL) scores, which includes the SF-36 and 9 disease-specific scales in a group of 5 patients 5 years after they were determined to be at risk for CDMS. CHAMPIONS is an ongoing open-label study for participants from the earlier, 2-year, CHAMPS study of the benefit of intramuscular interferon beta-1a treatment for individuals at risk for CDMS. The MSQOL was administered to CHAMPIONS subjects 5 years after enrolling in CHAMPS. 196 of 203 patients enrolled in CHAMPIONS (97%) completed the MSQOL at their 5-year visit. Of the 196 patients, 82 had developed CDMS by the 5-year visit. There were significant differences (p < 0.001) in 7 of 11 MSQOL subscales in patients who had developed CDMS compared with patients who had not. Patients who had developed CDMS reported more fatigue (Modified Fatigue Impact Scale score 33.5 ± 18.0 vs. 21.5 ± 17.5) and pain (Pain Effects Scale score 12.9 ± 6.2 vs. 9.2 ± 4.8), more problems with bladder (Bladder Control Scale 3.2 ± 4.9 vs. 1.0 ± 2.8) and bowel control (Bowel Control Scale 2.8 ± 4.2 vs. 1.0 ± 1.9), and more cognitive impairment (Perceived Cognitive Deficits Questionnaire score 24.8 ± 17.2 vs. 18.0 ± 14.5) than patients without CDMS. Patients with CDMS also had lower scores for the SF-36 Physical Component (43.1 ± 11.8 vs. 50.5 ± 8.5) and the Mental Health Inventory (61.3 ± 18.4 vs. 68.2 ± 17.8). There were no differences between patients with CDMS and those without in SF-36 Mental Summary, Sexual Satisfaction Scale, Impact of Visual Functioning Scale, or Modified Social Support Scale. Based on the results of the MSQOL, patients with CDMS have decreased quality of life compared with patients who are at risk for, but have not yet developed, CDMS.

Quality of life in multiple sclerosis patients in Kaunas, Lithuania

R. Balnyte, L. Malciene, V. Matjosaitys, V. Pauza; Kaunas Medical University Hospital (Kaunas, LT)

Background: Multiple sclerosis (MS) is a chronic disease that does not reduce life expectancy but has a great impact on quality of life. Quality of life studies show patient’s physical, psychological and social state as well as one’s activity, relationship with family members and society. Aim: To establish quality of life in patients with MS older than 16 years in Kaunas city. Methods: In this study we used the general type QOL (quality of life) WHO (World Health Organization) – 100 questionnaire, that thoroughly describes the way patient feels and one’s subjective attitudes towards 7 topics. The validity of the questionnaire was assessed by Pirson correlation coefficient and the stability – by Cronbach alfa index. The cases included were all the patients whom MS diagnosis was established according McDonalds diagnostic criteria from January 1, 2001 till December 31, 2002. Sources of information were obtained from outpatient clinics and neurology departments of city hospitals and Kaunas Medical University Clinic’s department of radiology. Results: 172 patients (54 men and 118 women) had a first diagnosis of MS. At first, by using the general type QOL WHO – 100 questionnaire, the eligibility of the questionnaire itself was assessed. The deterministic values of questionnaire’s validity and stability revealed that QOL WHO – 100 can be used to evaluate MS patients’ quality of life. The general evaluation of MS patients’ quality of life was compared with the respective data in the control group. The were no patients in MS group to evaluate this field as “very good”. In the control group QOL was evaluated as very good by 1.4%. The leading QOL evaluations in MS patients were “neither good nor bad” and “bad” – 34.8% and 33.3%, respectively. Only 11.8% patients in the control group evaluated their QOL as “bad”. The difference among QOL assessment in MS and control group was statistically significant (p < 0.001; Δ = 32.43). Conclusions: The established validity (moderate ant strong correlation among the topics) and stability (very strong connection among the questions) of the QOL WHO – 100 allow using the questionnaire for MS patients QOL assessment. In comparison with the random control group, the MS patients evaluated their QOL, physical state, the degree of indepenedency and working capacity worse.

Validity and reliability of Persian version of the MSQol-54 questionnaire

A. Borhani Haghighi, H. Gharem, P. Jafari, A. Niskeresht; Shiraz University of Medical Sciences (Shiraz, IR)

Background: The MSQol-54 questionnaire is one of the most widely used instruments for evaluation of the quality of life in patients with multiple sclerosis. Objective: To translate and test the reliability and validity of 54-item multiple sclerosis quality of life questionnaire (MSQol-54) in Iranian MS patients. Methods: Using a standard “forward-backward” translation, cognitive debriefing and cultural adaptation procedure; the English version of the questionnaire was translated to Persian language. The reliability and internal consistency of the questionnaire were assessed by Cronbach’s alpha coefficient and Spearman, s correlation, respectively. Validity was evaluated by convergent validity and factor analysis. Results: 141 MS patients entered into the current study. The mean ± SD age of respondents was 32.2 ± 9.8 years. Reliability analysis showed satisfactory result (Cronbach, s = 0.962). There were no significant differences between each item and mean physical and mental scores of MSQol-54 by sex, marital status, education, The scaling success rates were 100% for convergent validity of each scale. Conclusion: The Persian version of the MSQol-54 questionnaire is a reliable and valid instrument for measuring the effect of MS on quality of life in Iranian patients.

Perceptions of MRI research in patients with clinically isolated syndromes

R. Lanyon, C. Dalton, J. Swanton, G.T. Plant, A.J. Thompson, D.H. Miller; Institute of Neurology (London, UK); National Hospital of Neurology and Neurosurgery (London, UK); Moorfields Eye Hospital (London, UK)

Introduction: We have been involved in longitudinal MRI research in multiple sclerosis for many years, during which time the McDonald diagnostic criteria have been introduced. These criteria have particular relevance for research subjects who had initially been recruited with clinically isolated syndromes (CIS). In this survey, we explore CIS patients’ experiences of our research, including; (i) their reactions to the scientific and clinical information with which they were provided, given its relevance to a possible diagnosis of MS; (ii) their response to the actual MRI procedures involved. Methods: An anonymous questionnaire was used to survey a group of CIS patients who were research subjects in a longitudinal MRI study. Of the 172 patients in the MRI study, the questionnaire was given to the ~ 60 who attended for follow-up between Feb-Sep 2005. Results: A total of 38/ ~ 60 questionnaires were returned. The response to the research study was positive-none of the subjects felt it had put them off taking part in future research. Most participants wanted more information both about the science of the study and about its meaning for them as patients: 34/37 subjects requested more information about the...
Treatment of specific symptoms

P409

Auditory feedback for improvement of gait in patients with multiple sclerosis

Y. Baran, A. Miller; Technion (Haifa, IL)

Objective: To study the use of auditory feedback for gait management and rehabilitation in patients with Multiple Sclerosis (MS). Methods: An auditory feedback cue, responding to the patient’s own steps in closed loop, was produce by a wearable motion sensor and delivered to the patient through ear phones. On-line (device-on) and residual short-term therapeutic effects on walking speed and stride length were measured in fourteen randomly selected patients with gait disturbances predominantly due to cerebellar ataxia. Results: Patients showed an average improvement of 12.8% on-line and 18.75% residually in walking speed. Average improvement in stride length was 8.30% on-line and 9.93% residually. The improvement results are particularly noteworthy when compared with the lack of change in healthy control subjects. Conclusions: Patients with MS using auditory feedback cues showed improvement in walking abilities.

P410

Long-term open-label treatment with Sativex® in patients with multiple sclerosis

C.S. Constantinescu, N. Sarantis; Queens Medical Centre (Nottingham, UK); GW Pharma Ltd (Salisbury, UK)

Background: Randomised placebo-controlled trials have shown standardised whole plant cannabis medicine (Sativex®) to have a positive effect on many symptoms associated with Multiple Sclerosis (MS). On completing these trials patients could enter long term open label safety studies, the results of which are presented alongside the safety results from acute short term trials. Methods: Sativex was administered as an oromucosal spray and was self titrated by patients. Symptom status was assessed through questionnaires and patient diaries. The safety endpoints were the incidence of adverse events (AEs), serious adverse events (SAEs) and clinical laboratory parameters. Results: A total of 930 patients entered the comparative placebo-controlled study, of which 444 were eligible and chose to enter a long term open-label study with a positive experience of the research project. Where this was not the case, the main reason was lack of information about the link between CIS and MS. To improve patient perceptions of research it may be helpful to provide additional information, and to ensure all information is delivered in the most appropriate manner. Verbal discussion may be the best means of delivering sensitive information.

P411

Randomised controlled study of cannabis-based medicine (Sativex®) in patients suffering from multiple sclerosis associated detrusor overactivity

D. de Ridder, C.S. Constantinescu, C. Fowler, R. Kavia, N. Sarantis; UZ Gasthuisberg (Leuven, B); Queens Medical Centre (Nottingham, UK); The National Hospital of Neurology & Neurosurgery (London, UK); GW Pharma Ltd (Salisbury, UK)

Bladder problems are a common feature of Multiple Sclerosis (MS), with up to 80% of MS subjects experiencing voiding dysfunction. Methods: A 10 week double blind, randomized, placebo controlled parallel group trial was conducted. After a 2 week baseline period, 135 subjects with MS and detrusor overactivity were randomized to receive either Sativex (a standardised whole plant cannabis medicine), or placebo. The primary endpoint was a reduction in the daily number of episodes of urgency incontinence. Other end points included incidence of nocturia and urgency, overall bladder condition (measured on an 11-point numerical rating scale), daytime frequency, quality of life, patient’s global impression of change (PGIC) and volume voided. Results: For the primary endpoint, the decrease from baseline in incontinence episode frequency per day was in favour of the Sativex treated group but was not statistically significant (−1.08, p = 0.57). Of the secondary/tertiary end points, 10 of the 11 were in favour of Sativex. Conclusions: Sativex showed improvement in incontinence episode frequency per day was in favour of the Sativex treated group but was not statistically significant (−1.08, p = 0.57). Of the secondary/tertiary end points, 10 of the 11 were in favour of Sativex. In 4 out of 7 secondary end points there was statistical significance in favour of Sativex. These were reduction in nocturia episodes (−0.28, p = 0.010); highly statistically significant improvement in patient’s opinion of bladder symptom severity (−1.16 points, p = 0.001); reduction in the number of voids per day (−0.85, p = 0.007) and PGIC where 83.6% of subjects receiving Sativex compared with 58.2% receiving placebo considered the status of their bladder condition had improved (odds ratio 2.56, p = 0.005). The decrease in number of urgency episodes in Sativex treated subjects just failed to reach statistical significance (−0.76, p = 0.071). Of the tertiary end points, the number of daytime voids was statistically significantly in favour of Sativex (−0.57, p = 0.044). There was a trend in favour of improvement in Quality of Life but which did not reach statistical significance. Conclusions: Sativex treatment had a positive impact on the symptoms of overactive bladder in multiple sclerosis patients. It provides qualitative and quantitative symptomatic improvement and a normalisation of the symptoms of urinary frequency for many subjects with MS and further research is warranted.

P412

A randomised controlled study of Sativex® in patients with symptoms of spasticity due to multiple sclerosis

C. Collin, Z. Ambler, R. Kent, R. McCalla; Royal Berkshire and Battle NHS Trust (Reading, UK); Neurologica Klinika FN (Pizen, CZ); Pinderfields General Hospital (Wakefield, UK); GW Pharma Ltd (Salisbury, UK)

Muscle spasticity is a common clinical problem in about 60% of patients with multiple sclerosis (MS) often leading to considerable
In the present study we wanted to determine whether

Objective: (Ancona, I)

R. De Sisto, B. Viti, D. Minardi, G. Muzzonigro; Villa Adria Santo Stefano

significant. This did not extend to the ITT populations but in this, and
treatment with the best available

levels of spasticity despite ongoing treatment with the best available treatment.

in favour of Sativex with a treatment
difference of −0.23 points (p = 0.219; 95% CI: −0.59, 0.14). In the PP population 36% of patients achieved at least a 30% improvement in spasticity NRS with an odds ratio of 1.74 (95% CI: 0.005, 0.266). This trend was also observed in the ITT population with an odds ratio of 1.34 in favour of Sativex. These findings were supported by the CGIC assessment which was strongly in favour of Sativex (Odds ratio 1.25, p = 0.270; 95% CI: 0.84, 1.85). The following secondary endpoints showed trends in favour of Sativex: spasticity and sleep assessments at clinic visits, Modified Ashworth Scale; timed 10-metre walk, quality of life questionnaire EQ-5D, sleep quality, review of pain, tremor and fatigue, spasm severity and bladder symptoms. When the data from this and a previous study were pooled, a statistically significant difference in spasticity in favour of Sativex was seen in the ITT population (−0.34, 95% CI: −0.64, −0.04, p = 0.027).

Conclusions: The patients randomized in this study exhibited severe levels of spasticity despite ongoing treatment with the best available anti-spasticity treatments. In the PP population the reduction in symptoms of spasticity in the Sativex-treated group was statistically significant. This did not extend to the ITT populations but in this, and other secondary endpoints, the outcomes were in favour of Sativex.

P413
Lower urinary tract disorders and multiple sclerosis: role of sacral neuromodulation
R. De Sisto, B. Viti, D. Minardi, G. Muzzonigro; Villa Adria Santo Stefano (Ancona, I); University of Ancona (Ancona, I); University of Ancona (Ancona, I)

Objective: In the present study we wanted to determine whether sacral neuromodulation benefits patients with bladder symptoms caused by multiple sclerosis (MS).

Methods: Ten patients with MS underwent unilateral implantation of a sacral neuromodulation system, InterStim (Medtronic Inc.), between April 2001 and October 2004; the mean follow-up was 28.6 months (range 24–36). The following parameters were evaluated before and after implant of the neuromodulator device: number of daily voidings, number of incontinent episodes, residual urine, Wexner score, quality of life (Qol), and psychological impact. Result: There was an overall 81.4% decrease of urgency and frequency with a significant decrease in the number of upper urinary tract infections and fever; there was an overall 51.8% improvement in the Qol. and a discernible improvement emotional well-being. Conclusion: Unilateral chronic sacral neuromodulation can be a valuable treatment for neurogenic bladder disorders associated with MS.

References

P414
Plasma exchange for severe and refractory optic neuritis
L. Ramí-Torrenta, M. Aguirregomozcorta, J. Tarrés, N. González, M. Vallet, V. Medina; Hospital Universitari Dr. Josep Trueta (Girona, E)

Introduction: Optic neuritis (ON) may occur in isolation or associated to systemic autoimmune disorders. It usually has a good prognosis but in some cases reduced visual acuity (VA) persists despite treatment with high doses of glucocorticosteroids (hd-GC). Plasma exchange (PE) may be a therapeutic option for these severe unresponsive cases. Objective: To describe two cases of severe ON which responded poorly to hd-GC but showed a good functional recovery after PE. Methods: The clinical features and results of blood tests and cerebrospinal fluid (CSF) analyses, cranial, spinal and orbital MRI, visual evoked potentials (VEP), and evolution before and after PE of two patients are described. Results: Both patients were women (18 and 26 years old) and had clinical features of ON. Patient A presented a VA of 20/40 with an atidirectional defect, afferent papillary defect (APD), positive Ishihara test and swollen optic disc. Cranial, orbital and spinal MRI, CSF analysis and complete blood tests were all negative or normal. No oligoclonal bands (OCB) were found. VEP were strongly abnormal in the affected eye. She was treated twice with hd-GC with no clinical response. 78 days after the initial symptoms, PE was performed. Her VA started to recover during the treatment and 4 months later was 20/20 with small non-significant scotoma and normal VEP. Patient B started with left ON with VA <20/400. She was treated with hd-GC. One week later she began to have right ON with VA 20/400. She had APD, bilateral positive Ishihara test and normal optic discs. She was treated again with hd-GC. Complete blood tests were normal including IgG neuromyelitis optica autoantibody and their VEP were both strongly affected. CSF analysis showed high protein levels, 10 mononuclear cells and positive OCB. MRI showed enlargement of both optic nerves, three non-specific subcutical bilateral hyperintense lesions, and demyelinizing lesions in the left cerebellar peduncle, cervical spine and conus medullaris. 15 days after the second episode of NO she had right VA 20/400 and left VA 20/200 leading us to perform PE. She started to recover during the treatment and 1 month later her VA was 20/70 on the right and 20/40 on the left. Conclusion: Plasma exchange may be indicated in the treatment of severe optic neuritis which has previously failed to respond to standard hd-GC therapy. Clinical features, short term evolution after hd-GC, and VEP results may help in deciding on whether to use this treatment.

P415
Activity of levetiracetam on multiple sclerosis cerebellar symptoms evaluated by kinematic analysis: a single-blind placebo cross-over study
C. Solaro, G. Brichetto, E. Capello, S. Albuqurk, G.L. Mancardi, P. Tanganelli, V. Sanguineti; ASL 3 (Genoa, I); University of Genoa (Genoa, I)

Introduction: In multiple sclerosis (MS) patients symptoms and signs referable to cerebellar impairment occur in about one-third of subjects leading to static and kinetic tremor that might preclude preclude effective use of the extremities. Unfortunately medical and rehabilitation therapies are of limited efficacy. Levetiracetam (LEV) is a novel anti-epileptic drug whose efficacy and safety have recently been demonstrated in patients with partial seizure as add-on medication or mono-therapy. Furthermore, recently, an improvement of severe cerebellar tremor with LEV in three MS patients has been. The aim of the study was to evaluate the activity, tolerability and efficacy of LEV, measured by kinematic analysis, in MS patients affected by cerebellar symptoms, in a single blind, placebo controlled cross-over study. Materials and Methods: The study used was a single blind controlled crossover design with four assessments at T0 (baseline – day one), T1 (after 21 days of treatment), T2 (after wash our period – day 35) and T3 (after 21 days of treatment – day 56). The medication used was levetiracetam standardized to 250 mg capsule or an identical
Oxcarbazepine for treating paroxysmal painful symptoms in multiple sclerosis: a pilot study

C. Solaro, D. Restivo, G. Bricchetto, P. Tanganelli; ASL 3 (Genoa, I); Garibaldi Hospital (Catania, I); University of Genoa (Genoa, I)

Oxcarbazepine (OXC) is a keto-analog of carbamazepine recently approved as monotherapy for partial onset seizure. Open-label studies have demonstrated efficacy in the treatment in several neuropathic pain. In multiple sclerosis (MS) patients painful paroxysmal symptoms (PPS) affect approximately 10% of patients. We performed an open-label pilot study of OXC at a dosage range of 600–1200 mg/die in a group of MS patients with PPS. Twelve MS patients with PPS, previously treated with gabapentin or carbamazepine, participated at the study. Eight subjects were female and 4 male, with a mean age of 43.6 years, mean disease duration of 7.3 years, mean score at the EDSS of 3.2. Ten patients had a relapsing-remitting disease course, 1 had secondary progressive and 1 had primary progressive course. PPS were defined as transient painful symptom at any body area, with abrupt onset, brief duration, from a few seconds to a few minutes, repetitive and stereotyped features. The subjective level of the PPS was scored using a three-point scale previously described. The mean dosage of OXC was 1033 mg daily. Nine patients experienced a complete and sustained recovery within 1 month from treatment initiation. Two patients (8.5%) dropped out of the study due to adverse effects: 1 case of nausea and dizziness, 1 case of Hyponatriemia (serum Na lower than 125 mmol/l). The medication was well tolerated in the majority of the subjects. The study results provide a new possibility for treating painful pictures in MS and efficacy on PPS must be confirmed in a larger study.

Menopause in multiple sclerosis

M. Valití, I. Sornóvá, R. Talád; MS centrum FN HK (Hradec Králové, CZ); Faculty Hospital Charles University (Hradec Králové, CZ)

Introduction: The effect of hormones on the course of multiple sclerosis is still one of the shadiy areas around this severe neurologic disease. Correct evaluation of the effect of hormones on the disease progression and appropriate indication of hormone replacement therapy is another of available therapeutic options. Method: The analyzed sample included 16 female patients with MS who started hormone replacement therapy in postmenopausal period. The main indications was severe combined osteoporosis and symptoms of estrogen deficiency, which may sometimes be masked by MS symptoms. All patients underwent gynecologic examination and their hormone profiles were tested. Analyzed parameters included age, CGI- Clinical Global Impression and EDSS (Expanded Disability Status Scale) after the first year of therapy and the type of therapy. Results: Analysis of therapeutic response based on global improvement in CGI showed no worsening in any of the patients, 60% improved significantly, 20% improved slightly and 20% did not change. No significant effect on EDSS was detected after a year of treatment. The most common treatment used was combined HRT (estrogens and progestins) in 75% of patients, 15% were treated with estrogens only (patients after hysterectomy). 10% of patients were given local therapy. Discussion: How can menopause affect MS? Very significantly, since even in healthy women is this period often associated with a decrease in quality of life. Symptoms of climacteric syndrome appear in up to 75% of women and more than half of female MS patients show worsening of their condition after menopause. There are currently no reasons to hesitate with starting hormone replacement therapy in indicated cases of patients with MS, but the problem is, however, that symptoms of estrogen deficiency may overlap with symptoms of MS. Analysis of CGI scores showed no worsening in any of the patients and only 20% showed no reaction to the therapy. No significant change in EDSS was detected after a year of therapy. Treatment was very well tolerated and without any negative influence on MS. Conclusion: Based on scientific evidence, hormone replacement therapy is currently considered a good treatment option for climacteric syndrome and prevention and therapy of postmenopausal osteoporosis. Early start of therapy improves significantly the quality of life of patients and sometimes has beneficial effects on MS.
Final analysis of combination therapy (Provigil® + Avonex®) in the treatment of cognitive problems in patients with relapsing-remitting multiple sclerosis

J.A. Wilken, M.T. Wallin, C.L. Sullivan, R.L. Kane, H. Rossman, S. Lawson, J. Simssarian, C. Saunders, R. Shin, J. Mikszewski, D. Kerr, M.E. Quey; Veterans Affairs Medical Center (Washington, USA); Veterans Affairs Medical Center (Baltimore, USA); Michigan Institute for Neurological Disorders (Farmington Hills, USA); Neurology Center at Fairfax (Fairfax, USA); Northern Virginia Neurology Associates (Arlington, USA); Johns Hopkins University Medical Center (Baltimore, USA)

There is evidence that treatment of multiple sclerosis (MS) with interferons can slow the progression of cognitive dysfunction associated with the disease. Scant data exist, however, regarding the treatment of breakthrough cognitive symptoms (e.g., attention, processing speed, and memory problems). Although studies have recently demonstrated the benefits of using modafinil as an adjunctive therapy to treat fatigue caused by MS, there are no data on whether this treatment translates into improved functioning in patients with cognitive impairment that progresses even while on a disease-modifying agent. The objective of this study was to determine whether combination therapy (modafinil plus interferon beta-1a, Avonex) was safe and effective in treating the progression of cognitive deficits in MS. Preliminary data from this study was presented at prior scientific conferences (AAN, CMSC). The current pilot study had a multi-centered, randomized, parallel-group design and was intended to assess safety and provide preliminary data regarding efficacy of adjunctive treatment with modafinil. MS patients already taking interferon beta-1a (Avonex) completed an attention screening battery. Those patients who demonstrated significant attention problems were randomized to receive modafinil (200 mg/day) or no additional treatment. Evaluators were blinded to medication status. Subjects underwent a complete neuropsychological battery (including measures of mood and QOL) at baseline and four months. Side effects were closely monitored to determine the relative safety of this combination therapy. 59 patients were enrolled: 29 in the combination group and 30 in the IFN-beta-1a alone group. 48 patients completed the 4-month evaluation. At baseline, mean age was 47.1 ± 10.03 years, mean years of education was 14.85 ± 2.19, mean EDSS score was 3.95 ± 2.06, and mean estimated Full Scale IQ was 107.72 ± 7.49. There were no significant differences in demographic variables between groups. Side effects of combination therapy were mild and no different from those described in the package inserts for both medications. Compared with the IFN-beta-1a alone group, patients in the combination therapy group demonstrated significant improvement from baseline on neurocognitive, fatigue, mood, and QOL measures at 4 months. In this pilot study, the use of modafinil in addition to interferon-beta-1a (Avonex) for breakthrough cognitive symptoms appeared to be safe and effective.

Comprehensive care & rehabilitation

A comprehensive, personalized and intensive neurorehabilitation program with a multidisciplinary team for persons with multiple sclerosis. A pilot study

Yanely Real Gonzalez, J.A. Cabrera-Gomez, A. Echenenda del Valle, A. Gonzalez-Quevedo, G. Penton-Rol, M. Cervantes, A. Aguiar-Rodriguez, G. Martinez-Aching, Y. Lopez-Perez, M. Lopez-Hernandez, J. Torres-Lizarrago, R. Gomez-Perez, C. Fernandez, A. Gonzalez-Calvo; International Neurological Restoration Center (CIREN) (Havana City, CUB); Institute of Neurology and Neurosurgery (Havana City, CUB); Institute of Psychology and Neurosurgery (Havana City, CUB); CBGB (Havana City, CUB)

Introduction: The recent disease-modifying therapies have not eliminated the need for Neurorehabilitation strategies in the management of MS. Objective: To evaluate the efficacy of a comprehensive, personalized and intensive Neurorehabilitation program for persons with MS. Methods: Persons with Multiple Sclerosis (McDonald et al) were included to receive an integrated, comprehensive and intensive Neurorehabilitation. The program was during 4–8 weeks (2 sessions daily), except on week-ends; each patient had a single physiotherapist (one to one). The evaluation of impairment-disability by means: Scripps Neurological Rating Scale (SNRS)/EDSS; Fatigue Impact Scale (FIS) to evaluate fatigue and quality of life (QoL) by MSQLI-54 instrument. Results: Seventeen persons with MS (11 progressive, 6 relapsing-remitting), 11 (64.7%) female with mean SD 8.76 ± 5.32 years of disease were included. All the cases completed 4–8 weeks of an Intensive Neurological Rehabilitation. The evaluation of impairment/disability: SNRS initial mean SD 54.29 ± 19.14 (25; 90), 4 weeks mean SD 74.6 ± 18.94 (36; 95) p = 0.000, 8 weeks mean SD 69.63 ± 11.31 (53; 86) p = 0.003. EDSS was: initial mean SD 5.4 ± 2.02 (2; 8.5), 4 weeks mean SD 4.55 ± 2.62 (1; 8.5) p = 0.01 and 8 weeks NS student t-test. FS demonstrated in pyramidal (initial mean SD 3.71 ± 1.10 (1; 5), 4 weeks mean SD 2.90 ± 1.59 (1; 5) p = 0.022, 8 weeks mean SD 3.08 ± 1.30 (1; 5) p = 0.006; in Cerebellar (initial mean SD 0.94 ± 1.14 (1; 5), 4 weeks mean SD 1.00 ± 0.816 (0; 2) p = 0.000, 8 weeks mean SD 2.38 ± 0.916 (1; 4) p = 0.002; in Brain Stem initial 1.88 ± 1.166 (0; 4), 4 weeks NS, 8 weeks mean SD 1.38 ± 1.302 (0; 4) p = 0.007; in Sensotorial initial mean SD 4 and 8 weeks NS; in Bowel and Bladder initial mean SD 1.41 ± 1.661 (0; 5), 4 weeks mean SD 0.70 ± 1.232 (0; 4) p = 0.022, 8 weeks NS; in Cerebral initial mean 4 and 8 weeks NS. There was reduction of fatigue by FIS but NS. QoL demonstrated and improvement in Physical Health initial mean SD 41.0 ± 8.12 (28; 54) and final mean SD 50.6 ± 12.8 (26.9; 66.7)
P422

Neurorehabilitation in progressive multiple sclerosis
J.A. Cabrera-Gomez, Yndely Real-Gonzalez, A. Gonzalez-Quevedo; International Neurological Restoration Center (Havana, CUB); Institute of Neurology and Neurosurgery (Havana, CUB)

Introduction: Progressive Multiple Sclerosis (P-MS) is the most frequent clinical form which presents with great impairment, disability, handicap and a poor quality of life (QoL). Objective: Evaluation of the efficacy of Neurorehabilitation in persons with P-MS. Methods: The data we are presenting are based on results from controlled clinical trials. Results: For primary symptoms, treatment of spasticity suggested that most of the patients need the combination of physical therapy and medication; interventions with exercising programs for equilibrium, balance and for strength have shown favorable results. Conversely, there is no proof of effectiveness for tremor. In occupational therapy (OT) the efficacy of educational courses on energy conservation was demonstrated with a positive impact on fatigue and QoL. In other measurements related to OT in the upper limbs have shown a moderate improvement in the coordination after a program of exercises. In speech-language therapy, the efficacy of rehabilitation interventions has been showed an improvement in some aspects of dysarthria. For swallowing disturbances most of the studies confirm the need to evaluate completely this function especially in patients with brain stem lesions and severe disability. Compensatory rehabilitation techniques are efficacious. In respiratory insufficiency a rehabilitation program for inspiratory muscles has a beneficial effect. For physical agents pulsed magnetic field therapy, with weak pulses and low frequencies, has been efficacious in the treatment of fatigue, QoL and spasticity. On the contrary hyperbaric oxygen therapy is not useful in MS. There are two approaches to cognitive rehabilitation: the use of compensatory strategies and cognitive retraining. At present, there is no confirmation of the efficacy of cognitive rehabilitation programs in P-MS. In advanced MS loss of comfort is the crucial problem. It should be analyzed how to make life more comfortable for a person in this phase and this is where neurorehabilitation could have an important role in the care of these persons. Conclusion: Neurorehabilitation in progressive MS forms does not improve the impairment, which continues to progress, it has a positive impact in many symptoms, disability, handicap and in QoL. The neurological rehabilitation process in progressive MS should be continuous throughout the evolution of the disease at specialized centers and especially in the community.

P423

Conventional physiotherapy in chronic progressive multiple sclerosis: effects on quantitative gait parameters
K. Gusowski, A. Kaiser, P. Flachenecker; NRZ Quellenhof (Bad Wildbad, D)

Despite the advances of disease-modifying therapies, treatment of patients with multiple sclerosis (MS) in the chronic progressive phase remains a challenge, particularly in the more advanced stages of the disease when spontaneous improvement is unlikely to occur. Physiotherapy has been regarded an essential part of symptom-oriented therapies, but its efficacy is still a matter of debate. Therefore, we retrospectively analysed the effects of conventional physiotherapy by means of quantitative parameters of gait and stance which were assessed before and after a 4-week multidisciplinary inpatient rehabilitation program. Patients and Methods: We retrospectively analysed an unselected group of 44 MS patients (31 female, 13 male, mean age 54.0 ± 9.7 years, median disease duration 17 years, median EDSS 6.0 [range 2.5 – 7.0]) who (1) suffered from primary or secondary progressive MS, (2) had not experienced a relapse within the last 12 months, nor were given steroids within the last 3 months, and (3) were evaluated by quantitative measures (10-m walking [10 MW], 2-min walking [2 MW], timed-get-up-and-go test [TGUG], Tinetti score [TS], and Barthel Index [BI]) both before and after a 4-week inpatient rehabilitation program that was individualized and tailored towards task-specific training using conventional physiotherapy according to the Bobath concept. The intensity of physiotherapy was 4 single sessions each lasting 30 min and 3 group sessions per week, accompanied by occupational, neuropsychological and speech therapy as necessary. Results: Median parameters significantly improved during the treatment period (10 MW velocity 43.6 vs. 39.1 m/min, stride length 95.2 vs. 87 cm, 2 MW 91 vs. 80 m, TGUG 12.0 vs. 15.2 s, TS 20 vs. 16, p < 0.001, Wilcoxon signed rank test). In the activities of daily living, patients significantly performed better after 4 weeks (median BI 90 vs. 85, p < 0.001). The effects were more pronounced in patients with more severe impairment of baseline scores. Conclusions: This retrospective study showed that multidisciplinary inpatient rehabilitation with conventional physiotherapy significantly affects the risk of falling, walking abilities, and the activities of daily living and should therefore be recommended to MS patients in the chronic progressive stage of the disease on a regular basis. Further studies are warranted to determine the long term effects and the factors that might influence the outcome of the intervention.

P424

Respiratory function in multiple sclerosis: correlation to clinical disability and effects of rehabilitation
A. Kaiser, K. Gusowski, P. Flachenecker; NRZ Quellenhof (Bad Wildbad, D)

Respiratory complications are common in the terminal stages of multiple sclerosis (MS), but involvement of respiratory muscles may also occur during the early course of the disease. This study aimed at determining the degree of respiratory dysfunction, its relationship to motor disability and the effects of an unspecified rehabilitation program on respiratory parameters in an unselected group of MS patients. Patients and Methods: Sixty MS patients (40 women, 20 men, age 50.2 ± 12.2, median 48 years), and 20 age- and sex-matched healthy controls (13 women, 7 men, age 43.5 ± 13.5, median 45 years) were studied. Respiratory function was evaluated by spirometry and included vital capacity (VC), forced vital capacity (FVC), inspiratory vital capacity (IVC), expiratory residual volume (ERV), and forced expiratory volume (FEV). Motor disability was assessed by the Expanded Disability Status Scale (EDSS) and the Rivermead Motor Assessment (RMA), with subscores for leg and arm/trunk function. Results were obtained at admission and repeated (2) after a 4–5 week rehabilitation program that was tailored towards functional disability, but not specifically designed to improve respiratory function (“unspecific” treatment). Results: Median SVC (86 vs. 102%) of predicted values), FVC (74 vs. 90%), and IVC (77 vs. 109%) were significantly lower in MS patients than in controls (p < 0.001, Mann-Whitney rank sum test), but not FEV and ERV. Thirty-six patients (60%) but none of the controls showed SVC values below 90% of predicted (p < 0.001). In patients with visible respiratory dysfunction (n = 7, 11%), median SVC was further reduced to 52% (range 43–64%). Respiratory dysfunction was significantly correlated to RMA, particularly to the subscore of arm function (r = 0.38), but not to EDSS and disease course. After rehabilitation, RMA but not respiratory function tests were significantly improved. Conclusions: Respiratory dysfunction is common in MS patients pointing to restrictive but not obstructive deficits. Vital capacity is correlated to arm function, but not to disability measures of leg function. The observation that an “unspecific” rehabilitation program did not increase respiratory parameters suggests that exercises specifically designed to improve pulmonary function are needed to treat respiratory dysfunction in MS patients.
Coping and quality of life in multiple sclerosis: long-term effects of a newly developed self-management programme

H. Meissner, T. Blessing, B. Rexinger, A. Immosberger, K. Gusowski, P. Flachenecker; NRZ Quelletalhof (Bad Wildbad, D)

P425

REMUS is an interactive self-management program that was developed for patients with multiple sclerosis (MS) and designed to deliver information about the disease, develop coping strategies, prevent psychological maladaptations and somatic complications, and eventually increase quality of life in these patients. Herein we report the long-term effects of this program in a large sample of MS patients. Patients and Methods: All patients were included who were (1) admitted to inpatient neurological rehabilitation and (2) given recently a diagnosis of MS or experienced MS-related deficits requiring psychological and somatic adaptations to the disease. The REMUS program was administered over three weeks in addition to the individual, goal- and deficit-oriented rehabilitation program and included 11 hours per week with single therapies, group treatments, lectures and discussion rounds dealing with MS-relevant issues. Self-efficacy, i.e. one’s belief to have the ability to overcome challenges (Rigby et al., Mult Scler 2003; 9: 73–81), and quality of life (SF-36) were assessed at baseline, at the end of the three-week program, and at follow-up after 6 months. Results: During January to December 2005, 113 patients participated. From these, 49 patients (37 women, 12 men, mean age 37.8 ± 10.3 years, mean disease duration 6.4 ± 7.1 years, median EDSS 3.0) were treated during the first 6 months, and follow-up data were available in 30 patients (61%). The psychological subscale of the SF-36 was significantly (p < 0.001, Friedman repeated measures analysis of variance on ranks) improved (median 47.7 vs. 41.8), with only a slight decrease after 6 months (46.3), whereas the physical subscore was only marginally improved at either time point (p = 0.08). Self-efficacy increased significantly during the treatment period (median 62.5 vs. 51.0), but again decreased to 58.0 after 6 months. Conclusions: With our three-week self-management program, long-lasting effects on the psychological but not the physical dimension of the SF-36 were observed, whereas self-efficacy was only temporarily increased. The fact that only the specifically treated component showed significant changes suggests that the administered program had long-lasting effects on the quality of life in MS patients.

Assessment of coping in patients with multiple sclerosis: general versus vs. specific measurement

A. Apel, B. Greim, T. Klauer, N. Koenig, U.K. Zettl; University of Rostock (Rostock, D); Marianne-Strauss-Clinik (Berg, D)

P426

Objective: The objective of the study was to evaluate coping behaviour of patients with multiple sclerosis (MS) and to differentiate between general and MS-specific coping behaviour. Methods: Data were collected from 243 patients with multiple sclerosis (MS). The objective of the study was to evaluate coping behaviour of patients with multiple sclerosis (MS) and to differentiate between general and MS-specific coping behaviour. The Coping with Information (SI) and Search for meaning in religion (SR) were used. Results: For the evaluation of MS-specific coping behaviour the Coping with MS scale (CMSS) from Pakenham was used, which contains 43 items. Results: The evaluated MS patients were on average 44.0 years old, had been diagnosed for 8.2 years and a mean EDSS from 4.0. 72.8% of the investigated patients were women. Factor analysis (principle component analysis, varimax rotation) of the CMSS extracted five factors: problem solving and search for information (PS), energy conservation (EC), personal health control (PHC), emotion-focused coping (EFC) and lacking acceptance (LA). When comparing the results from TSK and CMSS for different main problems MS patients are confronted with (e.g. physical, emotional, cognitive or financial problems), the scales EC and PHC were able to differentiate between main problems MS patients had reported. In contrast to TSK the CMSS showed differences for course of disease on the scales PS (F = 9.2; p < 0.001) and PHC (F = 37.7; p < 0.001). Post hoc multiple comparisons showed that patients with SPMS reported higher scores for PS than patients with RRMS or PPMS. Further more, patients with SPMS and PPMS were using more often active therapy attempts (PHC) than patients with RRMS. CMSS was also closer related to EDSS than TSK. While only the scale SA from TSK was correlated to EDSS (r = −0.131), three scales of CMSS were correlated to EDSS (PS: r = 0.194; EC: r = 0.177; PHC: r = 0.600). Conclusion: MS specialized assessment of coping gives specific insight in the complexity of coping in MS considering individual differences and unique problems in MS patients.

Coping and quality of life in multiple sclerosis: long-term effects of a newly developed self-management programme

H. Meissner, T. Blessing, B. Rexinger, A. Immosberger, K. Gusowski, P. Flachenecker; NRZ Quellentalhof (Bad Wildbad, D)

P425

Effects of cognitive rehabilitation in multiple sclerosis

I. Galán, I. Gich, J. Vendrell; Fundación Esclerosis Múltiple (Barcelona, E); Escola de Patologia del Llenguatge (Barcelona, E)

P428

Background: Impairment of attention and slow information processing are frequent in persons with multiple sclerosis (MS). Efficacy of drill and practice computerised programs to improve these functions...
has been proposed but remains controversial. One of the possible limitations of this approach would be the lack of generalisation. **Aim:** To present the results on attention and several other cognitive domains of a controlled clinical trial of a computer-based, drill and practice, tailor-made, hierarchical cognitive rehabilitation program in persons with MS (PwMS). **Method:** 50 PwMS were recruited from the MS Foundation Day Hospital in Barcelona, stratified and randomly assigned to a control (n = 23) and a rehabilitation (n = 27) group. During the study, an assessment protocol including neuropsychological, psychosocial and medical parameters was administered four times at six-monthly intervals. Between the second and the third assessment, the treatment arm underwent the six-month long cognitive rehabilitation program. Hierarchical criteria were followed in the sequencing of rehabilitation tasks. **Results:** Control subjects were younger at baseline (41.5 vs. 47.8; p = 0.017), but similar on years of schooling (11.4 vs. 10.8) or gender distribution (female/male: 16/7 vs. 16/11). Statistically significant improvements pre- versus post-rehabilitation (paired t-test) were found in the treatment group: reaction times in tonic alertness (p = 0.035), phasic alertness (p = 0.017) and divided attention (p = 0.015) became faster; also, standard deviation in tonic alertness decreased significantly (p = 0.048). In contrast, a significant worsening in reaction times in tonic alertness (p = 0.021) was found in the control group, with no significant changes in the remaining attentional functions. Less consistent changes were found for executive functions subtests, memory and language domains, as well as emotional and quality of life indicators. **Conclusion:** These preliminary results may indicate that the computer-based drill and practice cognitive rehabilitation program herein applied could be useful to improve attentional functions in PwMS. The most basic attentional function (tonic alertness) seems to be the most sensitive. Its effect on other cognitive domains such as executive functions, language and memory is less well defined, as well as its generalization to quality of life and emotional aspects.

**P429**

Cognitive rehabilitation of memory processes and processing speed to improve daily functioning of multiple sclerosis patients

J. Bacon, J.T. Fromon, J. Herbert; NYU Hospital for Joint Diseases (New York, USA)

**Objective:** To develop a cognitive rehabilitation program that focuses on improving processing speed in multiple sclerosis (MS) patients. **Background:** Cognitive rehabilitation has emerged only recently as a treatment opportunity for individuals with MS. Cognitive impairments, present in as much as 60% of the MS population, most often affect working memory, attention, and speed of processing (Thorton & Raz, 1997; Kail, 1998). The few studies that have addressed MS and cognitive rehabilitation, have been limited by treatment plans that either did not target specific impairments or utilized imprize outcome measures derived primarily from self-report (DeLuca, 2005). Moreover, rehabilitation has been primarily directed at memory and attention, even though evidence suggests that the primary deficit in MS is not working memory, but processing speed (DeLuca et al., 2004). **Method:** Patients are screened to exclude those with severe cognitive impairment. Cohorts of 10 patients participate in 14 biweekly, hour-long group sessions and three individual assessment sessions. Over the course of the study, multiple domains of cognition are addressed and specific interventions are introduced to help improve speed of processing. In the group sessions, rehabilitative and compensatory memory strategies are presented, from a functional and psychological perspective, followed by speed of processing exercises. After each exercise, patients graph their accuracy. At the conclusion of the program, each participant is evaluated to determine if there has been significant improvement in speed of processing and to evaluate its more general impact on cognitive processing. **Results:** The outcome measures that are being used, which, from a functional and sensitive to memory functioning and speed of processing, include the CVLT-II, the Processing Speed Index and Working Memory Index subtests of the WAIS III, and the Adjusting-Paced Serial Addition Test. The data collected is currently being analyzed and will be presented. **Conclusion:** We believe that focusing on improving the speed of processing will improve the overall cognitive functioning of MS patients. Directing rehabilitation both to the more effective use of encoding strategies and to increasing processing speed will provide a more comprehensive approach to rehabilitation and has the promise of helping participants translate the rehabilitation experience into meaningful, real life gains.

**P430**

Motivational styles as a predictor of adherence to injection therapy for multiple sclerosis

J. Bacon, L. Riber, J.T. Fromon, M. Safier, J. Herbert; NYU Hospital for Joint Diseases (New York, USA); Yeshiva University (New York, USA)

**Objective:** To investigate the role of motivational style as a predictor of adherence to injection therapies for Multiple Sclerosis. **Background:** Previous literature has focused primarily on personality variables such as self-efficacy, and locus of control as predictors of adherence to injection therapy for MS. The present study investigates the role of motivational styles. The Treatment Self-Regulation Questionnaire (TSRQ) identifies three modes of motivational self-regulation, Autonomous, Controlled, and Amotivated. An autonomous style reflects an internalization of goals independent of external pressure or particular outcomes, whether positive or negative. Controlled motivation is driven by consequences and amotivation is passive and resigned. We predicted that an autonomous motivational style would be an effective counter to the negative constellation of factors that are associated with injection therapy and would be a significant predictor of adherence behavior. **Method:** Eighty patients with clinically definite MS were recruited from the NYU HJD MS Care Center. Ten of these patients had stopped taking injection therapy or had regularly skipped taking a dose. After a brief, semi-structured interview, participants rated on a ten point scale frequency of thoughts about stopping injection therapy (1 = rarely, 10 = constantly). They then completed three scales, the Multidimensional Health Locus of Control (MHLC), Multiple Sclerosis Self Efficacy (MSSE), and the TSRQ. A content analysis of the interview by blind readers for positive and negative attitudes towards injection therapy provided an overall rating of attitude to injection therapy on a ten point scale (10 = most positive). **Results:** A regression analysis with MHLC, MSSE and TSRQ scores as independent variables and frequency of thoughts to stop as the dependent measure showed the Autonomous variable to be the only significant predictor (p < 0.005). The Autonomous variable was again the only significant predictor with interview ratings as the dependent measure (p < 0.05). On a Logistics regression with adherers vs. those who stopped or frequently skipped dosages as the dependent measure categories, the Autonomous variable was the most significant predictor (p < 0.005) while self efficacy on the physical dimension was a second significant factor (p = 0.05). **Conclusion:** An autonomous motivational style supports a more positive attitude to taking injection therapy and is a significant predictor of maintained adherence.

**P431**

Efficacy of neurological inpatient rehabilitation measured by Fatigue Severity Scale and Multiple Sclerosis Functional Composite in multiple sclerosis patients

S. Bamborschke, D. Weigt, P. Scherer; Brandenburg Klinik Bernau (Bernau, D); Berlin (Berlin, D)

**Objective:** To assess the efficacy of neurological rehabilitation in MS patients we measured the functional deficit using the Multiple Sclerosis Functional Composite (MSFC) (Cutter 1999) and fatigue using the Fatigue Severity Scale (FSS) (Krupp 1989) before and after rehabilitation. We also looked for parameters which possibly could...
predict the improvement of fatigue. Patients and methods: 138 patients (35 m, 103 f, age 22 – 61 years, mean 44.1 years) were studied. Disease course was primary progressive in 4%, relapsing remitting in 69%, secondary progressive in 24%. EDSS ranged from 1 to 7 to 0.9 with a mean of 4.1. All patients were in a stable phase of the disease. 27% of patients received interferon b 1b, 17% interferon b 1a subcutaneously, 11% interferon b 1a intramuscular, 16% glatiramer-acetate, 4% azathioprine, and 2% mitoxantrone, respectively. In 103 of the 138 patients the FSS, and in 128 of the 138 patients the MSFC was performed at the beginning and the end of rehabilitation. Fatigue was diagnosed in FSS values higher than 23 (possible range 9 – 63). Additionally in all patients the Beck questionnaire for assessment of depression, and SF36 quality of life questionnaire were performed at the beginning of rehabilitation. All patients underwent individually modified treatment using physiotherapy, ergotherapy, aerobic physical exercise, and if applicable neuropsychological training during 4 weeks of neurological inpatient rehabilitation. Mean values and standard error of the mean (SEM) were calculated for FSS and MSFC values at both time points and the significance of improvement was determined using the Wilcoxon test for paired samples. Gender, age, quality of life (SF36), EDSS, initial functional deficit (MSFC) or depression (Beck's questionnaire) were checked for correlation with the improvement of fatigue (FSS) using Pearson's method. Results: The FSS value (mean value ±SEM) was 45.0 ± 1.5 (beginning) and 42.2 ± 1.6 (end of rehabilitation), the MSFC value (mean value ±SEM) was −0.38 ± 0.07 (beginning) and −0.24 ± 0.09 (end of rehabilitation), respectively. The improvement of fatigue (p = 0.002) and MSFC (p = 0.000035) both were significant. There was no correlation between the parameters mentioned above and the improvement of fatigue. Conclusions: Fatigue and functional deficit were significantly improved during 4 weeks of inpatient rehabilitation. The improvement of fatigue could not be predicted by gender, age, EDSS, initial MSFC values, quality of life measurement or extent of depression.

P432
Is the fatigue management programme effective?
I. Jansa, Z. Sichler, U. Rot, A.J. Horvat Ledinek; University Medical Centre Ljubljana (Ljubljana, SVN)

Background: The main purpose of occupational therapy (OT) is to enable individuals to participate in self-care, productivity and leisure activities they want and need to perform. Fatigue, commonly seen in multiple sclerosis (MS) may negatively interfere with performing meaningful daily tasks. Fatigue management is a traditional OT intervention elsewhere, yet, within our department it was introduced in a systematic way for the first time. The aim of this study was to explore the efficacy of OT energy conservation course in a group of patients with MS (PwMS). Methods: Patients: there were 7 female PwMS treated by Interferon beta 1b, with mean age of 37 years (range 26 – 50), mean MS duration of 9 years (range 4 – 22), mean EDSS score of 3.6 (range 1 – 6.5) and mean Beck Depression score of 9.8 (range 4 – 18). Assessment Tools: following assessment tools were used: Canadian Occupational Performance Measure (COPM), Assessment of Motor and Process Skills (AMPS)and Modified fatigue impact scale (MFIS). COPM is client-centred, semi-structured interview; it is standardized, valid and reliable. AMPS is the measure of activities of daily living (ADL); it is an observational assessment providing information about the quality of ADL motor and ADL process skills. AMPS is valid and reliable with no ceiling or floor effect. MFIS evaluates the impact of fatigue on physical, cognitive and psychosocial functioning. Procedure: PwMS were invited to participate and those who agree were assessed prior and after the programme with the COPM, AMPS and MFIS. Fatigue management programme was based on the work of Packer (1995) and consisted of 6 educational meetings. It emphasized the value of rest, planning rest periods, introducing ergonomics principles and adaptive equipment, separating fatiguing task into components, learning to make priorities in one's life. Results: Mean MFIS scores decreased from 37.14 to 29.7 (p = 0.045). AMPS motor scores improved with statistical importance (p = 0.03; AMPS process scores improved with statistical importance (p = 0.000). COPM performance and satisfaction scores improved in 5/7 PwMS, however this improvement was not statistically important. Conclusions: The results support the efficacy of fatigue management programme in this group of PwMS treated with Interferon beta 1b. Further work is needed to show the long term benefit of such a programme.

P433
Treadmill training vs. overground gait training in subjects with multiple sclerosis: a pilot study
M. Aringolo, M. Capecci, V. Bombace, V. Cardinali, G. Cecacci, S. Pittillo, M. Danni, L. Provinciali, M.G. Caravolo; Department of Neurosciences (Ancona, I)

Background: Impaired walking ability and fatigue are common and disabling symptoms in Multiple Sclerosis (MS) patients. Although treadmill training proved effective at improving both gait and aerobic capacity in stroke patients, there isn't yet any evidence of its efficacy in MS subjects. Objective: to assess feasibility and efficacy of treadmill training in improving gait speed and endurance in ataxic MS patients. Methods: Seven patients met the following inclusion criteria: a) Relapsing-Remitting MS as defined by Poser; b) age: 30 to 60 years; c) gait impairment mainly due to cerebellar ataxia; d) Expanded Disability Status Scale (EDSS) score ≥ 3.0 – 5.5; e) steady clinical condition since at least 3 months. Study Design: replicated single-case experiments with a multiple phase (ABACA) design, where A = no treatment, B = treadmill training and C = over ground gait training are scheduled with a random sequence in different subjects, allowing an in-between test between B and C. Treatment phases consisted of 3 sessions per week for 3 weeks. During B phase, patients underwent 5’ warming-up, 20’ treadmill training (at the maximum tolerated speed) and 5’ cool down. During C phase, patients were pressed to reach the maximum achievable gait speed. Outcome Measures: a) timed 10 meter test (t10 mT), b) endurance task (6 MinuteWalkingTime-6 MWT), c) Timed Up & Go (TUG) d) spatial-temporal gait parameters, e) Tinetti scale. The Nine Hole peg test (NHPT) was also applied to control for disease evolution. Results: In all subjects, TUG, Tinetti score and 6 MWT improved after either treatment with respect to no treatment phase, whereas t10 mT and spatial temporal gait parameters did not show any change. A trend towards step cadence increase was observed after treadmill though not over ground gait training. The occurrence of persistent dizziness limited gait speed raising over treadmill in 2/7 cases. No EDSS score variations were observed in any subject during the study. Performances at the NHPT remained unchanged. Conclusions: Treadmill training is a feasible approach to gait disability in MS subjects, although it may produce a little discomfort possibly hindering patients' compliance and affecting functional outcome. It allows to improve postural control and endurance to a similar extent with respect to standard over ground gait training.

P434
Evaluation of trunk control and muscular effects in ambulatory multiple sclerosis patients
K. Armfield, N. Cettisi Korkmaz, I. Keser, R. Karabudak; Hacettepe University (Ankara, TR)

Background and Aim: A frequently occurring problem in Multiple sclerosis (MS) is postural incompetence. During activities of daily living, proper functioning of the trunk depends on a variety of interacting factors. Problems at the level of impairment, such as loss of muscle strength can reflect in the functional performance of the trunk. The aims of this study were, to observe the trunk control and the muscular effects that affect the trunk control in ambulatory MS patients.

Method: 21 MS patients were included to the study. Their mean age was 39.29±9.93 years, mean of EDSS was 4.88±1.56 (between 2 – 6.5)
and mean of disease duration was 9.05 ± 6.15 years (range 1–25 years). Trunk control was assessed with Trunk Impairment Scale (TIS), which has got dynamic and static sitting balance and coordination parts. Manual muscle tests were applied to trunk and lower extremity muscles. Results: Patients’ mean TIS scores was 15.10 ± 4.40. The mean of manual muscle tests were 3.59 ± 0.58 for back extensors, 3.94 ± 0.81 for m. rectus abdominis, 3.47 ± 1.04 for right and 3.49 ± 1.03 for left trunk rotators, 2.93 ± 0.87 for both right and left side trunk lateral flexors. The mean of right and left side of lower extremity muscles’ manual muscle test were for m. gluteus maximus 3.55 ± 1.01 and 3.63 ± 0.98, m. iliopsoas 3.54 ± 0.96 and 3.58 ± 0.86, m. quadriceps femoris 4.19 ± 1.03 and 4.41 ± 0.93, m. hamstrings 3.86 ± 1.04 and 3.78 ± 0.89, respectively. It was determined that there was a significant correlation between scores of TIS and m. nucruss abdominus, trunk rotatoes and lateral flexors and sol m. iliopsoas (p < 0.001), left m. gluteus maximus, right m. iliopsoas and hamstrings (p < 0.05). However the correlation between TIS and back extensors, right m. gluteus maximus, left hamstrings and both sides of m. quadriceps femorii was insignificant (p > 0.05). Conclusion: In accordance with these results we obtained that ambulatory MS patients’ trunk control was affected significantly. Anterior, rotational and lateral abdominal muscles have major support on trunk control. In addition to these abdominal muscles lower extremity muscles have got assists in trunk control by stabilizing pelvis. There wasn’t any contribution of back extensors to trunk control and this was an interesting and contrary of expected finding. The deterioration of balance to the posterior supports this finding. We conclude that the special exercises for abdominal muscles have to be planned for the MS patients physiotherapy and rehabilitation programs.

P436
To use or not to use: exploring factors that predict which patients who have registered for a Web-based communication system with their MS clinicians actually use it.
D. Miller, R.A. Radick, K. Nichols, J.C. Lee; Mellen Center (Cleveland, USA)

Background: Telemedicine is an increasingly common aspect of health care delivery. Electronic Patient Health Record (PHR) are growing a rate consistent with other Telemedicine applications. (See S.N. Weigant, 2006, JAMIA and europa.eu.int/information_society/quali health/index). PHR’s vary in functionality but typically include a secure messaging system between patients and health care providers. Benefits of PHP’s include efficiency in communication, a record of the information exchange that is available for patients’ review. Little is understood about factors that influence patient adoption of these systems. Such knowledge may help to develop or modify PHR’s to increase patient adoption. Methods: Mellen Center Care On Line (MCCO) is a MS-specific PHR available since 1998. It allows patients to communicate with their clinicians about changes in symptoms, medication questions, prescription renewals and other matters. Patients who register for the system must have an active e-mail account. They are provided user names and password to activate their account. They, however, are not considered users until they have used the system at least once. This report includes all patients registered for MCCO between April, 1998 and March, 2006. Demographic characteristics (age, race, gender, marital status, employment status, insurance payor and number of active diagnosis) of patients who registered for but never used the system (n-users) are compared to patients who used the system at least once (users). Logistic regression assessed which variables contributed to computer use status. Results: 1081 MCCO registrants were assessed; 815 were users and 566 were never users. Only marital status (p = 0.0051) and number of active medical problems (p = 0.0014) distinguished between the two groups. While married individuals were evenly distributed between groups, 64.7% of those divorced/separated and 57.7% of single/widowed were n-users. Of those who had > 10 active diagnoses, 62% were users. Individuals with more than 10 active diagnoses had odds ratio of 1.83 of using the system compared to those with 1 to 5 active diagnoses (95% CI 1.05, 3.17). Conclusion: Based on these data having a marital partner and having a large number active diagnoses encourages MCCO use. In order to better understand factors that influence use of PHP it is recommended that qualitative methods also be considered.

P437
Urinary rehabilitation effectiveness on multiple sclerosis patients
M.L. Lopes de Carvalho, R. Motta, M.A. Battaglia; Italian MS Society (Genoa, I)

Background: Over 80% of MS patients have symptoms of lower urinary dysfunction during the disease course. Urinary dysfunction can have a significant impact on patient quality of life. Comprehensive evaluation is essential for MS specialists to effectively manage these potentially life-disrupting symptoms. Goals: This study evaluated the effectiveness of a rehabilitation programme for MS patients with urinary dysfunction being followed in a specialised rehabilitation centre. Subjects: Thirty female MS patients with urinary symptoms consecutively referred for the first time to the rehabilitation centre were enrolled in the study. Methods: Data collected at Time 0 (pre-treatment) included: age, EDSS, course and duration of disease, mobility status, urinary symptoms, current pharmacological therapies, uro-dynamic investigation, pelvic floor evaluation. Primary outcomes are Post Void Residual with bladder ultrasound for patient with retention; Wagner Test and mean number of episodes of leakage with 5-day bladder diary for patients with incontinence; frequency evaluated with 5-day bladder diary and
secondary outcomes are MSQoL-54, Visual Analogue Scale and Grading Test of pelvic floor muscles. The rehabilitation programme was chosen based on uro-dynamic feature and pelvic floor dysfunction and could included:hydration and nutrition counseling, self-catheterisation training, pelvic floor muscle re-education, biofeedback, electro-stimulation, Posterior Tibial Nerve Stimulation (FTNS). At the end of the rehabilitation programme (mean duration:12 sessions) all patients were assessed with primary and secondary outcomes. Results and Conclusion: The results showed that the rehabilitation programme for urinary dysfunction had a positive impact, statistically significant, on post void residual and on pelvic floor muscle function (expecially for Strenght and Resistance). MSQoLS4 doesn’t seem an appropriate outcome for urinary dysfunction. VAS doesn’t change, probably due to an improve of awareness about urinary dysfunction on pwMS during the treatment. The other outcomes scores improve but the results were not statistically significant. The outcomes are different for each type of urinary dysfunction (retention, incontinence, frequency), for this reason our sample is too small if we consider each group. It could be usefull to keep on the study.

P438

It’s important to follow the disability of multiple sclerosis people: the Italian MS Society Rehabilitation Centre
M.L. Lopes de Carvalho; Italian MS Society (Genoa, I)

The MS Rehabilitation Centre of Genoa–Italy–is managed by the Italian Multiple Sclerosis Society (A.I.S.M.)in agreement with the National Healthcare System. At the present time this service has almost 900 patients under treatment (87% MS patients and 13% with another rare CNS pathologies). This is an outpatient service with 250 MS patients and, an in-home rehabilitation service with 650 patients, all over Liguria region. The outpatient service is dedicated to Genoa area patients. The home service covers all Liguria region and out of Genoa it’s the only alternative. In Genoa, it is dedicated to the patients with high disability or to complete and integrate the outpatient rehabilitation program. In our service work about 100 health professionals:77 Physiotherapist,16 Rehabilitation Doctors,10 Speech Therapist,7 Psychologist,2 Occupational Therapist,1 MS Nurse,1 Neurologist,1 Social Worker. Rehabilitation is an interdisciplinary comprehensive treatment regimen centred around the person with MS. The members of the interdisciplinary team have to have special knowledge and training in all aspects of MS. The Centre organises special courses and seminars for the staff training. The healthcare activities done on a centre included:medical visits, physiotherapy, occupational therapy, advice on technical aids and home modifications, speech and swallowing therapy, psychological support, evaluation and training for the individual and family by the nurse, bladder and bowel dysfunction therapy, hydrotherapy, manual lymphatic drainage. Network with other healthcare services includes: Neurological MS Centers, Urological and Proctological Department, Phoniatic Department, Vascular Surgery Department. In 2005 the Rehabilitation Service did:

- 46.529 home treatments
- 5.496 outpatient treatments
- 2.200 medical visits
- 1.151 psychological visits

Advantages of service: creates better opportunities for involving family members in the rehabilitation plan; training is performed in the person’s real-life situation and not in an artificial healthcare setting, allows employed people with MS more flexible access according to their individual needs; allows significantly disabled individuals to receive rehabilitation; provides the ideal setting for testing of and training in the use of assistive devices. The last aspect concern the cost of one-hour rehabilitation treatment at home that have almost the same cost of one-hour rehabilitation treatment in the centre if we consider also the transportation.

P439

Effects of a resistance training on strength manifestations and hypertrophy in multiple sclerosis patients
F. de Souza-Teixeira, S. Costilla, D. García-López, C. Ayán, R. Jiménez, J.A. de Paz Fernández; León University (León, E)

Introduction: Impaired muscle function like weakness, fatigue and decrease ambulatory ability are common in multiple sclerosis [1]. The well known beneficial effects of exercise in both emotional and physical aspects can also improve the pharmacological treatments. In this line, systematic physical activity reduces perceived fatigue, improves depression states and slows down the functionality decline [2,3]. However resistance training effects in patients with MS remains unclear [2] and studies focused on hypertrophy response following resistance training are few [4]. Although in healthy population strength gains after resistance training result from neural and structural adaptations, it is not clear if MS patients, whose muscle weakness has a central cause, keep a neural adaptation capacity in this line. Thus, we aimed to study the effects of a resistance training program on strength manifestations and muscular hypertrophy, in MS patients. Methods: Thirteen MS patients clinically defined with mild to moderate disability (EDSS scores 1.0 to 6.0) participated in a 8-week supervised strength training for the knee extendors two times a week. They were evaluated two months before the program training (control period), immediately before (baseline) and immediately after. Outcome assessments performed included Magnetic Resonance of the right and left thighs and knee extensor strength manifestations (maximal voluntary isometric contraction (MVIC), maximal force, strength-endurance and power). Results: We did not find any change between control period and baseline conditions. Significant gains were found in MVIC (16%), maximal force (30%), strength-endurance (84%) and power (51%). The magnetic resonance results shown a significant hypertrophy (from slice 6/27 to slice 11/27) in both thighs. Improvements in cross sectional area were not correlated to improvements in strength manifestations. Conclusion: A progressive resistance training induces significant gains on isometric and dynamic strength in MS patients. This improvements are probably due to neural adaptations. The results confirm that MS patients can improve muscular function and indicate a promising therapy to delay the functional decline which characterizes MS.

References

Economic burden

P440

The costs and quality of life of multiple sclerosis in Europe
G. Kobelt, J. Berg, P. Lindgren, S. Fredrikson, B. Jonsson; European Health Economics (Spårvägen, F); Stockholm Health Economics (Stockholm, S); Karolinska Institute (Stockholm, S); Stockholm School of Economics (Stockholm, S)

Background: Multiple sclerosis (MS) is a chronic and debilitating disease producing high economic burden for affected individuals and society. Objective: This project assessed the resource consumption,
work capacity, and quality of life of patients with MS in nine European countries. **Methods:** A patient-completed questionnaire containing items on health care resource consumption (inpatient and outpatient visits, medications, tests, aids and appliances), informal care by relatives, productivity losses and the EQ-SD as a measure of quality of life (utility) was developed and pre-tested. Disease information collected included disease duration, self-assessed disease severity, and relapse experience. A total of 13,186 patients enrolled in national MS societies or followed in neurology clinics were recruited to the survey. Mean annual costs per patient ($, 2005) were estimated from the societal perspective. **Results:** Across the countries, mean age ranged from 45.1 to 53.4 years, and all levels of disease severity were represented. Between 16% and 29% of patients reported having experienced a relapse in the preceding 3 months. The proportion of patients in early retirement due to MS ranged from 33% to 45%. The use of direct medical resources (e.g. hospitalisation, consultations, drugs), varied considerably across countries, while the use of non-medical resources (e.g., walking sticks, wheelchairs, modifications to house and car), and services (e.g., home care, transportation) was more comparable. Informal care use was highly correlated with disease severity, but was further influenced by health care systems and family structure. All types of costs increased with worsening disease. The total mean annual costs per patient (adjusted for GDP purchasing power) were estimated at $18,000 for mild disease (EDSS <4.0), $36,500 for moderate disease (EDSS 4.0－6.5) and $62,000 for severe disease (EDSS >7.0). Utility was similar across countries at ~0.70 for a patient with an EDSS score of 2.0 and ~0.45 for a patient with an EDSS score of 6.5. Intangible costs were estimated at around $13,000 per patient. **Conclusions:** This study provides estimates of the costs of MS treatment in Europe and highlights the importance of interventions that delay or avoid the accumulation of costs and burdens associated with the higher levels of MS disability.

P441

The cost of multiple sclerosis in Norway—and how certain can we be?**

B. Svendsen, K. Myhr, H. Nyland, J.H. Aarseth; MS National Competence Center (Bergen, N); Haukeland University Hospital (Bergen, N); Multiple Sclerosis National Register (Bergen, N)

The research question initially formulated for this study was to attempt to set a numerical target for the total yearly cost of multiple sclerosis to the Norwegian society, and relate the cost and patients’ experienced quality of life to illness severity. As work progressed, the question of how much confidence may be put in this kind of information in Norway as for today turned into another main question. Our study may be classified as a combined top-down/ bottom-up cost-of-illness study with the human-capital method used to set a numerical target for the cost of sick absence from work, early retirement and premature death. No special attempt was made however, to make the study fit perfectly with any given such classification scheme. Information from a wide range of sources was been gathered and attempted combined in a way that may hopefully provide the best possible results. Still it turned out, however, that much of the information that could be used for our study was so imprecise or unreliable that giving an impression that the information could be used to give an acceptably precise single estimate of the cost of multiple sclerosis to the Norwegian society would be seriously misleading. Therefore both “conservative” and “best” estimates are given. A conservative estimate of the yearly cost of MS to the Norwegian society around year 2002 is NOK 1836 million. A best estimate is NOK 4033 million, more than twice the conservative estimate. Mainly three factors account for the difference: Uncertainty on what elements should be included in cost-of-illness studies, uncertainty on how some cost elements should be valued, and differences in information gathered from different sources. Uncertainty is a major problem as decisions making purposes the last one is most grave since it will usually go unrecognized. When related to illness severity, the total cost per patient to society seemed to increase in a close to linear fashion with increasing EDSS-levels. The patients experienced quality of life seemed to decrease in mostly the same way with increasing EDSS-levels. Because of the uncertainties mentioned, however, Norway has probably a long way to go before studies like ours in general might be regarded as providing acceptable information for decisions as important as those that have to be made in the health sector.

P442

Cost of an multiple sclerosis relapse: the patient’s perspective

K. Johnson, M. Olen-Burkey, J. Abdalla, J. Haley, M. Lage; University of Maryland (Baltimore, USA); Teva Neuroscience (Kansas City, USA); HealthMetrics (Gronont, USA)

**Background:** In Relapsing Remitting Multiple Sclerosis (RRMS) the cost of relapses can be measured not only in terms of functional disability but also in direct medical costs, productivity losses and declines in quality of life. **Objectives:** The objective of our survey was to characterize the current total burden of the disease for patients with RRMS who were in relapse vs. remission. **Methods:** A cost of illness survey was designed to capture inpatient care, outpatient care, community services, and device/adaptation purchases. The Work Productivity and Activity Impairment (WPAI), EQ-SD and Goodin Neurological Impairment Questionnaires measured productivity losses, health-related quality of life and functional disability, respectively. Following IRB approval, information about the survey was sent via electronic mail to members of MSWatch.com who in turn linked to 1 relapse screener. Qualitative respondents completed a web-based survey. Responses were downloaded to an Access database and analyzed by HealthMetrics Outcomes Research. **Results:** There were 711 qualified respondents of 1601 screened. Two-thirds of respondents (67%) reported having a relapse during the last year; the mean number of relapses was 2.2 (SD 2.25). Subjects in relapse used significantly more resources than subjects in remission (p<0.02). Costs increased by $3,276 or 16%. All categories of resources increased; differences were only significant for hospitalizations, ambulatory care and drugs. Relapses resulted in significant work and activity impairment outside and inside the home. This impairment was estimated at $2,331, a 70% increase over those in remission during the entire year (p<0.01). The mean self-reported EDSS score increased from 3.9 to 4.6 (p<0.01) with relapse while mean quality of life decreased from 0.68 to 0.53 (p<0.01). **Conclusions:** Relapses do result in significant costs to patients in the form of direct medical costs, losses in productivity and quality of life as well as functional ability.

P443

Impact of relapses on total costs of care for patients with multiple sclerosis

K. Akras, M. Cisternas, A. Foreman, D.H. Miller, R. Bennett, A. Al-Sabbagh; Serono, Inc. (Rockland, USA); Ovation Research Group (San Francisco, USA)

**Background:** Relapses in multiple sclerosis (MS) are a major burden on patients’ welfare and related healthcare costs, and have been shown to impact residual disability (1). While relapse costs have been reported previously (2), no publication has examined the impact of recurrent relapses on total healthcare costs. **Objective:** We investigate the impact of recurrent relapses on short- and long-term healthcare costs in the United States. **Methods:** We used medical (International Classification of Diseases-9 diagnoses) and pharmacy claims from a large, US National Health Plan database to identify MS patients with a relapse who had experienced different numbers of relapses and who had continuous enrolment 6 months pre- and 12 months post-index relapse. Costs were estimated based on claim charges, and were
adjusted to project the amount in 2005 US dollars. Analyses were stratified by whether patients were newly diagnosed or previously diagnosed, and by the number of relapses they had experienced (1 or ≥ 2). Costs were analyzed in 90-day intervals in reference to the index relapse period (days 0 – 30). **Results:** Newly diagnosed patients with ≥ 2 relapses had higher monthly costs compared with patients with 1 relapse only at days 0 – 30 (index relapse) ($26,890 vs. $16,121), 31 – 90 ($3597 vs. $1506), and $3768 vs. $1074. Although previously diagnosed patients with ≥ 2 relapses had costs similar to those of patients with 1 relapse only at index relapse at days 0 – 30 ($21350 vs. $21015), monthly costs were higher for patients with ≥ 2 relapses at days 31 – 90 ($3792 vs. $2712) and remained higher at days 271 – 360 ($3636 vs. $1676). Monthly costs were generally higher for previously diagnosed patients; however, the cost of the acute phase of relapse (days 0 – 30) in the ≥ 2 relapses subset was lower for previously diagnosed patients compared to their newly diagnosed counterparts ($21,350 vs. $26,890). **Conclusion:** Recurrent relapses are associated with increased costs, both in the acute phase of managing a relapse and during the follow-up year in both newly diagnosed and previously diagnosed patients.

**References**


**P444**

**Multiple sclerosis and employment in Europe: results from Italy**

M. Messner Uccelli, C. Specchia, M.A. Battaglia, A. Kemppi, D.M. Miller; Italian Multiple Sclerosis Society (Genoa, I); Mario Negri Institute (Milan, I); University of Siena (Siena, I); Masko Neurological Rehabilitation Center (Masko, FIN); Mellen Center for MS (Cleveland, USA)

**Premise:** Early published studies of the employment situation of people with MS have focused on identifying factors that differentiate the employed from the unemployed for predicting which individuals are at risk for leaving the workforce, which have included disease and demographic characteristics, pre-morbid personality, coping style, workplace characteristics and social support. In a resolution adopted by the European Parliament in December, 2003, specific priorities were delineated that directly pertain to Europeans with MS (an estimated 400,000), including improving the employment situation of these individuals through implementing legislation that encourages autonomy and job security. Currently there are no comprehensive data available on the employment situation of people with MS in Europe. **Objectives:** outline the general employment situation of people with MS in a sample of European countries; identify factors that influence choice/ability to maintain employment; identify demographic and disease characteristics that differentiate employed from unemployed. **Methods:** A comprehensive, self-administered questionnaire, formatted as a checklist of factors that can either facilitate or hinder job maintenance, divided into three major categories (personal, MS-related, work-related), with six sub-categories, is currently being administered in 15 European countries to approximately 1,000 people with MS. **Results:** A statistically significant difference between employed subjects and unemployed subjects was demonstrated in the categories of Workplace environment, Attitudes toward work and Financial considerations. No statistical differences were found in MS-related factors, including symptoms. **Conclusions:** While MS symptoms are often reported to influence work status, they are not the factors that differentiate employed from unemployed individuals. Preliminary data supports the hypothesis that factors that influence the employment status of people with MS are more related to societal issues (accessibility and financial) than to a person’s individual impairment, symptom or disease course. These results are relevant in that often disease-related issues are difficult to address, for example, symptom management. On the other hand, focusing on the barriers created by society that marginalize people with MS and all disabled people, and exclude them from the workforce, can be addressed.

**P445**

**Employment and multiple sclerosis: an assessment of discrimination issues in Italy**

S. Bazzzone, A. Moretti, M. Messner Uccelli, M.A. Battaglia; Italian Multiple Sclerosis Society (Genoa, I)

Studies involving people with MS have reported high numbers of unemployment and job loss. The reasons for premature retirement are disputable, but are likely related to a number of factors including discrimination, which may be compounded for women with MS. Italian legislation and culture create situations that can both hamper and promote the participation of disabled people in the workforce. Laws are in place that permit disabled people certain accommodations that facilitate their employment. Although, many disabled people are unprotected from losing their jobs after disclosing a diagnosis of MS or other illness or impairment, face innumerable architectural barriers and are often unaware of their rights as disabled citizens. These factors combine to convince many people with MS that receiving a disability pension is preferable to facing the challenges of finding and/or maintaining a job. A retrospective study was performed on data collected through the Italian MS Society’s employment consultancy toll-free telephone number between January 2002 and January 2005. The calls were from persons with MS (77%), partners (12%), relatives (8%), others (3%). Among persons with MS, 4% were newly diagnosed, 9% within one year from diagnosis, 22% in the interval 1 – 5 years, 64% after 5 years from diagnosis. 80% of the total number of calls were from employed callers. One thousand three hundred calls were received, 70% of which were from females. Twenty-eight percent of callers were seeking information regarding the rights of disabled workers within the workplace, 22% regarding job protection when taking a leave of absence, 17% were looking for information about finding a job through agencies for disabled people, 9% for early retirement, 5% regarding communicating their MS to their employer and 5% with actual complaints of direct discrimination in gaining, changing or maintaining employment. An assessment of the types of information sought out by people with MS related to employment serves to determine the optimal direction in which to take lobbying activities and service provision, two major roles of a national MS.

**P446**

**Health status, utilisation and access to care among community-dwelling Canadians with multiple sclerosis**

S. Warren, A. Jones, S. Pohar, K. Turpin, K. Warren; University of Alberta (Edmonton, CAN)

**Background:** Few studies on health status, utilisation and access to care among community-dwelling persons with MS have been published, and those which have typically do not include comparisons to the general population. **Methods:** Data for this study came from the 2000/01 cross-sectional Canadian Community Health Survey conducted by Statistics Canada. A representative sample of community-dwelling Canadians was interviewed. Questions on demographics and health-related experiences in the past year were asked, including the Health Utilities Index Mark 3 (HUI3) that measures health-related quality of life (HRQL) with scores up to 1.0 (perfect health). Of the 131,535 respondents, 335 reported having MS. Descriptive statistics were used to summarize information on persons with/without MS. Chi-squared and t-tests

Multiple Sclerosis 2006; 12: 51 – 528

www.sagepub.co.uk
onset was 32 years (ranging from 7 to 64 years) with male to female ratio 0.85:1. Relapsing-remitting multiple sclerosis (RRMS) was the commonest type (77.5%). 86% of the subjects had definite MS and 14 had probable MS. Twenty-five patients had pure optico-spinal form. More than 80% patients followed a relapsing-remitting course. Treatment and outcome of patients with definite multiple sclerosis (MS) from Pakistan

Methods: A total of 217 patients were analyzed at five centers in Pakistan. Patients were only included in the study if they had clinically definite MS (CDMS) and Magnetic Resonance Imaging study suggesting MS. We used Poser criteria for diagnosis of CDMS and Thompson’s criteria for Primary Progressive MS (PPMS).

Results: 142 patients were included in study (84; 60% women, 58; 40% men). Age range was 15–54 years (Mean 31 years). Mean age at onset was 27 years. The onset was poly symptomatic in 107 (75%) patients. Symptoms at presentation included motor weakness; 99 (70%), sensory motor impairment; 85 (60%), visual symptoms; 39 (29%), ataxia; 31 (22%), dysarthria; 30 (21%), Diplopia; 21 (15) and bladder symptoms; 20 (15%) patients. Brain MRI was performed in all patients. 137 (95%) had abnormal Brain MRI. Spine MRI was done in 37 (26%) patients showing abnormalities in 22 (15%) patients. 25 (18%) patients showed contrast enhancing lesions. CSF analysis was performed in 92 (65%) patients, of which 75 (84%) were positive for oligoclonal bands. The course was relapsing-remitting in 115 (81%) patients, Primary-progressive in 21 (15%) and secondary-progressive in 6 (4%) patients. The topography showed cranial only; 99 (70%) patients, spinal only; 5 (3%), opticospinal; 4 (3%), optico-cranial; 20 (15%) and cranial-spinal; 11 (7%) patients. During the course 135 (95%) patients received at least one course of pulse steroid therapy. Only two patients received beta interferon, 16 (11%) patients received Methotrexate and 14 (10%) patients received Mitoxantrone. Seven (5%) patients did not receive any disease specific therapy. At most recent follow up 33 (24%) were normal functioning, 63 (45%) required some assistance for ambulation or activities of daily living, 45 (31%) were chair or bed bound. One patient died due to sepsis after pulse steroid treatment. Conclusions: MS is not uncommon in Pakistan. Cranial MS was more common than spinal or opticospinal form. More than 80% patients with MS follow a relapsing-remitting course. Use of beta-interferon is extremely low in our patient population. More than 75% were moderately or severely disabled at the time of evaluation.

Epidemiology

Multiple sclerosis in Pakistan

M. Wasay, S. Ali, A. Hassan, A. Asif, A. Malik, A. Ahmed, A. Haq, S. Frederikson, M. Wasay; The Aga Khan University (Karachi, PAK); Jinnah Postgraduate Medical Center (Karachi, PAK); Shifa International Hospital (Islamabad, PAK); Baqi Medical University (Karachi, PAK); Liaquat National Hospital (Karachi, PAK); Al Amin Hospital (Peshawar, PAK); Karolinska Institute (Stockholm, S)

Objective: To analyze clinical characteristics, imaging findings, course, treatment and outcome of patients with definite Multiple Sclerosis (MS) from Pakistan

Methods: A total of 217 patients were analyzed at five centres in Pakistan. Patients were only included in the study if they had clinically definite MS (CDMS) and Magnetic Resonance Imaging study suggesting MS. We used Poser’s criteria for diagnosis of CDMS and Thompson’s criteria for Primary Progressive MS (PPMS).

Results: 142 patients were included in study (84; 60% women, 58; 40% men). Age range was 15–54 years (Mean 31 years). Mean age at onset was 27 years. The onset was poly symptomatic in 107 (75%) patients. Symptoms at presentation included motor weakness; 99 (70%), sensory motor impairment; 85 (60%), visual symptoms; 39 (29%), ataxia; 31 (22%), dysarthria; 30 (21%), Diplopia; 21 (15) and bladder symptoms; 20 (15%) patients. Brain MRI was performed in all patients. 137 (95%) had abnormal Brain MRI. Spine MRI was done in 37 (26%) patients showing abnormalities in 22 (15%) patients. 25 (18%) patients showed contrast enhancing lesions. CSF analysis was performed in 92 (65%) patients, of which 75 (84%) were positive for oligoclonal bands. The course was relapsing-remitting in 115 (81%) patients, Primary-progressive in 21 (15%) and secondary-progressive in 6 (4%) patients. The topography showed cranial only; 99 (70%) patients, spinal only; 5 (3%), opticospinal; 4 (3%), optico-cranial; 20 (15%) and cranial-spinal; 11 (7%) patients. During the course 135 (95%) patients received at least one course of pulse steroid therapy. Only two patients received beta interferon, 16 (11%) patients received Methotrexate and 14 (10%) patients received Mitoxantrone. Seven (5%) patients did not receive any disease specific therapy. At most recent follow up 33 (24%) were normal functioning, 63 (45%) required some assistance for ambulation or activities of daily living, 45 (31%) were chair or bed bound. One patient died due to sepsis after pulse steroid treatment. Conclusions: MS is not uncommon in Pakistan. Cranial MS was more common than spinal or opticospinal form. More than 80% patients with MS follow a relapsing-remitting course. Use of beta-interferon is extremely low in our patient population. More than 75% were moderately or severely disabled at the time of evaluation.
Clinical-epidemiological study on multiple sclerosis in Saudi Arabia: evidence of a recent epidemic!

A. Ait Ahan, A. Daf, M. Alnoaimi; King Saud University (Riyadh, SA)

Introduction: Epidemiological studies in multiple sclerosis (MS) have provided a strong evidence for the importance of population movement, in increasing the incidence of MS, or even introducing it for the first time. Despite an earlier impression that a similar process is occurring in Saudi Arabia (SA), there has been no attempt yet to answer this question. Objective: This study aims to assess objectively recent changes in the incidence of MS in SA, by studying all MS patients seen at a main national referral center over 2 decades.

Methodology: Files of all Saudi MS patients seen at Khalid University Hospital from 1984 to 2005 were studied. Patients when necessary were contacted or reevaluated in the clinic. Diagnosis was reassessed utilizing Mcdonald criteria. Results: From a total of 160 patients 142 (89%) were Saudis, 48 (33.8%) were males and 94 (66.2%) were females, with a mean age of onset of 28.2 ± 7.7 years. Male to female ratio was 0.51:1. 108 (76%) had definite MS, 24 (16%) had probable MS and 10 (7%) had possible MS. Among males the mean age of onset was 29.31 ± 7.9 yrs (range: 11 – 54 yrs), compared to 27.68 ± 7.5 yrs (range: 14 – 52 yrs) in females. There were 5 confirmed cases of positive family history. Weakness was the most common clinical feature in both males and females followed by unsteady gait and numbness of the lower extremities. In males, numbness of the upper extremities is a more common presenting feature than diplopia and blurring of vision which is rather more common among females. Incidence was studied meticulously and found to be clearly increasing with time. Using a five year interval periods, the number of new cases was: 5 before 1984, 13 between 1985 – 1989, 22 between 1990 – 1994, 46 between 1995 – 1999, 56 between 2000 – 2005. Furthermore the onset in the first patient was found to date back to 1978. Conclusion: First, considering the limitations of this hospital based study, the change in the rate of incidence over time probably reflects national figures, as it is the main referral hospital in central region. MS incidence in Saudi population increases to as much as 160% from 1984 to 1989, and 109% between 1990 – 1994 to 1995 – 1999. Second, considering the natural history of MS, the absence of any MS patients with onset before early 1970’s, imply that this disease was either very rare or was actually introduced to the country around that time. This date coincide well with the high influx of expatriates from higher risk countries after 1973 oil price boom.
P453


Background: Repeated epidemiological surveys almost invariably show an increase in incidence and prevalence of MS. A better case ascertainment with time has its share in the increase, and has been regarded as the major contributor, whereas a true increase in incidence has been questioned. The problem is important, as an increase in incidence indicates a response to stronger or more widespread exposure to environmental factors. Purpose: to ascertain whether the incidence of MS in Denmark has increased during the period 1973–2002.

Material and Methods: Information on virtually all patients has been collected since 1948 in the Danish MS Registry from multiple sources as a continuous project. The registry is linked to the Civil Registration System, the National Patient Registry, and the MS Treatment Register. Results: During the twenty-year period 1983–2002, the incidence of onset (per 105 person years) was 4.41 in 1973–1977 (CI: 4.15–4.67); 5.39 in 1978–1982 (CI: 5.11–5.68); 5.53 in 1983–1987 (CI: 5.25–5.83); 6.21 in 1988–1992 (CI: 5.91–6.52); 6.54 in 1993–1997 (CI: 6.26–6.84); and 6.47 in 1998–2002 (CI: 6.17–6.78), a significant increase. However, the increase in incidence is almost exclusively owing to an almost 70% increase in incidence in females, especially in the age group 25–44, in contrast to males for whom the incidence of onset has remained almost constant from 1978 through 2002. In women, the annual incidence of diagnosis rose even higher in the study period: From 4.61 to 11.85; in males, it rose from 3.18 to 5.79. The figures may be slightly adjusted at the final presentation. The true rates for incidence of onset in recent years may be even higher, as an unknown number of patients with onset before January 2003 may not yet have received a final diagnosis. Conclusion: There is a true increase of incidence of MS among Danish women which cannot be explained by a better case ascertainment, indicating increasing exogenous risk-factors.

P454

The epidemiology of multiple sclerosis in the northeast region of Northern Ireland – A high and rising prevalence O.M. Gray, G.V. McDonnell, S.A. Hawkins; Royal Victoria Hospital (Belfast, UK)

Objective: To estimate the incidence and prevalence of multiple sclerosis (MS) in Northern Ireland (NI). Background: NI has been recognised to be an area of high risk for MS. The original study of Allison and Millar in 1951 found a prevalence of 41 per 100,000. Subsequent studies in 1951, 1961, 1986 and 1996 suggested prevalence rising serially-57, 104 and 168.2 per 100,000. Methods: We surveyed the North-East of NI (population 160,446, area 2,030 km²). Sources of cases included the Northern Ireland Neurology Service records, general practitioners, hospital discharge coding, MS charities, MS specialist nurses and respite facilities. Cases complied with the Poser criteria for definite or probable MS or the McDonald criteria. Results: From a provisional list of 469 cases, 370 (123 males, 247 females) were identified with definite or probable MS. The prevalence was 230.6 per 100,000 (95% CIs 207.0–255.4) with a significantly higher prevalence in females (308.8/100,000) than males (157.0/100,000). In 1996, incidence was 9.3/100,000/ year. Mean age on prevalence day was 50.3 years (SD 14.0). Mean age at onset was 32.6 years (SD 10.5). Mean delay between onset and diagnosis was 4.6 years. Conclusions: At 230.6/100,000, NI continues to have a rising prevalence of MS. An increase in incidence to 9.3/100,000/year confirms a true increase in the disease.

P455

Multiple sclerosis mortality in South Wales: a 20-year prospective population-based study C. Hirst, R. Swingler, A. Hennessy, D.A.S. Compston, N. Robertson; University Hospital of Wales (Cardiff, UK); Ninewells Hospital (Dundee, UK); Morriston Hospital (Swansea, UK); Addenbrookes Hospital (Cambridge, UK)

Introduction: Multiple Sclerosis (MS) affects both quality of life and life expectancy. Mortality studies are important to determine effect of MS on survival and cause of death. A number of mortality studies have been reported but many have been of short duration and/or selected populations. Studying death certificates in a prospective cohort of patients known to have MS is a valuable data source and provides information on disease duration and death certificate accuracy. We present 20 year prospective population based mortality data of MS in South Wales. Methods: In 1985 a prevalence study in South Wales identified 441 patients, 86% of which had clinically definite or probable MS by Poser criteria and 14% had suspected MS. Cases were registered with the Office of Population Censuses and Surveys and death certificates collected prospectively. Data was examined for cause of death, whether death was related to the disease and age at death. Comparison was made with available national statistics, and standardised mortality figures determined. Expanded disability status scale (EDSS) scores in 1985 were analysed for subsequent time to death.

Results: Of 441 patients identified, 220 patients had died, 214 were alive and 7 untraceable. Mean age at death was 64 in females and 66 in males, compared with UK population 79 in females and 73 in males. 102(46%) deaths were not related to MS and in 30% MS was absent from certificates. Crude death rate was 32.16 deaths/1000/year, age standardised mortality ratio was 127.9(95% CI 111.0, 144.8), and excess death rate 5.4/1000/yr. The most common cause of death was respiratory tract disease or infection in 43%. No suicides were identified. Mean disease duration was 39.21 years (95% CI 37.48, 40.92) and compared to a matched population survival was significantly reduced in patients with MS (p<0.0001). In those with EDSS of < or equal to 3 in 1985 mean survival from this point was 19.13 years (95% CI 18.31, 19.96) compared with a mean survival of 8.89 years (95% CI 7.23, 10.54) (p<0.0001) for those patients with EDSS > or equal to 8. Discussion: We identified a 28% increase in deaths in patients with MS compared to an age and sex matched Welsh population, with males dying 7 years and females 15 years earlier than national average. Mean disease duration at 39.21 years is longer than quoted in earlier studies and suicide rate is low. In nearly half of all patients cause of death is unrelated to the disease.

P456

Differences in prevalence of multiple sclerosis between groups of non-Western immigrants in Oslo, Norway C. Smestad, L. Sandvik, T. Holmøy, H.F. Harbo, E.G. Celius; Ullevål University Hospital (Oslo, N); University of Oslo (Oslo, N)

Scandinavia is a high-risk area for multiple sclerosis (MS), whereas a low prevalence is reported among the indigenous people of Asia and Africa. Substantial immigration to Norway of people of non-Western origin (defined as a person with both parents born in Asia, Africa or South- and Central America) started in the early 1970s. Oslo is the capital of Norway with a total population of 529,846 by 1 January, 2005, including 18% non-Western immigrants. The objective of this study was to compare the prevalence of MS between native Norwegians and different ethnic groups migrating from regions with lower risk of MS to a high-risk area. Our primary data source was the registry of MS patients at Ullevål University Hospital, Department of Neurology and only patients with definite MS according to the Poser criteria were included. The non-Western immigrants were divided into three categories depending on ethnic origin: Asia, Africa and the Middle
East. Western immigrants were defined as persons originating from Europe, North-America and Oceania. The numerator of the crude prevalence rates was the number of persons in each ethnic group resident in Oslo with a diagnosis of definite MS on prevalence day, 31 December, 2005. The denominator in the calculations was the number of individuals in the respective ethnic groups alive and resident in Oslo on prevalence day. A total of 786 patients with definite MS were alive and resident in Oslo, yielding a crude prevalence rate of 148/10³. The prevalence cohort included 27 non-Western patients; Middle East n = 13, Asia n = 10, Africa n = 4. In the Norwegian/Western cohort the prevalence was 170/10³, in the Middle East group it was 116/10³, while there was a lower prevalence in the Asian (21/10³) and African (20/10³) group. These differences in MS frequency remained unchanged when adjusting by the immigrants’ time of residence in Norway. The age at which the non-Western patients migrated to Norway was scattered. The observed pattern of MS frequency in the different ethnic groups is in accordance with the known distribution of MS worldwide, although the prevalence is higher among the immigrants living in Oslo compared to their countries of origin. The majority of the Asian patients are second-generation immigrants, and this trend combined with the strikingly high number of MS patients from the Middle East, despite their shorter time of residence in Norway, are compatible with both environmental and genetic causes of MS.

P457
A multicentric study of 230 Italian patients affected with progressive multiple sclerosis
F. Martellini Boneschi, F. Esposito, M. Rodegher, A. Ghezzi, V. Pilato, G. Congiolo, R. Capra, D. Tonoli, P. Rossi, P. Amovazzi, L. Bernardoni, L. Collinaedaglia, L. Moiola, V. Martinelli, H. Abderhamin, G. Coni; Scientific Institute San Raffaele (Milan, I); Azienda Ospedaliera S. Antonio Abate (Gallarate, I); Ospedale S. Carlo (Potenza, I); MS Regional Centre (Brescia, I); Ospedale Maggiore della Carità (Novara, I); Serono Genetics Institute (Geneva, I)

Objective: To characterize from a clinical and genetic point of view a sample of MS patients affected with progressive form of disease from onset. Methods: Patients were recruited from the in-patient and out-patient facilities of 5 different MS centres as part of a multicentric case-control genetic association study. A semi-structured questionnaire has been developed to collect retrospective informations on demographic, disease related, and familial aggregation data. Results: A total of 217 MS patients were recruited between 1/2002 and 12/2005 with an history of progressive symptoms from onset, 73% of whom were classified as pure PP with no relapses, 17.5% as progressive-relapsing, and the remaining 9.2% was classified as a transitional course. More information will be given by genetic association studies, which are ongoing at the present time.

P458
Increasing incidence of multiple sclerosis in Lorraine, eastern France is not related to better ascertainment of patients with mild disability
M. Deboverie, S. Pittion-Vayovitch on behalf of the Lorsep Group

Objective: To describe prevalence and incidence rates of multiple sclerosis (MS) in Lorraine, France and evaluate its temporal profile to assess a possible increase in the MS risk in our study population comparing incidence between 1990 and 2000. Methods: We studied the frequency of MS in Lorraine, France (2,311,000 inhabitants as reported in the 1999 census). The source for the case ascertainment was the regional network of MS health-workers in Lorraine. Only the cases, with definite or probable MS according to Poser’s classification, were integrated in the cohort. Results: We found 2718 subjects with MS and the prevalence rate (January 2006) was 117/100,000 (95% CI: 116 to 118), higher in women (166/100,000 (95% CI: 164 to 167) than in men (57/100,000 (95% CI: 56 to 58). During the period 1990–2000, the age and sex adjusted annual incidence rate was 5.4/100,000 (95% CI: 4.3–6.5), higher in women (7.5/100,000 (95% CI: 5.8–9.2) than in men (3.2/100,000 (95% CI: 2.4–4.0). During this same period, total and female incidence increased (p=0.0006 and 0.002 respectively). The male incidence seemed stable (p=0.41). Increasing incidence of MS was not related to better ascertainment of patients with mild disability because the disability (EDSS) at five years after MS onset, number of relapses during the five first years, proportion of primary progressive MS, proportion of first attack with sequelae or polysymptomatic were not significantly different during the more recent years. The mean age at MS onset seemed stable (p=0.14). Conclusions: Prevalence and incidence rates of multiple sclerosis were higher than those expected according previous studies in France. Incidence increased during the period 1990–2000 and this increasing of incidence was not related to better ascertainment of patients with mild disability.

P459
Prevalence of multiple sclerosis in Isfahan, Iran
V. Shaygannejad; Isfahan University (Isfahan, IR)

Background: The prevalence of multiple sclerosis (MS) has a considerable variability all over the world. According to Kurtzke, Iran is considered to have low prevalence. Objective: To estimate the prevalence and risk factors of MS in Isfahan, central part of Iran. Methods: A cross-sectional case register study conducted from 2004 to 2005. In the province of the Isfahan, Iran, all patients known to have definite multiple sclerosis during 2004 to 2005 and alive and resident within the Isfahan and were member of Isfahan MS Association were included in the study. Demographic and case-related information was recorded. 1391 definite MS patients (308 men and 1083 women) from Isfahan MS Association, Iran have been identified. The disease was confirmed according to clinical information and magnetic resonance imaging findings by a neurologist and radiologist. The patients were evaluated by interview and a questionnaire. The mean (SD) age of participants was 32.5 (9.3) years with a mean (SD) duration of disease of 6.4 (5.1) years for men and 6.9 (5.3) years for females. Results: The period prevalence of MS was 35.5 per 100,000 [95% confidence interval (CI) 33.6, 37.3] in a population of 3,923,255, with higher rate in women than men (54.5 (95% CI: 51.1, 57.8) women and 14.9 (95% CI: 13.3, 16.6) men). The female/male ratio was 3.6 (95% CI: 3.2 to 4.1). MS rates were highest among 30–39 year olds and decrease with increasing age. Sensory and visual disturbance were the most common initial presentation with a prevalence of 51.1% (95% CI: 48.4, 53.7) and 47.0% (95% CI: 44.4, 49.7) respectively.
Conclusion: The Isfahan could be considered as an area of medium-high risk. This is in sharp contrast with the gradient hypothesis.

P460

Stronger disability of North Africans compared with Caucasians with multiple sclerosis in France
S. Jeannin, C. Lebrun, S. Pittion-Voyouitch, M. Debouverie; Hôpital Central (Nancy, F)

Background: Previous studies suggest a strong ethnic effect on the incidence, clinical presentation, and course of multiple sclerosis (MS). The aim of the current study was to compare the clinical disease progression in Caucasian (C) and North African (NA) MS patients in France. Methods: The clinical features of MS were compared in 211 NA patients and 2945 C patients in a French population-based cohort with definite MS according to McDonald's criteria. Results: Considering MS patients of NA and C populations respectively, female MS patients accounted for 66.4% and 72.9% (p < 0.04), proportion with the primary progressive form of MS was 15.6% and 11.7% (p = 0.08) and the mean ages at baseline were 29.9 ± 9.8 and 32.9 ± 10.6 years (p < 0.0001). The predictive factors for disease progression were stronger in NA patients, including a higher proportion of patients with incomplete recovery from the first relapse (p < 0.0001), a shorter time between the two first relapses (p = 0.02), a higher number of relapses in the five first years (p = 0.03), and a shorter time to reach the Expanded Disability Status Scale score of 4.0 (p = 0.001) or 6.0 (p < 0.0001). There was no statistical difference in these factors between NA patients born in France and those born in North Africa except that the mean age at first onset of symptoms was earlier in NA patients born in France (p < 0.0001). A higher proportion of NA patients encountered Barkhof's criteria on baseline MRI (NA: 71.8%; C: 60.1%; p < 0.001). Conclusions: The course of MS is more aggressive in NA than in C patients.

P461

Multiple sclerosis in North African migrants to southern France
S. Jeannin, F. Berthier, V. Boug, C. Lebrun; Hôpital Pasteur (Nice, F); Hôpital Chimiez (Nice, F)

Background: Most workers agree that environmental and genetic factors both play a role in developing Multiple Sclerosis. Three French epidemiological studies concluded that MS in North African migrants to France is primarily an environmental disease acquired after childhood requiring prolonged exposure (mean of 3 years) followed by prolonged incubation period between acquisition and symptom onset (mean 10 years). Clinical forms seem to be more aggressive with mostly primary progressive forms and cerebellar symptoms. Objective: Collect and study patients from North Africa with multiple sclerosis among the 958 MS patients gathered in our MS clinic. Methods: Descriptive study of North African MS patients with a documented MS according to McDonald’s diagnostic criteria. Data were crossed with expected age- and gender-matched characteristics available in our EDMUS database for the period 1990–2005. Results: 76 patients (representing 8% of the patients included in the database, 53.9% from Algeria, 25% from Tunisia, 15.1% from Morocco) with definite MS were identified: 47 women, 29 men, mean age at the first documented symptom: 29.5 yrs; mean age at MS diagnosis: 32.9 yrs; MS forms were RR 61.8%, SP 19.7%, PP 18.4%. 36.8% came in France before 15 years and 32.9% after 15 years. Others (30.8%) were born in France. 90.8% of the migrants acquired their MS in France with mean interval of 7.5 years between immigration and MS onset. For the 7 migrants with a presumed MS acquisition in North Africa, there was no difference in age at diagnosis and evolution compared with migrants after 15 yrs. Statistical analysis shows a significant result for age at the first symptom and at diagnosis with North African patients born in France younger at MS onset (mean 27.2 yrs) than French born MS (32.2 yrs; p < 0.003). NA patients also have short interval between the two-first relapses, with a CIS characterized by cerebellar or motor involvement (p = 0.03) and most of them fulfilled Barkhof’s criteria at presentation (p < 0.001). The progressive clinical phase, with or without surimposed relapses occurred earlier than of patients born in France. Conclusions: The course of MS is more aggressive in NA MS patients compared with Caucasians patients. Results were statistically significant for gender, age at the first symptom, short time between the 2-first relapses, sequelae after the first demyelinating event, and important burden of disease on the first MRI.

P462

Determinants of residual disability after multiple sclerosis relapses: a multivariate analysis
M. Vercellino, A. Merola, C. Maragò, F. Plano, S. Masera, C. Piacentino, A. Chiò, R. Mutani, P. Cavallì; University of Turin (Turin, I)

The recovery from Multiple Sclerosis (MS) relapses is very variable, and the factors which can influence the persistence of residual disability after a relapse have not been yet thoroughly investigated. The aim of our study is to ascertain the extent of residual disability after MS relapses and to determine which factors are associated with a higher or lower risk of persistence of residual disability after a relapse. Data were collected for all relapses in a population of relapsing-remitting MS patients followed by a MS referral center in Turin, Italy, during three years, reaching a number of 100 relapses (each for a different patient). Severity of the relapse and residual disability after one month and one year were calculated basing on the Expanded Disability Status Scale (EDSS). A multivariate analysis for factors influencing residual disability was also performed, considering variables such as age, sex, duration of disease, number of previous relapses, severity of the relapse, presence of oligoclonal bands, presentation with optic neuritis or with corticospinal involvement. Persistence of residual disability after one year was observed in 47% of the relapses. Mean residual disability after one year was 0.55 EDSS units; severe residual disability (EDSS change one year after the relapse > 1, if compared to baseline EDSS) at one year was present in 33% of the relapses. The presence of residual disability at one month was highly predictive of persistence of residual disability at one year. Factors associated with a higher risk of persistence of residual disability one year after a relapse were a severe relapse (EDSS change at the relapse > 1, if compared to baseline EDSS) (Exp(B) = 6,933; 95% CI 2,633–18,257; p < 0.0001) and a relapse presenting with optic neuritis (Exp(B) = 8,902; 95% CI 1,992–39,775; p = 0.004). Risk of a severe relapse (EDSS change at the relapse > 1, if compared to baseline EDSS) was lower in patients with age > 30 years at the time of the relapse (Exp(B) = 0,169; 95% CI 0,066–0,429; p < 0.0001). Duration of disease did not seem to influence the risk of persistence of residual disability after a relapse. In conclusion, relapses are an important determinant of accumulation of disability in MS patients. The persistence of residual disability after a relapse is common, and severity of the relapse is the main factor associated with the risk of residual disability, probably due to more severe tissue damage in severe relapses.

P463

Atlas of Multiple Sclerosis, WHO/MSIF
A.J. Thompson, M.A. Battaglia, R. Porter, T. Dua, P.J. Rompani, I. Douglas; Institute of Neurology (London, UK); AISM (Genoa, I); WHO (Geneva, CH); MSIF (London, UK)

Introduction: Multiple sclerosis (MS) is the most common primary neurological disorder of young adults in most parts of the world. Although some patients experience little disability during their lifetime, up to 60% are no longer fully ambulatory 20 years after onset. Such functional decline often interferes with patients’ opportunities

www.sagepub.co.uk

Multiples Sclerosis 2006; 12: S1–S228

Abstracts 5127

S127

Downloaded from msj.sagepub.com by Shula Edelkind on October 1, 2010
to perform customary roles, and has negative consequences for their quality of life. Because the onset of MS is typically at about age 30, patients’ loss in productivity can be substantial and the financial cost to society is great. Both patients and their family members also bear a financial burden. The information about resources available within countries worldwide to tackle the huge medical, social and economic burden caused by MS is lacking. To fill this information gap, a survey of country resources available to diagnose, inform, treat, support and care for people with MS is being conducted within the framework of the World Health Organisation’s (WHO) Project Atlas. It represents a major collaborative effort involving WHO and the Multiple Sclerosis International Federation (MSIF) and its members. Methodology: Data is being collected in the form of a questionnaire from key persons identified as the WHO and MSIF in every country with a significant prevalence of MS. Countries are grouped into the six WHO regions and four World Bank income categories.

Data Collection and Presentation: The Atlas of MS presents information in 4 broad sections; (1) MS: the disorder; (2) services; (3) care-providers; (4) public health aspects. The data included is organized in themes and is presented as graphs, world maps and written text. The results are presented as global, WHO regions and income categories within each theme. Selected limitations specific to each theme are highlighted and need to be considered when interpreting the data and their analyses. The Atlas also includes brief reviews of selected topics summarizing the medical, lifestyle, social and economic issues surrounding people with MS.

Conclusions: The results confirm that resources for MS diagnosis, treatment, care and support vary widely between countries, and in many cases appear grossly inadequate compared to the needs exhibited in most countries. The value of the Atlas is in replacing impressions and opinions with facts and figures. We hope that the realities uncovered by the Atlas will motivate governments and health care providers to improve MS treatment and care.

P464

Is the real level of disability due to multiple sclerosis hidden to neurologists and other health professionals?

R. Simmons; Canberra Hospital (Canberra, AUS)

The Australian MS Longitudinal Study (AMLS) tracks a nationwide sample of >2,700 people with MS using interdisciplinary survey methodology and a relational database. In one longer-term project, both patient self-report and neurologist-derived data are collected on disease status in relation to use of specific immunotherapies, as well as self-reported use of complementary and alternative therapies. In order to validate the patient self report on medication usage and disease status, data were compared with neurologists’ reports where both were available in the same year (2005). Using the same scales (Disease Steps, Hohol et al 1995), it was found that for severe disability (Disease Steps 6 and 7) there was good agreement between patient and neurologist assessment of disability level (Cohen’s Kappa for inter-rater reliability = 0.789). However, for less than severe MS there was poor agreement between neurologists and patients, with neurologists more likely to rate as mild impairment (Disease Steps 1, 2) what patients themselves rated as moderate impairment (Disease Steps 3 – 5) (K = 0.521). It appeared that MS patients with less than severe disability were “putting their best foot forward” when visiting the doctor compared with the difficulties they admitted to in an anonymous survey. Follow-up questioning of patients substantiated this phenomenon and resulted in qualitative data of potential relevance to the clinical consultation. The main categories of patient concern were: (1) the neurologist looks at my MS differently, and doesn’t see the evolution. Whatever the outcome measure, the date of origin was

P465

Recombinant interferon-beta efficacy in preventing conversion of clinically isolated syndromes to clinically defined multiple sclerosis: a Cochrane meta-analysis

M. Clerico, F. Faggiano, J. Palace, G. Rice, M. Tintore Subithana, L. Durelli; San Luigi Gonzaga University Hospital (Orbassano, I); Amedeo Avogadro University Hospital (Novara, I); Radcliffe Hospital (Oxford, UK); London Health Sciences Center (London, CAN); Val d’Hebron Hospital (Barcelona, E)

Objective: To perform a Cochrane meta-analysis based on published data on the efficacy of Interferon (IFN) beta in preventing conversion of clinically isolated syndromes (CIS) to clinically defined multiple sclerosis (CDMS). Background: IFN beta for multiple sclerosis (MS) is approved either at low-dose once-weekly or high-dose multiple weekly injections. A Cochrane meta-analysis showed a limited efficacy in reducing disease activity. In all studies treatment started in patients with a long disease history. Design/method: Relevant articles have been identified attempting different search strategies (medical literature, data bases, abstracts books). Following the Cochrane methodology we established the criteria for considering studies (types of studies, types of participants, type of intervention, types of outcome measures). Results: 454 papers identified; 430 not eligible; 6 of adequate quality all referred to two studies, CHAMPS and ETOMS, both using once-weekly low-dose IFN beta 1a. At first, since the two studies had different outcomes (occurrence of a second clinical episode or of disease progression in CHAMPS; occurrence of a second clinical episode in ETOMS) the studies have been analysed separately. Per protocol analyses showed that IFN beta treatment significantly prevented the outcome. Sensitivity analyses (“best”, “worst” and “likely” scenarios) have been done. Results of CHAMPS study were not statistically significant in both “worst” and “likely” scenarios at 1 year (analyses at 2 years were not possible because not all the enrolled patients completed the 2-year follow up). ETOMS results were statistically significant in all scenarios at 1 year; not significant in worst scenario at 2 years. As second step, we merged the data in a meta-analysis only at 1 yearsince at 2 years was not possible. Those patients in CHAMPS study whose conversion was due to the progression (5 patients) have been taken off. The metaanalysis was significant in “per protocol” analysis and in “sensitivity analysis” in every scenarios but the “worst” one. Conclusion: The efficacy of early IFN beta treatment in preventing conversion to MS was modest at 1 year. Both the separated analysis as well the metaanalysis provided no firm evidence that once-weekly low-dose IFN beta prevent conversion to MS beyond 1 year.

P466

Benign multiple sclerosis?


Multiple sclerosis (MS) is characterized by a great heterogeneity in its long term prognosis. It is worthy, for patient’s counselling but also for a better understanding of the natural history of MS, to consider whether patients without substantial disability (less or equal to an irreversible score of 3 on the Kurtzke’s Disability Status Scale) after 5, 10, 15 or 20 years, may develop significant disability afterwards. We explored therefore the Lyon MS database to evaluate the median time to an irreversible score of 4, 6, 7 and the onset of the progressive phase, after 5, 10, 15 or 20 years of so-called benign evolution. Whatever the outcome measure, the date of origin was

Multiple Sclerosis 2006; 12: 51 – 528
the date of MS onset plus 5, 10, 15 or 20 years respectively for each step of interest. Patients were censored at the date of the last visit whenever they did not reach the endpoint. For the purpose of this study, we analyzed only data before April 1997. They correspond to the natural history of MS in the cohort, as the first immunoactive drugs (Interferons) with a proven efficacy on disability progression were made available in France only after this date. Out of the initial 1844 patients, we found 811 patients with an irreversible score of 3 or less 5 years after MS onset, 482 after 10 years, 258 after 15 years and 115 after 20 years. During the consecutive follow-up, 381, 211, 106 and 50 reached an irreversible score of 4, respectively. According to the Kaplan-Meier survival analysis, the median time to an irreversible score of 4 was 12.1 years [95% confidence interval: 10.7 – 13.5], 11.8 years [10.1 – 13.4], 10.1 years [7.5 – 12.7] and 10.0 years [8.2 – 11.8] after respectively 5, 10, 15 and 20 years of a benign course. Similar analyses are also available for irreversible scores of 6 and 7, and for the onset of the progressive phase. Our data demonstrate that a so-called benign MS may start to deteriorate even after many years without significant disability. Even if the medians might have been underestimated because of a differential follow-up bias towards more severe cases, those results lead to reconsider the definition of a benign course and to remain cautious in advising patients.

### P467

**Benign multiple sclerosis**

S. Glad, H. Nyland, J.H. Aarseth, K.M. Myhr; MS National Competence Centre (Bergen, N)

**Background:** Studies dealing with factors influencing clinical course and survival in multiple sclerosis (MS) have frequently defined a benign course as minimal or no disability, equivalent to a score on the Expanded Disability Status Scale (EDSS) ≤ 3.0 at least 10 years after disease onset. Patients with benign MS may later experience disease progression and thus develop a more non-benign course. The EDSS is heavily weighted toward motor function while non-motor symptoms have less impact on the EDSS.

**Objective:** Study longitudinal trend of benign MS, evaluate the impact of non-motor symptoms on function and identify potential clinical or demographic predictors of a benign course.

**Material and Methods:** MS patients with disease onset during 1976–1986 in Hordaland County, Western Norway were included. Patients still remaining in the benign MS group or changing to benign MS status were followed for a median period of 5 yrs (range 1 – 9 yrs). At enrollment, 29.7% of patients with MS were classified in group I, 19.2% in group II and 20.8% in group III (p < 0.0001). Normal MRI at enrollment (p < 0.00001), female gender (p < 0.0005) and younger age at onset (p = 0.0048) were predictive of benign MS course. Having SP (p < 0.0001) and PP (p = 0.029) MS, and being in ENBP group (p < 0.0001) determined a higher loss at follow-up. Of those in the original benign cohort with follow-up visits, 66.5% in group I, 64.2% in group II and 65% in group III were classified as benign MS after 5 yrs (p = NS). 17.4% of patients in PB group, 18% in ENBP group and 9.1% in LNBP group were classified as benign after 5 years. RR disease course (p < 0.00001), younger age at enrollment (p = 0.003) and cumulative use of disease-modifying treatment (p = 0.014) increased the likelihood of remaining in the benign MS group or changing to benign MS status over 5 years in NYSMSC cohort. **Conclusions:** Classification criteria for benign MS are essential in establishing its frequency, with definition II (EDSS ≤ 2/DD ≥ 10) being most conservative. The choice of classification criteria did not affect conversion to non-benign MS after 5 years. Use of disease-modifying treatment increased the proportion of benign MS in the NYSMSC cohort.

### P468

**Evolution of benign multiple sclerosis in the New York State Multiple Sclerosis Consortium according to different classification criteria**

R. Zivadinov, B. Weinstock-Guttman, D.L. Cookfair, L. Knopp, S. Schwind, A.E. Miller, N. Luna, P. Coyle; A. Goodman, C. Granger, C. Milham, C. Christodoulou, B. Jubelt, M. Lenihan, J. Herbert, M.H. Gottesman, D.H. Snyder, N. Garg, A.T. Frontera, B. Apostoli, R. Holub, A.B. Perel, K. Patrick, D.P. Singh, F.E. Munschauer; University of Buffalo (Buffalo, USA); University of Stony Brook (Stony Brook, USA); University of Rochester (Rochester, USA); Maimonides Medical Center (Brooklyn, USA); Albany Medical College (Albany, USA); University of Syracuse (Syracuse, USA); Glens Falls Neurology (Glens Falls, USA); The Hospital for Joint Diseases/Orthopedic Institute (New York City, USA); Winthrop-University Hospital (Mineola, USA); New York Hospital Medical Center of Queens (Flushing, USA); Kingston Neurological Associates (Kingston, USA); Cornell Medical Center (New York City, USA); Neurological Associates of Albany (Albany, USA); Alpha Neurology (Staten Island, USA)

**Background:** The New York State Multiple Consortium (NYSMSC) is a regional affinity group of 17 multiple sclerosis (MS) centers.

**Objectives:** To monitor the proportion of benign MS in NYSMSC at enrollment and to evaluate the long-term evolution of benign MS during 5-year follow-up, according to the 3 most commonly used classification criteria of benign MS.

**Methods:** This was a historical cohort-study in which clinical and demographic information was taken from the centralized NYSMSC database. Three different definitions were used to classify patients as benign MS at baseline and at follow-up: I) EDSS ≤ 3 and disease duration (DD) ≥ 10 yrs, II) EDSS ≤ 2 and DD ≥ 10 yrs, and III) EDSS ≤ 3 and DD ≥ 15 yrs. Within these definitions, patients were placed in one of 4 categories: benign, potentially benign (PB) group (EDSS ≤ 3/DD < 15 yrs), early non-benign progression group (ENBP) (EDSS > 3 or 2, DD > 10 or 15 yrs) and late non-benign progression group (LNBP) (EDSS > 3 or 2, DD > 10 yrs or 15 yrs). **Results:** Out of 7706 at enrollment, 6258 fulfilled the inclusion criteria and entered the study. Of these, 59.6% were followed for a median period of 5 yrs (range 1 – 9 yrs). At enrollment, 29.7% of patients with MS were classified in group I, 19.2% in group II and 20.8% in group III (p < 0.0001). Normal MRI at enrollment (p < 0.00001), female gender (p < 0.0005) and younger age at onset (p = 0.0048) were predictive of benign MS course. Having SP (p < 0.0001) and PP (p = 0.029) MS, and being in ENBP group (p < 0.0001) determined a higher loss at follow-up. Of those in the original benign cohort with follow-up visits, 66.5% in group I, 64.2% in group II and 65% in group III were classified as benign MS after 5 yrs (p = NS). 17.4% of patients in PB group, 18% in ENBP group and 9.1% in LNBP group were classified as benign after 5 years. RR disease course (p < 0.00001), younger age at enrollment (p = 0.003) and cumulative use of disease-modifying treatment (p = 0.014) increased the likelihood of remaining in the benign MS group or changing to benign MS status over 5 years in NYSMSC cohort. **Conclusions:** Classification criteria for benign MS are essential in establishing its frequency, with definition II (EDSS ≤ 2/DD ≥ 10) being most conservative. The choice of classification criteria did not affect conversion to non-benign MS after 5 years. Use of disease-modifying treatment increased the proportion of benign MS in the NYSMSC cohort.
Abstracts

2. To examine disease progression and associated risk factors that did/ did not form part of the selection criteria. Methods: Controls were selected from the British-Columbia MS population based natural history database. 'Study entry' was defined as the first EDSS score in 1988. Inclusion criteria at study entry were: definite MS; age 18–50 years; 2+ relapses in the last 2 years and EDSS 0–5.0. Outcomes were: time from study entry to secondary-progressive MS (SPMS) and sustained EDSS 3.6 (confirmed at 6 months with no lower subsequent EDSS scores). Prognostic factors at study entry were examined individually in a Kaplan-Meier analysis and simultaneously in a Cox proportional hazards model: age; onset age of MS; relapses in last 2 years; EDSS; disease duration* and gender.* (*characteristics not explicit in the selection criteria). An 'intention to treat' approach was taken; those subsequently starting an IMD were included to minimize bias. Results: 101 patients met the inclusion criteria; 77% were female, mean age at study entry = 35.0 years and onset of MS = 26.2 years; mean relapse count in last 2 years = 2.9 (range 2–8) and median EDSS = 2.0. Mean follow-up from study entry = 11.3 years (range 1.3–15.4 years). Although 35/101 (34.7%) were prescribed an IMD, in this group, the first 9.1 years (range 2.5–13.1) were IMD-free. Median time to SPMS from study entry = 10.2 years (95% CI: 6.4–14.1); to EDSS 3 = 7.2 years (95% CI: 2.8–11.5); one-quarter reached EDSS 6 by 5.7 years (95% CI: 3.2–8.3). From the Kaplan-Meier curves, the study entry EDSS affected all three outcomes (p < 0.008). The multivariate analysis found time to EDSS 3 and 6 were only affected by the entry EDSS (p = 0.019 and p = 0.001, respectively), as was SPMS (p = 0.021) which was additionally affected by gender (p = 0.021) and entry age (p = 0.006). Conclusions: Selection of historical controls based on published selection criteria from clinical trials is feasible; however factors not specified by the criteria could influence subsequent progression. Close matching of individuals from each clinical trial is probably the optimal approach; we found the study entry EDSS score the most crucial factor in RRMS.

P471

Age-related conversion to secondary progression in relapsing-remitting multiple sclerosis
I. Bosca, B. Casanova, A. Pascual-Lozano, M. Magnuner, F. Coret; Hospital Universitario La Fe (Valencia, E); Hospital Clinico Universitario (Valencia, E)

Objective: to study the influence of age at the beginning of relapsing-remitting multiple sclerosis (RRMS) and the current age over conversion to secondary progression (SPMS). Methods: All originally RRMS patients, followed for a minimum of one year, from the Hospital Universitario La Fe and Hospital Clinico Universitario MS Units (Valencia) were recruited for this study. Scheduled visits every three or six months and whenever patients had a relapse were performed. MS cases were defined using the Poser criteria. Course of MS was categorized as relapsing-remitting (RRMS) and secondary-progressive (SPMS) according to Lublin criteria. Secondary progression was prospectively diagnosed when a patient had an EDSS increase of 1.0 points, over 6 months, sustained 3 months after (0.5 points if previous EDSS was 5.0 or higher). We recorded demographic data (gender, date of birth); date of onset; date and number of relapses; date of conversion to SPMS; and use of immunomodulatory treatment. Depending on the age at the beginning of MS, patients were grouped into four age-groups: < 20, 21–30, 31–40, > 40. Kaplan-Meier survival curves, with the end-point ‘conversion to SPMS’, were performed with two different basal times: ‘evolution time’ and ‘age’. Hazard ratio was calculated comparing the < 20 group with the rest of groups.

Results: Since 1995, 399 RRMS patients have been followed in our MS Units. Demographic characteristics: 68.4% females, mean age at the beginning of MS 28.2 (SD 8.82). Clinical characteristics: mean disease duration 10.26 years (SD 7.29), conversion to SPMS 18.3% of patients, mean age at conversion 41.2 (SD 8.3). Kaplan-Meier survival estimates (considering the time: evolution time) showed that patients in 31–40 and > 40 groups reached sooner the conversion to SPMS (Hazard ratio 31–40: 2.47, p = 0.021; Hazard ratio > 40: 6.74, p = 0.001). When performing Kaplan-Meier survival estimates considering the time: age, no differences were shown between age-groups.

Conclusions: Younger patients take longer to reach the progressive phase of MS, which has traditionally been considered as a good prognostic factor. But these results suggest that the conversion to SPMS is, at least to some extent, age-dependent and not substantially affected by the age at the beginning of the disease.

P470

Is late onset multiple sclerosis associated with a worse outcome?
H. Tremlett, V. Devonshire; University of British Columbia (Vancouver, CAN)

Background: Typically, late-onset MS (LOMS) is associated with a poor prognosis, however the population characteristics and natural history of LOMS have been explicitly described in only a few studies. Aims: To describe the characteristics of late-onset MS (LOMS, 50+ years) compared to adult-onset MS (AOMS, 16–<50 years) and examine disease progression and associated prognostic factors. Methods: We selected patients from the British Columbia MS (BCMS) longitudinal population-based database who are followed prospectively from the beginning of MS (Poser criteria), onset prior to July 1988 (to maximize the possibility of a substantial and meaningful follow-up time), registered with a BCMS clinic before July 1998 (to allow the disease course to be established), with at least one Expanded Disability Status Scale (EDSS) score. No minimum follow-up time was required. Disease course was classified clinically into either a primary progressive (PP) or relapsing (R) course from onset. Clinical and demographic characteristics were compared between LO and AOMS. Progression was measured as time to reach sustained EDSS 6 and potential risk factors examined were: gender, disease course (PP v R) and onset symptoms. Results: Of those eligible (n = 2837), LOMS comprised 32 (4.7%), with PPMS predominating (54.5% v 10.6% in AOMS, p < 0.0005). Motor onset symptoms were more prevalent in LOMS and sensory and optic neuropathy more prevalent in AOMS (p < 0.0005). AOMS averaged 27.7 years (95% CI: 26.3–29.1) to EDSS 6 from onset v 16.9 years (95% CI: 9.0–24.8) in LOMS, p < 0.0005. However, AOMS was associated with a younger age at EDSS 6, (58.4 years (95% CI: 57.1–59.6) v 71.2 years (95% CI: 65.2–77.3) in LOMS, p < 0.0005). There were no differences in progression between AOMS or LO for those with PPMS (p = 0.373) or R-MS (p = 0.438), although considerable variation was observed.

Conclusions: We found that late-onset MS was not necessarily associated with a worse outcome: firstly, progression in the PPMS or R-MS patients differed little between late-onset versus adult-onset; secondly, those with late-onset MS were older when reaching EDSS 6 than adult-onset MS. The disease course has a greater implication for disease prognosis than actual presentation of MS late in life.

P472

Childhood onset multiple sclerosis (the KIDMUS study): course and prognosis as compared to adult onset multiple sclerosis

There are limited data on the natural history of childhood onset MS and wether it differs from adult onset MS in terms of its course and prognosis is still unknown. The Kidmus multicenter study addresses these issues. Patients have been selected from pediatric and adult departments of neurology. We report the part of the study concerning patients selected from adult departments. The cohort was gathered through the European Database for Multiple Sclerosis (EDMUS) users network from France and Belgium and originated
Long-term effects of childbirth in multiple sclerosis
M.B. D’hooghe, G. Nagels; National MS Center (Melsbroek, B)

Background: In a large European study the short term influence of pregnancy on the relapse rate in multiple sclerosis (MS) has been demonstrated. No effect on disability progression was found. A beneficial effect of childbirth after the onset of MS on the time to progression has been suggested in 2 earlier studies. Recently, the Multiple Sclerosis Severity Score (MSSS) was shown to be a powerful method to detect differences in rates of progression in patient groups. Using single assessment data, the MSSS reflects the progression of the EDSS over time and may detect an influence of childbirth on disease severity. Goal: To study whether delivery of a life infant after the onset of MS affects disease progression as measured by the MSSS and the time to secondary progression in heterogeneous population of MS patients seen at our MS-centre. Methods: Informative data about MS history, last neurological status and childbirth were obtained from the medical records of 233 female patients with definite MS. MSSS and time to secondary progression were determined. To analyse the effects of childbirth, 2 groups of patients were made: one with no children after the onset of MS and one with children born after the onset of MS. Results: Data from 233 MS patients (99 relapsing remitting (RR), 119 secondary progressive (SP), 14 primary progressive (PP) and 1 progressive relapsing (PR) were analysed. The first group (n = 173) included 54 patients with no childbirths and 119 patients with children, born before the onset of MS. In the second group (n = 60), 14 patients had children born both before and after the onset of MS and 46 patients had children born after the onset of MS. The mean MSSS in the first group was 6.4 ± 2.6 compared to 4.6 ± 2.5 in the second group. In an ANOVA with the MSSS as dependent variable, the presence or absence of childbirth after MS onset as fixed factor and age at onset as covariate, the overall effect was clearly significant (df = 2, F = 10.013, p < 0.001). The effect of childbirth after MS onset was significant (df = 1, F = 11.681, p = 0.001) while there was no significant effect of age at onset (df = 1, F = 1.407, p = 0.237). Time to progression was clearly shorter in the first group (8.2 ± 6.8 years) compared to the second group (16.6 ± 10.5 years). This difference was confirmed and significant in a survival analysis (Cox proportional hazard model, p = 0.001). These results support a beneficial effect of childbirth after the onset of MS on disease severity.
and frequencies had no specificity compared with non MS patients, excepted for the age incidence. Free interval from MS diagnosis to cancer was 7.3 yrs. (-23+/+34). Female patients had more cancers than males (RR: 2.35 vs; p = 0.0002). PP and SP patients had a higher cancer risk than RR patients (RR: 2.03; p = 0.0004). Standardized incidence rates calculated for all MS associated with cancer were 0.11 for men and 0.67 for women. Conclusions: According to the recent literature, overall incidence of cancer in our MS population is lower than expected in the general population. Matched to age, gender, MS form and histologies of French Cancer Registry, cancer in MS is associated with young age, female gender, SPMS form and familial history of cancer.

P476
Impact of modifying disease’s treatments on the cancer incidence in multiple sclerosis: annual report of the CARIMS cohort
C. Lebrun, M. Debouverie, P. Vermeersch, P. Clavelou, L. Rumbach, J. Deseze, G. Defer, D. Aufauvre, R. Deschamps, F. Berthier, A. Danzon on behalf of the Club Francophone de la SEP

Background: Recent data demonstrated that female MS patients has an increased risk of breast cancer. All except one published studies did not consider potential risk factors such as medication, smoking and diet. Female MS patients treated with glatiramer acetate (GA) showed an elevated rate of breast cancer and all MS patients treated with beta interferons (IFN) showed an elevated risk of non-breast cancers though not statistically significant. Objective: To collect and study patients profile with an history of cancer and multiple sclerosis among the 7322 MS patients gathered from 8 French MS centers. To evaluate impact of disease’s modifying therapies (IM: IFN or GA; IS: cyclophosphamide, methotrexate, azathioprine, mitoxantrone) on cancer’s incidence. Methods: Prospective study on MS patients with or without history of cancer and their MS treatments. Data from the FRANCIM network of French population-based Cancer Registries (available from period 1975-2000) were used as reference. 3 groups of patients were identified: A: patients without history of IM or IS treatments; B: patients with only IM treatments; C: patients with IM+IS treatments. Results: 117 patients (1.6%) with definite MS and cancer were identified: 100 women, 17 men, mean age at MS diagnosis: 37.9 yrs; Age at cancer diagnosis was group A: 58 patients; 47.7 yrs; B: 29 yrs; 41.9 yr and C: 30 yrs; 47.3 yr (p = 0.02). Mean duration of IM treatments before cancer was 38 months (1-84) with one to 4 different lignes of treatments. Compared to the 2793 patients who had only IM treatment in our database, patients with cancer were younger at MS onset (31.4 vs. 34.5 yrs) and had a longer exposure to IM (22 vs. 38 months; p < 0.05). 62.7% patients had cancer diagnosis during IM or IS treatments. 44 patients with cancer history had IM (26) or IS (18) treatments after cancer remission. Conclusions: Matched to age, gender and histologies of French Cancer Registry, cancer in MS is associated with young age at MS onset, IM treatments with younger age at cancer diagnosis and time to exposure to IM (p < 0.0001). No association was found between cancer’s incidence, gender and IS treatments. History of cancer does not influenced therapeutic strategy and choice of IM or IS drugs. It is important to follow and collect in MS centers, patients with history of cancer, to document histologies, and potential relations with repeated or long-term IM or IS treatments.

P477
Descriptive epidemiology of multiple sclerosis in la Coruña (northwest Spain)
D.A. García-Estévez, M. Marín-Sánchez, A. López-Real, I. Urrutia-Díaz, T. Lema-Facal, Hospital Juan Canalejo (La Coruña, E)

Introduction: The latest epidemiological prevalence studies have placed Spain in a moderate-high risk area for developing Multiple Sclerosis (MS). The latest studies on its incidence in our country suggest that this has almost doubled in the last decade. The social and health impact of MS is determined by the major disability that it produces at early stages of life and by the high cost of the immunomodulating treatment, so that knowledge of the epidemiology of the disease is important. Aims: The aims of our study were to determine the prevalence (P) and the accumulated incidence (AI) of MS in the city of La Coruña, as well as to ascertain the degree of disability of our patients. Methods: La Coruña is a city of Northwest Spain that has a latitude of 43°23’N. It is a very well defined geographic area with a satisfactory healthcare system. We carried out a prospective study from 1-1-2004 to 31-12-2005 in order to determine the AI of MS in the different care districts of the city. The P date was 31-12-2005. The population census at 1-1-2005 was 243,349 inhabitants. All clinically defined and clinically probable cases of MS according to Poser’s clinical criteria were included. The diagnosis was always performed by a neurologist. Imported cases and non-native patients were excluded as, depending on the critical period of exposure, they could bring the risk from their area of origin. All the patients had a brain MR scan compatible with the diagnosis. Disability was assessed by applying the EDSS. Results: 165 cases were recorded at the prevalence date, 28 of these were incident cases. MS was 2.17 times more frequent in women (113F:52M). The age at diagnosis was 33.5 ± 11.0 years. The AI in the period was 11.5 cases/100,000 inhabitants (CI95%: 7.25 – 15.75). The value of P was 67.80 cases/100,000 inhabitants (CI95%: 57.48 – 78.12). As regards disability, 71.2% had an EDSS <3, and 12.3% an EDSS >6.5. The distribution of the clinical course forms was as follows: relapsing-remitting 79.6%, primary-progressive 9.6%, secondary-progressive 7.6% and progressive-relapsing 3.2%. Conclusions: The accumulated incidence of MS in La Coruña is one of the highest reported to date in Spain, with a prevalence that makes our city a high risk area for the disease. Secondary-progressive MS in our population is low, and we believe that in the coming years we will be witnessing an increase in the degree of disability of our patients and, therefore, of the social and health costs.

P478
Incidence of multiple sclerosis in Auvergne (French county): a population-based study
F. Taithe, C. Tilgner, L. Rieu, C. Murat, P. Clavelou on behalf of Reseau SEP Auvergne

Introduction: An epidemiological study on multiple sclerosis (MS) was performed between 1 January 2003 and 31 December 2005 to ascertain the prevalence and the incidence of this disease in Auvergne (French county at 45° northern latitude; 1,309,000 inhabitants in 1999). Methods: The incident cases were identified by centers of rehabilitation and all neurologists, together in a regional network which allowed this general population-based study. Then, each case was classified by 3 independent neurologists according to Poser’s and MacDonald criteria and clinical form: clinically isolated syndrome with oligoclonal bands in CSF and more than 2 hyperT2 on MRI (CIS), clinically definite MS (CD) and primary progressive forms (PP). This survey concerned only the patients with a disease onset occurred in Auvergne and during the studied period. Results: For the three studied years, 166 patients were included: 26 CD [19 women and 7 men (sex ratio = 2.71); mean age at clinical onset 30.2 ± 3.5 years], 130 CIS [104 women and 26 men (sex ratio = 4); mean age 35.86 ± 2.9 years] and 10 PP [4 women and 6 men (sex ratio = 0.6); mean age 41.7 ± 2.9 years]. Clinical data were similar with published databases. The evaluated mean annual clinical onset adjusted incidence (with definite and probable MS according to the Poser criteria) was 4.22 per 100,000. Evolution of CIS are still in progress. Conclusions: Auvergne seems to be one of the high-incidence regions noted in Europe. However incident cases ascertainment with a disease onset during these 3 years must be prolonged at least 2 years to take in count the delay between clinical onset and diagnosis.
Cigarette smoking and risk of multiple sclerosis in multiplex families

N. Jafari, I.A. Hoppenbrouwers, R.Q. Hintzen; MS Centre ErasMS (Rotterdam, NL)

Objective: To determine whether persons with multiple sclerosis (MS) more often have a history of cigarette smoking before the age of onset of the disease than their unaffected siblings. Background: Previous studies suggest a history of cigarette smoking as a risk factor for MS and perhaps even a worse disease course. A family study has not yet been performed. Within a family, excellent matching can be established for both environmental and genetic factors. Methods: In a matched case-control study of 73 multiplex MS families, individuals with MS (n = 153) were compared to sibling controls (n = 232). Participants were selected from Erasmus MS family cohort (GEMS), which counts more than 125 families. We enrolled MS probands with one or more unaffected siblings, all participating in a nationwide study on genetics of MS. Self-report questionnaires were mailed to the participants. For clarifying the association between cigarette smoking and risk of developing MS, we used conditional logistic regression. We controlled for confounding by sex, age at time of study and age of onset as a continuous variable. Because both age and sex are associated with MS, we conducted analyses stratified by age and sex. Results: In this preliminary analysis of 75% of the questionnaires, we found that smokers had an increased risk of MS (odds ratios were 2.90 (95% CI 0.68–2.90) p < 0.084 and in the group with age of onset below 28.3 years old odds’s ratios for ever smokers were 2.43 (95% CI 0.89–6.63) p < 0.084 and in the group with age of onset above 28.3 years old, odds’s ratios were 1.40 (95% CI 0.68–2.90) p < 0.362. Additional data on the effect of duration of smoking, dose of smoking (pack years) and their relation to disease course will be presented. Conclusions: Interim analysis in these multiplex MS families demonstrates a higher, but non-significant risk for MS after smoking before age of MS onset.

Nutrition and multiple sclerosis: an ecological study

K. Lauer (Griesheim, D)

Besides infectious agents, climate, tobacco smoking, and a low amount of vitamin D, also diet is considered as possibly relevant in multiple sclerosis (MS). Prior epidemiological studies suggested a role of animal fats and proteins, meat, pork, and smoked meat, whereas vegetables, fish and seafood, fibres, juices, potassium, calcium, and vitamins E and C were negatively associated. In the present study nutritional data from the Food Balance Sheets (FBS) of the Food and Agriculture Organisation (FAO) of the United Nations were evaluated on three steps. The MS prevalence 1980–2006 in 58 countries was subdivided in group A (more than 60 per 100,000; n = 19), group B (31–80 per 100,000; n = 10), and group C (less than 31 per 100,000; n = 29). The annual intake (in kg) 1984–1986 of 79 items from macro-nutrients and individual food commodities, given in a standardized formate, was taken for comparison. All items (excluding calories) were divided by caloric intake and compared with the MS prevalence by the Mann-Whitney U test. Significance was assessed at p < 0.05. When group A and group B countries were compared, after first comparing groups (A+B) vs. C countries, the following positive associations remained: animal calories (p = 0.0002), animal proteins (p = 0.01), total fat (p = 0.005), animal fat (p = 0.0001), rye (p = 0.01), oats (p = 0.002), peas (p = 0.04), coffee (p = 0.02), cocoa (p = 0.03), barley beer (p = 0.005), all meat (p = 0.04), pigmeat (p = 0.04), milk (p = 0.049), copra oil (p = 0.03), all animal fat products (p = 0.0004), butter/ghee (p = 0.0007), and cream (p = 0.001). Vegetable calories, vegetable proteins, all cereals, rice, maize, all pulses, and beans were all negatively related to MS. Between Italy (MS prevalence: 34.4 per 100,000) and Malta (15 per 100,000) the ratio was 2.3 for rice, coffee, cocoa, all animal fat products, and cream, and lower than 0.67 for all pulses and beans. Smoking and nitrate-curing of meat and sausages that was not included in the FBS, is practised, according to numerous ethno-geographic sources, in Italy (as in Spain, Portugal and Greece), but is virtually absent from Malta. Neither calories nor any fish or seafood item was associated with MS. From an additional consideration of historic perspectives of food, coffee, cocoa, and smoked and nitrate-cured meat are among the first candidates to be studied further by individual-based approaches.
that limit physical activity. The objective of this study is to estimate the prevalence of overweight and obesity in veterans with MS enrolled in the Veterans Health Administration (VHA), compared to gender specific published rates for the US population, veterans receiving outpatient care at VA medical facilities, and male veterans in the VHA with spinal cord injury (SCI). Setting: A retrospective study of 9,688 veterans with MS enrolled in VHA identified by linking the VA MS Centers of Excellence Data Repository to the VA Office of Quality and Performance 1999 Large Health Survey and the Survey of Healthcare Experiences of Patients (2002-2004). Methods: Body mass index (BMI = kg/m²) was calculated from self-reported weights and heights. Subjects were then classified into four weight categories (95% CI) based on Center for Disease Control guidelines: underweight (BMI < 18.5), normal weight (18.5 ≤ BMI ≤ 29), and obese (BMI ≥ 30). Findings: The proportion of males with MS (N = 8,536) in the four weight classifications is: 2.9% (2.55, 3.27) underweight, 32.3% (31.29, 33.29) normal weight, 42.1% (41.02, 43.12) overweight and 22.7% (21.84, 23.64) obese. The proportion of females who are overweight and obese is 28.5% and 33.4% for the US population, 31.0% and 27.2% for SCI. The proportion of females who are overweight and obese is 38.7% for veterans and slightly decreased rates of obesity.

Neurophysiology

P484

Relationship of core body temperature to saccadic latency profiles in multiple sclerosis

A. Saltel, S. Davis, C. Crandall, P. Kramer, T. Frohman, E. Frohman; UT Southwestern Medical Center (Dallas, USA); New Jersey Neuroscience Institute (South Orange, USA)

Objective: To characterize the effects of core body temperatures changes on the saccadic latency profiles in MS patients. Background: MS patients often exhibit transient deterioration in neurologic function in response to changes in body temperature. The quantitative analysis of saccadic eye movements, provide an objective model for characterizing the relationship between changes in core body temperature and physical function. These studies have implications for understanding the pathophysiology of demyelination in MS.

Methods: Twenty-four subjects (8 normal subjects; 8 MS patients with INO; 8 MS patients without INO) were heated with water passed through a tube lined suit (NASA). Core body temperature was monitored utilizing an ingestible temperature probe and telemetry. During heating and cooling conditions, saccadic eye movements were recording using 2-D infrared oculography. The latency of saccadic eye movements was analyzed across the subject groups, and with respect to stimulus characteristics (simultaneous, gap, and overlap conditions). Results: There was a significant stratification in the saccadic latency profile across the different subject groups and across stimulus conditions. Increases in core body temperature were associated with a corresponding prolongation in saccadic latency in MS patients. Conclusion: MS patients exhibited prolongation in saccadic latency in response to increases in core body temperature. Latencies were most significantly affected in those with INO (demyelination within the MLF). Our observations objectify the Uhthoff’s phenomenon and provide a quantitative model of MS pathophysiology that may be useful for confirming the efficacy of treatment interventions focused on preventing heat induced changes in axonal conduction mechanisms.

P485

Temperature-induced worsening of saccadic velocity in multiple sclerosis patients with internuclear ophthalmoplegia

T. Frohman, S. Davis, C. Crandall, P. Kramer, E. Frohman; University of Texas (Dallas, USA); New Jersey Neuroscience Institute (South Orange, USA)

Objective: To determine whether INO is a useful model to document objective neurophysiologic changes in response to elevations in core temperature. Background: Many patients with MS experience transient exacerbations of clinical symptoms as a result of increases in body temperature. The transmission of electrical impulses by nerve fibers is exquisitely heat sensitive and demyelination can compound this phenomenon. Internuclear Ophthalmoplegia (INO) is the most common ocular motor abnormality observed in MS patients and results from myelin loss in the medial longitudinal fasciculus (MLF). INO is characterized by slowing of adduction in the eye ipsilateral to the side of the lesion. Infrared oculography has been established as an effective tool for identifying and quantifying the severity of INO, and may also be used to characterize the effects of increased core temperature on the saccadic velocity profiles in MS patients with INO. Methods: Twenty-four subjects (eight MS patients with INO; eight MS patients without INO, and eight healthy controls) underwent whole-body heating (increased internal temperature ~1.0 °C) via a water perfused tube-lined suit. An ingestible telemetry pill provided continuous internal temperature recordings throughout the heating process. 2D infrared oculography (500 Hz sampling rate) was used to record 20 deg saccadic eye movements...
every 10 minutes during baseline (perfusing 34°C water) and whole-body heating (perfusing 46–48°C water). Saccadic eye movements were analyzed using the versinal disconjugacy index (VDI), the velocity ratio of abducting to adducting eye movements. Results: There were no significant changes observed in VDI between baseline and peak heating for the healthy control group or the MS non-INO group (p = 0.770 and p = 0.900, respectively). Significant VDI differences were observed in the MS with INO group at peak heating when compared to baseline (p < 0.007). Conclusions: INO is an objective model for corroborating the hypothesis that changes in core body temperature are associated with decay in axonal conduction. Ocular motor measures may constitute a useful paradigm to detect and monitor responses to therapeutic strategies that stabilize nerve cell membranes in response to temperature induced decay in axonal conduction mechanisms.

P486

Central motor conduction examined with the triple stimulation technique correlates with pyramidal tract dysfunction and disability in patients with multiple sclerosis U. Hofstätt-van Oy, D. Hagenburger, C. Klawé, K. Schröder; Krankenhaus der Barnhuisen Brüder (Trier, D)

Inflammatory lesions and axonal degeneration of central motor conduction pathways result in gait disturbance and result in increasing disability in patients with multiple sclerosis (MS). Conventional transcranial magnetic stimulation can be used to estimate central conduction times, but is a less sensitive method to detect axonal damage or conduction block and correlates weakly with clinical disability. The triple stimulation technique (TST) examines the corticospinal tract by magnetic stimulation of cortical motor areas followed by sequential distal and proximal peripheral nerve electric stimulation. The TST potential is a result of the collision of the sequential stimuli. The TST potential is compared with sequential stimulation of peripheral nerves alone, and central conduction failure can be quantified with the resulting amplitude ratio. Methods: Upper limb (to abductor digitii minimi muscle) and lower limb (to abductor hallucis muscle) TST was applied to 50 healthy volunteers to establish normal values and 60 patients with relapsing remitting, secondary progressive and primary progressive MS. Patients also underwent neurological examination including the Expanded Disability Status Score (EDSS) according to Kurtzke and in the Multiple Sclerosis Functional Composite Score (MSFC) to quantify clinical upper and lower limb function. Results: The EDSS of patients varied between 0 to 8.5. Central conduction failure quantified with TST correlated significantly with the EDSS (r = 0.45 for the TST to upper limb, r = 0.6 for TST to lower limb) and with the upper and lower limb motor function scores of the MSFC. Conclusion: TST is a useful method for examining central conduction failure in different stages of disability in patients with MS and correlates well with clinical deficits and disability scales reflecting motor involvement. TST could serve as a surrogate marker for axonal degeneration of motor pathways in MS.

P487

In asymptomatic multiple sclerosis subjects, detectable motor disorders are present but do not impair the ability to adapt to unfamiliar dynamic environments C. Solaro, M. Casadio, G. Brichetto, V. Sanguineti, P.G. Morasso; ASL3 (Genoa, I); University of Genoa (Genoa, I)

A prerequisite for motor rehabilitation is that patients preserve their ability to adapt to novel dynamic environments, an ability attributed to the cerebellar system. In this study, we use a robot manipulandum to test the ability of Multiple Sclerosis patients with subclinical sensorimotor symptoms to adapt to an unfamiliar dynamical environment, which consisted of a speed-dependent curvy force field. We used a planar robotic manipulandum (Braccio di Ferro), specifically designed for robot therapy and for the evaluation of motor learning and control. We studied 11 right-handed subjects with clinically defined, relapsing-remitting MS, according to Poser criteria (Poser et al., 1983), participated in this study. The inclusion criteria were: (i) Extended Disability Status Scale (EDSS) less or equal than 1 (ii) ‘normal’ score for the ‘arm’ portion of the Scrpps Neurological Rating Scale (NRS) (Sipe et al., 1984) for the sensory, motor and cerebellar systems. All the patients were examined by the same neurologist. The exclusion criteria were: (i) relapses within the last three months; (ii) treatment with corticosteroids within the previous three months; (iii) Mini Mental State Examination (MMSE) less than 24. The performance of these subjects was compared with 11 age-matched controls with previous history of neurological dysfunction. Movements analysis of the reaching trajectories was carried out by estimating the following indicators: Movement duration, Linearity, Aiming Error; Symmetry, Jerk index. Jerk ratio We found that mild MS subjects do indeed display incoordination problems (movements more curved, less smooth and greater aiming error) but have an intact ability to adapt to the force field. In general, this suggests that in spite of the motor deficits the subjects are capable of building internal dynamical models that are necessary for carrying out feedforward motor control actions.

P488

Kinematic analysis of cerebellar multiple sclerosis subjects and relationship between incoordination and lesion load G. Brichetto, V. Sanguineti, P.G. Morasso, G.L. Mancardi, C. Solaro; University of Genova (Genoa, I)

Introduction: Cerebellar symptoms in Multiple Sclerosis (MS) are common and their frequency varying in different study but could be estimated around 30% of subjects. In MS the assessment of sensorimotor impairment is difficult due to the dissemination in space and time of the central nervous system lesions and the lack of correlation between neuroimaging and neurological status. Kinetic analysis, using biorobotic devices, allows to identify and quantify arm movements alteration and to propose a cybernetic framework of interpretation. The aim of the study is (i) to evaluate “pure” cerebellar MS subjects utilising a kinematic approach in order to quantify upper limb movement and to (ii) to correlate MRI with kinematic data. Materials and Methods: Seven subjects with MS and prevalent cerebellar involvement (5 female, 2 male, ages 34–64 years, mean 46.6 ± 10.7 years; disease duration 6–43 years, mean 16 ± 13.1 years) were included in the study. Seven healthy age matched subjects were taken into consideration as controls. Each patients underwent clinical examination with the following scales: the EDSS score, Scrpps NRS and the nine-hole peg test (9HPT). Brain MRI scans were performed and infratentorial brain T1 and T2 lesion load, supratentorial brain T1 and T2 lesion load and total brain T1 and T2 lesion load were calculated. All subjects (control and cerebellar) performed center-out reaching movements (in eight randomly chosen directions) with their dominant hand on a digitizing tablet under VISION and NO VISION condition. Kinematic analysis estimated a number of indicators: aiming error, path curvature, symmetry and smoothness. Data were analysed utilizing an univariate analysis for kinematic parameter. Relationship between MRI parameters (T2 and T1 total lesion load), kinematic parameters and 9HPT were assessed using Spearman’s Rank Correlation. Results: We found a significant effect of disease and direction in all kinematic parameters considered. Regarding the effect of vision on trajectories we found a significant effect in symmetry, linearity and jerk. No correlations were found between 9HPT and kinematic parameters. T1 and T2 total lesion load showed significant correlation with smoothness and linearity parameter. Discussion: Kinematic analysis showed that in MS cerebellar subjects trajectories tend to be less smooth and less linear than controls and that kinematic parameter such as smoothness and linearity correlate with anatomical damage measured by MRI.
**P489**

**Long latency reflexes in multiple sclerosis: clinical, electrophysiologic and radiologic relationship**

H. Ertasoglu, B. Ismihanoglu, F. Seleker, E. Uysal, H. Forta, M. Çelik; Sisli Etfal Education and Research Hospital (Istanbul, TR)

**Background:** Long Latency Reflexes (LLR’s) tests both afferent and efferent pathways as transcortical ones. In this study we studied the diagnostic value of LLR’s and the relationship of them with the corpus callosum volume in patients with multiple sclerosis (MS).

**Material and Methods:** We studied 32 MS patients having ‘definite MS’ and 27 healthy controls prospectively, using Magnetic Resonans Imaging (MRI) and LLR’s. Expanded Disability Status Scale (EDSS) was obtained in all MS patients. Corpus callosum volume was measured in T1 weighted sequence in midsagittal plane, on the intercommissural line by drawing the corpus callosum contours manually and calculated automatically as mm³. The LLR’s recorded from the m. abductor pollicis brevis by stimulating the median nerve at the wrist. LLR II was recorded in all healthy controls. The lowest and upper limit of normal corpus callosum volume and LLR II latency were defined as ±3 standard deviation of the average values of them in the control group.

**Results:** Only the absence of the LLR II accepted pathologic. In 50% of the MS patients LLR was pathologic. EDSS significantly correlated with LLR II latencies (p<0.000). There was significantly negative correlation with EDSS and corpus callosum volume (p<0.000). The correlation between LLR II latencies and corpus callosum was also significant (p=0.049). The difference between the LLR latencies of the right and left hand approached statistical significance (p=0.035).

**Conclusion:** Easily performed LLR and corpus callosum volume measurement, showing concordance with the disability may be helpful in the MS diagnosis.

---

**P490**

**Olfactory dysfunction in patients with multiple sclerosis: correlation with clinical variables**

J. Kosti, E. Stefanova, T. Pekmezovic, J. Drulovic; Institute of Neurology, CCS (Belgrade, CS)

Multiple sclerosis (MS) is a chronic disease with a broad spectrum of neurological signs and symptoms. Studies using sensitive, objective and reliable standardized test of odor identification ability designed at the University of Pennsylvania (UPSIIT), have pointed out not only that a considerable number of MS patients have olfactory dysfunction, but also that the olfactory disturbances can be troublesome symptom, rarely detected by the individuals themselves. The aim of this study was to test odor identification ability in patients with MS and to examine possible correlations between the smell identification test scores and various clinical variables. A case-control study included 50 patients with MS diagnosed according to McDonald criteria (2001) and the same number of age-, sex- and smoking habit-matched healthy controls. The Pocket Smell Test (PST) has been used in this study as a brief test to identify olfactory loss in the groups. Furthermore, examination of: 1) neurological disability using Expanded Disability Status Scale (EDSS), 2) psychological functioning by Hamilton Depression Rating Scale (HDRS) and Hamilton Anxiety Rating Scale (HARS), and 3) cognitive impairment with Brief Repeatable Battery of Neuropsychological Tests (BRB-N), was performed. Our results demonstrated that olfactory dysfunction was significantly more frequent in MS patients (44.0%) than in the control group (11.1%) (p<0.001). Inverse correlation was found between PST and EDSS (r = -0.160, p = 0.266), duration of the disease (r = -0.092, p = 0.526), HDRS (r = -0.092, p = 0.525) and HARS (r = -0.081, p = 0.577). Positive correlation was demonstrated between PST and PASAT (r = 0.40, p = 0.049). However, none of these relations reached the level of statistical significance. Our findings support the notion that reduced sense of smell is frequent in patients with MS. The observed weak association between odor identification ability and PASAT, as part of BRB-N test, warrants further investigation of the relation between olfactory and cognitive dysfunctions in MS patients.

---

**P491**

**Motor-evoked potentials as a biological outcome measure in multiple sclerosis? Relation to disability and MRI**

N.F. Kalkers, R.L.M. Strijers, M.M.S. Jaspere, V. Neacu, F. Barkhof, C.H. Polman, C.J. Stain; VU Medical Center (Amsterdam, NL)

**Introduction:** Several outcome measures for measuring disability and progression are used in Multiple Sclerosis (MS) trials. Clinical outcome measures include the Expanded Disability Status Scale (EDSS) and the MS Functional Composite (MSFC), the latter comprising tests for cognitive function, hand-function and leg function. Biological outcome measures include Magnetic Resonance Imaging (MRI) techniques. However, none of these appear to be ‘gold standards’ for measuring in vivo pathology and disability. Therefore, Motor Evoked Potentials (MEP) were proposed, providing data on the function of the central motor pathways. We studied whether MEP can serve as a sensitive and reliable measure for evaluating neurological dysfunction in MS, by relating MEP to clinical and MRI measures. **Methods:** Fifty-two patients with MS underwent the MSFC and EDSS. We performed transcranial magnetic stimulation of the motor cortex on each patient to determine the conduction time to both arms and both legs. We recorded F-wave responses and used these to calculate the Central Conduction Time (CCT). Z-scores were computed (ZCCT) based on reference values matched for height. All patients underwent an MRI scan, of which T1 and T2 lesion volumes, brain volume and spinal cord volume were derived. For statistical analysis, Spearman rank correlation coefficients were calculated and stepwise linear regression analysis was performed with the MSFC and EDSS as dependent measure. **Results:** We found a significant correlation for the EDSS and leg function test with ZCCT of both arms and legs (r = 0.36 – 0.53; p<0.01). The correlation between ZCCT of the left arm and the MSFC (r = -0.29; p<0.05) and spinal cord volume (r = -0.31; p<0.05) was significant. T1 lesion volume correlated with ZCCT of the right arm, left arm and left leg (r = 0.33, r = 0.32 and r = 0.30 respectively; p<0.05), as T2 lesion volume correlated with ZCCT of the right arm only (r = 0.31; p<0.05). Linear regression analysis revealed that both the MSFC and EDSS were explained by both T1 lesion volume and ZCCT left leg adjusted R2 = 0.30 and 0.36 respectively. **Conclusion:** In this study we found a relation between MEP, brain and spinal cord MRI measures, and both the MSFC and the MSFC. Moreover, a model for explaining disability in MS revealed that MEP provides an added value above MRI measures, suggesting that MEP can serve as a biological outcome measure in MS.

---

**P492**

**Sympathetic skin response test in multiple sclerosis**

H. Ayronolu, M. Yazdchi Marandi, M. Mokhtary; Imam Hospital (Tabriz, IR)

**Background:** Multiple sclerosis (MS) is a demyelinating disease of central nervous system that involves it as a scattered pattern. One of the most prominent features of this disorder is autonomic nervous system involvement. Sympathetic skin response (SSR) is one of the most useful tests for evaluating integrity of sympathetic cholinergic functions. There are few studies about the SSR test and its relation with autonomic nervous system involvement in this disease, so we decided to detect SSR abnormality in our definite MS cases. **Methods:** In this case control analytical study 30 patients (17 female and 13 male, aged 18 – 55 years) with clinically definite MS diagnosis assessed with SSR in four limbs and compared the mean amplitudes of N1P1 and latency of P0 with normal persons (14 female and16 male with 19 – 50 years). All patients examined neurologically and were questioned about autonomic symptoms then registered latency and amplitude of SSR with Toennies Neuroscreen R plus machine. Then
we compared SSR parameters in MS cases with normal persons and correlation with autonomic symptoms, duration of disease, limbs weakness, disability scale and sweating disorder. T student test, Mann Whitney and analysis of variance were used for analysis and p < 0.05 considered significant. Results: The mean P0 latency amplitude of N1P1 in upper limbs of MS cases were 1.64 ± 0.22 s and 4.29 ± 5.12 mv respectively and 2.28 ± 0.47 s 1.61 ± 0.95 mv in lower limbs respectively but this parameters in control group for upper limb were 1.43 ± 0.07 s and 3.95 ± 1.5 mv and for lower limb were 1.82 ± 0.19 s and 2.4 ± 0.4 mv in order. P0 latencies in patient group was significantly longer than normal group despite amplitude parameters. We found significant correlation between P0 latency of a limb and weakness at that limb compared with normal persons. Also there was significant correlation between P0 latency of lower limbs disability severity. There wasn’t any correlation in age of disease onset, sex, duration of disease and sweat or other autonomic symptoms with SSR parameters (against some later studies). Conclusion: We concluded that SSR test has 80% sensitivity and 90% specificity in MS patients and we can use it as a useful test for studying autonomic nervous system in central demyelinating disorders.

P493

The study of sensitivity of evoked potentials in multiple sclerosis
M. Yazdchi Marandi, H. Ayromlou, M. Talebi; Emam Khomeini Hospital (Azadi, IR)

Introduction: Multiple sclerosis is an idiopathic inflammatory demyelinating disease of central nervous system. It is one of the most important neurologic diseases by virtue of its frequency, chronicity and tendency to attack young adults. Our purpose in this research was that assaysing the sensitivity of evoked potentials among the patients with multiple sclerosis. Materials and Method: We selected according to poser criteria, 36 patients with definite and 7 patients with probable multiple sclerosis (32 female and 11 male) that referred to neurodiagnostic ward of Tabriz Imam Khomeini hospital. We performed standard Visual, Somatosensory and Brainstem evoked potentials by Toeniss neuroscreen plus machine on them. Results: In this research, we found 88.8% sensitivity of visual evoked potentials in definite and 57.1% in probable multiple sclerosis, 83.3% sensitivity of tibialis somatosensory evoked potential in definite and 28.6% in probable multiple sclerosis and the sensitivity of auditory brainstem evoked potential in definite multiple sclerosis was 22.2%. We didn’t find any significant correlation between evoked potentials and sexuality or age. Conclusion: Visual evoked potential and somatosensory evoked potential are very useful methods for diagnosing multiple sclerosis. We don’t recommended auditory brain stem evoked potential for early diagnosis of Multiple sclerosis.

P494

Vestibular evoked neurogenic potentials are more sensitive in detecting abnormalities than vestibular evoked myogenic potentials in early stage multiple sclerosis
E. Papathanasiou, S. Papacostas, M. Pantzaris; Cyprus Institute of Neurology & Genetics (Nicosia, CY)

Introduction: Vestibular evoked myogenic potentials (VEMP) are a new method of examining the function of the vestibular system. It uses high intensity auditory clicks or tones to stimulate the saccule located close to the oval window, and are recorded from the tonically active sacculesternocleidomastoid muscle. Vestibular evoked neurogenic potentials (VENP) have been developed by our group using the same stimulus but recording from the scalp. A negative wave occurring about 5 msec after stimulus onset (labeled N5) is recorded from the parietal area and is believed to be specific to the vestibular system. The purpose of this study is to compare the two examinations in the diagnosis of multiple sclerosis (MS) using the established McDonald criteria. Methods: Patients under investigation for possible MS were included in this study. VEMP were performed by delivering a monaural 105 dB nHL auditory stimulus with contralateral masking noise via headphones, with recording from the tonically active sternocleidomastoid muscle. VENP were performed using a monaural 80–90 dB nHL tone-pip auditory stimulus with 2 cycles again via headphones with contralateral masking noise. Recording was performed using an ipsilateral parietal (P3 or P4) to Fpz montage. Results: Twenty patients have been studied. VENPs were abnormal in five patients (25%), with VEMP remaining normal in all patients. Visual evoked potentials were abnormal in four patients (20%). Five patients have been diagnosed with MS since the beginning of the study, with one patient having an abnormal VENP and which provided a diagnosis of MS based on the McDonald criteria before other paraclinical examinations. In fourteen patients still under investigation, an abnormal VENP resulted in the diagnosis of MS in one patient based on the McDonald Criteria. Conclusions: VENP can detect lesions in early stage MS better than VEMP, and compares favorably with visual evoked potentials which are considered in the criteria to be very useful in diagnosis. VENPs may aid in the early diagnosis of MS based on the McDonald criteria by providing objective clinical evidence of a lesion.
Neurobiology

P496

Cerebrospinal fluid of multiple sclerosis patients exert significantly weaker inhibition of kynurenine aminotransferase I activity in rat liver homogenate

B. Keplinger, H. Baran, A. Kainz, D. Zeiner, J. Wallner; Karl Landsteiner Institute Mauer (Mauer/Amstetten, A); VM University (Vienna, A)

Background: Kynurenine acid (KYNA) is a metabolite in the kynurenine pathway of tryptophan degradation and an endogenous antagonist of all ionotropic glutamate receptors and a7 nicotinic acetylcholine receptor. Kynurenine aminotransferase I and II (KAT I and KAT II) with different catalytic properties catalyze the biosynthesis of KYNA from L-kynurenine. Multiple sclerosis (MS), an autoimmune disease, is characterized by demyelination in the CNS accompanied by the disappearance of oligodendrocytes, neuronal/axonal degeneration and proliferation of astrocytes. The induction of MS is still not known. The aim of the study was to search cerebrospinal fluid (CSF) of MS patients and CSF of corresponding control subjects (CO) in respect to their ability to influence KYNA formation. The activity of KAT I and of KAT II in rat liver homogenates (RLH) in the presence of CSF of MS patient the KAT I activity of RLH reduced only by 38.6% (p <0.001) of RLH. No effect of CSF-CO on KAT II activity of RLH was observed. In the presence of CSF of MS patient the KAT I activity of RLH reduced only by 38.6% (p <0.001).

Methods: The KAT I and KAT II activities were measured using a radio-enzymatic method in the presence of 1 mM pyruvate and 100 mM [H3]-L-kynurenine. Results: The CSF of CO subjects significantly reduced KAT I activity by 71.5% (p <0.001) of RLH. No effect of CSF-CO on KAT II activity of RLH was observed. In the presence of CSF of MS patient the KAT I activity of RLH reduced only by 38.6% (p <0.001).

Conclusions: Obtained data indicate that the constitution of compounds and/or factors present in the CSF of MS is changed, compared to CSF of CO. Alteration of KAT I activity in the CSF of MS patients and also alteration of KYNA formation could be a transient event induced by the neuro-inflammatory process. KYNA might have anti-inflammatory and immuno suppressive activities. We suggest the presence of “KAT I activity depressing factor” in the CSF (and likely in the CNS) of CO subjects and the reduction of this factor(s) has been found in the CSF of MS patients, which might be of significance for MS diagnosis.

P497

Apoptosis inducing factor and JNK are indispensable for TNF-induced death of human adult oligodendrocytes

A. Jarzewicz, M. Matysiak, K. Tybor, C.S. Rainie, K. Selma; Medical University of Lodz (Lodz, PL); Albert Einstein College of Medicine (New York, USA)

Tumor necrosis factor (TNF) induces apoptotic-like cell death of oligodendrocytes, the cell type targeted in MS. The ligation of TNF receptors induces several signal transduction pathways including caspase cascade, MAP kinase cascade and mitochondrial derived factors like apoptosis inducing factor (AIF). The intracellular signaling pathways in TNF-induced death of mature human oligodendrocytes have not been well characterized. Oligodendrocytes (OLs) were prepared from human brain specimen. TNF-induced OLs death, was detected by flow cytometry with annexinV-FITC and PI staining, and was non-caspase dependent, as evidenced by: lack of generation of caspase-3 and -8, -9 and -3 active subunits on Western blot; lack of cleavage of caspase-1 and -3 fluorogenic substrates assessed by flow cytometry and lack of OLs death inhibition by the general caspase inhibitor, ZVAD-FMK. The intracellular signaling involved in TNF-induced death of OLs, JNK activation occurred prior to mitochondrial membrane dysfunction. Electrophoresis of TNF-exposed OLs DNA revealed large scale DNA fragmentation characteristic for AIF-mediated cell death, and co-localization experiments showed that AIF translocation to the nucleus occurred upon exposure to TNF. AIF depletion by an antisense strategy prevented TNF-induced OLs death but not mitochondrial membrane dysfunction. These data suggest that TNF stimulation induced JNK activation what lead to mitochondrial membrane dysfunction leading to AIF translocation into nucleus. Additionally, in brain sections, within the edge of MS lesion, some oligodendrocytes defined by anti-MBP staining showed nuclei positive for AIF, what might suggest effector role of AIF in OLs demise in MS. These results indicate that TNF-induced death of OLs depends on JNK activation, mitochondrial membrane dysfunction and AIF translocation into nuclei, information of significance for the design strategies to protect OLs during immune-mediated demyelination.

P498

Neurotrophic factors and neuroinflammation: experimental autoimmune encephalomyelitis in conditional BDNF-knock out mice and effective treatment with glatiramer acetate

D. Lee, R. Linker, I. Siglentli, F. Luehder, N. Kruse, M. Sendtner, R. Gold; Institute for Multiple Sclerosis Research (Gottingen, D); University of Wurzburg (Wurzburg, D)

Brain derived neurotrophic factor (BDNF) is involved in neuronal and glial development and survival. It is mainly produced by neurons, but also by leukocytes in vitro and in multiple sclerosis (MS) lesions. Yet, the functional relevance of BDNF expression by immune cells in autoimmun demyelination is still unknown, since conventional BDNF knockout mice die prematurely. We applied the Cre/loxP system to generate mice with a conditional deletion of BDNF in the T-cell lineage (lckCre BDNF flox/flox mice), in myeloid cells (lysMCre BDNF flox/flox mice) or in both lineages together (lysMcCre lckCre BDNF flox/flox mice). In these mice, experimental autoimmune encephalomyelitis (EAE) was actively induced with myelin oligodendrocyte glycoprotein peptide 35–55 (MOG 35–55). In a therapeutic approach, mice were injected with glatiramer acetate (GA), which is known to increase BDNF secretion and to exert beneficial effects in MS. Compared to wild type (WT) and lckCre control mice, lckCre BDNF flox/flox mice developed a similar disease course of MOG 35–55 EAE. Likewise, MOG 35–55 EAE in lysMCre BDNF flox/flox mice and lysMcCre lcktg BDNF flox/flox mice was of similar severity in the chronic phase of the disease. The immune reaction in conditional BDNF knock-out mice was attenuated with a decrease in interferon-gamma production and reduced inflammatory infiltration (Mac3 pos. macrophages: 338.3±268.1 per spinal cross section vs. 883.9±311.3 in WT). Despite less inflammation in chronic MOG-EAE, the extent of axonal damage was not different between lysMCre BDNF flox/flox mice and lckCre knock-out mice. These data support an unexpected role for BDNF for maintenance of axonal integrity, but also suggest a novel role of immune-cell derived BDNF in immunoregulation. Supported by SFB 581 TPA1 and TEVA Pharmaceuticals.

P499

Interferon-beta has a modular effect on de- and remyelination in the cuprizone model

C. Trebst, S. Heine, S. Lienenklaus, M. Lindner, W. Baumgärtner, S. Weiss, M. Stangel; Medical School Hannover (Hannover, D); German Research Center for Biotechnology (Braunschweig, D); Veterinary School Hannover (Hannover, D)

Background and Goals: Spontaneous remyelination and repair mechanisms in multiple sclerosis are mostly insufficient and...
contribute to clinical disability. Treatments improving these processes are not yet available. Interferon-beta (IFN-beta) has a known disease modifying effect in reducing the number of relapses in relapsing-remitting multiple sclerosis (MS). Here, we investigated whether IFN-beta has a potential in modifying the extent of de- and remyelination in the cuprizone model of MS. Methods: IFN-beta knock out (k/o) mice and wild type (C57Bl/6 mice) control mice were subjected to the cuprizone model. Extent of de- and remyelination was evaluated by scoring of LFB stainings and myelin protein immunohistochemistry at several different time points (at week 3 and 6 during demyelination; week 7, 9 and 12 during remyelination). In selected animals electron microscopy studies were done for quantitative analysis. Astrocytic and microglial response during de- and remyelination was quantified by immunohistochemistry for the non-phosphorylated neurofilament GFAP and MAC-3-positive cells within the corpus callosum. The number of oligodendrocyte precursor cells within the demyelinated lesion was assessed by quantitative analysis of NG2 immunohistochemistry. Immunohistochemistry for the non-phosphorylated neurofilament (SMI-32) served for the assessment of axonal pathology. Results: In IFN-beta k/o mice peak demyelination was significantly reduced as compared to the wild type control (p < 0.0019 for LFB at week 3; p = 0.001 for MOG at week 6). During the remyelination phase IFN-beta k/o mice showed a more rapid remyelination as compared to the controls (p = 0.03 for LFB at week 7). The overall amount of remyelination after 12 weeks did not differ between the two groups. Less demyelination in IFN-beta k/o mice was paralleled by a diminished astrocytic and microglia response (p = 0.025 for microglia at week 3, p = 0.019 for astrogocytes at week 6), whereas the more rapid remyelination was paralleled by an increased number of oligodendrocyte precursor cells within the demyelinated lesion (p = 0.046 at week 6). Evaluation of axonal pathology showed no differences between IFN-beta k/o mice and controls. Conclusions: IFN-beta has a modulatory effect on de- and remyelination in the cuprizone model.

Cannabinoid receptors 1 and 2 are highly upregulated in the blood RNA of multiple sclerosis patients

L. Jean-Gilles, A. Fahay, C.S. Constantinescu; University of Nottingham (Nottingham, UK)

The activation of cannabinoid receptor 1 (CB1), which is expressed predominantly in the central and peripheral nervous system, and cannabinoid receptor 2 (CB2), which is mainly localized in the peripheral immune system, strongly appear to reduce inflammatory and neuropathic pain as well as spasticity, muscle spasms, tremor and pain associated with multiple sclerosis. Although previous studies have demonstrated that such actions are mediated through both neuro- and immunoregulatory mechanisms of CB1 and CB2, respectively, few have investigated the regulation of these receptors in multiple sclerosis patients. Hence, this study examined the level of expression of each receptor in multiple sclerosis blood RNA. Blood was collected from normal (n = 19) and multiple sclerosis (n = 40) donors and RNA was extracted. CB1 and CB2 receptor expression was then measured by quantitative reverse transcriptase-polymerase chain reaction. The level of cannabinoid receptor expression revealed by each group (normal and multiple sclerosis) was then compared and analysed. The obtained results demonstrated that both receptors are highly expressed in multiple sclerosis patient blood when compared to normal subjects. More specifically, CB1 and CB2 were found to have an 8 to 10-fold increase in multiple sclerosis subjects as opposed to their counterparts. Such upregulation suggests an important role for both receptors in multiple sclerosis pathology. The underlying cause for the increased regulation of cannabinoid receptors in this disease has not yet been established. However, our studies have previously shown that a number of proinflammatory cytokines which were also found to be upregulated in multiple sclerosis, such as IL-12 and TNF-alpha, induce both CB1 and CB2 expression on peripheral blood mononuclear cells. Hence, the upregulation of cannabinoid receptors in multiple sclerosis may be induced as a protective mechanism against the detrimental effects of increased cytokine levels.
PS04

Serum and cerebrospinal fluid levels of neural cell adhesion molecule in multiple sclerosis
A.R. Massaro, G. Carbone; UCSC Medical School (Rome, I)

It was demonstrated that the neural cell adhesion molecule (NCAM) increased in the cerebrospinal fluid (CSF) of acute multiple sclerosis (MS) patients treated with corticosteroids who were improving after an attack. The aim of this study is to elucidate whether such an increase is a local phenomenon due to events taking place within the CNS or the reflex on the CSF of changes occurring in the blood. We studied serum and CSF NCAM of 77 acute MS patients and of 19 non-neurological controls. Serum and CSF specimens of MS patients were collected before and after steroid treatment, while specimens from non-neurological controls (patients who received a lumbar puncture for surgical reasons) were collected only once. NCAM was tested by an inhibition ELISA previously set up and described. The possibility of any influence of serum NCAM on CSF levels linked to a passage of NCAM through the BBB was evaluated by calculating the correlation between serum and CSF levels. Further, the CSF/serum NCAM ratio was calculated, the BBB was evaluated by calculating the correlation between serum NCAM on CSF levels linked to a passage of NCAM through the BBB. This reexpression of an oligodendroglial potassium channel in the diseased CNS might have an implication on OL cell cycle progression thus influencing remyelination in EAE and MS.

PS05

Re-expression of the embryonic morphogen BMP-6 in the lesions of experimental autoimmune encephalomyelitis
T. Seifert, J. Bauer, R. Weisert, C. Linnington, F. Fazekas, M.K. Storch; Medical University Graz (Graz, A); Medical University Vienna (Vienna, A); Heinrich Heine University (Dusseldorf, D); University of Aberdeen (Aberdeen, UK)

Bone morphogenetic proteins (BMPs) comprise members of the transforming growth factor beta superfamily. In central nervous system (CNS) development, they have been shown to direct neural progenitor cells to commit to the as trocytic rather than the oligodendroglial lineage and to regulate oligodendrocyte pre-cursor cell maturation. These proteins also exert neurotrophic activity in experimental models of traumatic, toxic and ischemic CNS injury. We investigated the expression of BMP-6 in experimental autoimmune encephalomyelitis induced by myelin-oligodendrocyte glycoprotein (MOG-EAE), a model closely mimicking multiple sclerosis and enabling thorough examination of different stages of demyelination and re-myelination. MOG-EAE was actively induced in DA rats. Histological evaluation was performed with light and confocal microscopy on paraffin-embedded CNS sections from days 20 to 120 after active immunization. In normal brain and spinal cord, BMP-6 was found in a punctuate pattern in the dorsal horn and the brainstem and in Schwann cells of the dorsal roots. In early active demyelinating lesions no BMP-6 immuno-reactivity (IR) was found. BMP-6 IR in few oligodendrocytes was found in late active demyelinating lesions. Strong immunostaining for BMP-6 was detected in inactive lesions as well as in very early remyelinating lesions. The vast majority of BMP-6 expressing cells in these lesions were oligodendrocytes. Additionally, BMP-6 IR in astrocytes was observed. Cell types were confirmed by double and triple labelling for confocal microscopy. BMP-6 IR was not found in fully remyelinated shadow plaques. In conclusion, the embryonic morphogen BMP-6 is re-expressed in lesion evolution in MOG-EAE. BMP-6 expression peaks in inactive and very early remyelinating lesions, and the majority of BMP-6 expressing cells are oligodendrocytes. The temporarily restricted pattern of BMP-6 expression in our animal model may suggest a putative role of this protein in remyelination following inflammatory demyelination.

Neuropsychology and fatigue

PS06

Impaired recognition of facial affect in multiple sclerosis
J.K. Berneiser, A.O. Hamm, J. Wendt, C. Kessler, A. Dressel; Universität Greifswald (Greifswald, D)

Background and Aim of the Study: The importance of cognitive and emotional disturbances in MS has been recognized in recent years. However, the comprehension of emotional clues which is important to maintain effective social interactions has rarely been studied. Therefore, we investigated the ability of MS patients to recognize and discriminate emotional facial expressions. Patients and Methods: 54 MS patients were recruited from the MS outpatient clinic of the University Greifswald. Patients had relapsing remitting MS (n = 41), secondary progressive MS (n = 11) or primary progressive MS (n = 2), the median age was 42.5 (range 19 to 68); the median Kurtzke’s Expanded Disability Status Scale score (EDSS) was 3.5 (1.0 to 8.0); and the median Multiple Sclerosis Functional Composite Index (MSFC) was 0.34 (–1.30 to 1.47); and the median Beck depression inventory score 8.5 (0 – 33). For this study the visual items of a standardized measure of emotional perception (Tübinger Affect Battery, (TAB)) were applied. Data were analysed using students t test and Spearman correlation. Results: There was no significant impairment
in the ability to discriminate facial identity (p = 0.136). In contrast, the patients had a reduced ability to discriminate facial affect (p = 0.003), correctly name (p = 0.005) select (p = 0.006) and match facial affects (p = 0.017). In addition, the total score of the TAB was inversely correlated with the BDI r = -0.435 (p = 0.002) and measures of physical disability EDSS r = -0.462 (p = 0.007) and correlated positively with the MSFC r = 0.581 (p < 0.001) and its subset for cognitive function (Faced serial addition test) r = 0.396 (p < 0.004).

Conclusion: The data presented here reveal that recognition of emotional facial expression is impaired in MS patients. Moreover, this cannot be explained by a reduced visual acuity since performance in a face recognition task was not altered. Therefore, deficits in social cognition may contribute to disability in MS. Studies to investigate the social cognition in MS in greater detail are needed to further elucidate the structural basis of these deficits.

PS07

Screening for cognition and callosal pathology in multiple sclerosis
A. Lombe, M. Kempa, M. Haupt, H. Shanib, P. Calabrese; Ruhr University Bochum (Bochum, D)

Background: The Corpus Callosum (CC) is the main interhemispheric commissure of the brain and thus represents an important hub for neuronal networks. Multiple Sclerosis (MS) may result in considerable regional and generalized brain atrophy including the CC. The objective of the present study was to assess callosal atrophy and cognitive abilities in patients with MS. Methods: 30 patients with confirmed first manifestation or RR-MS (mean age 38 ± 11 yrs., median EDSS 3.5, mean duration of MS 3 ± 3 years) were included. Callosal areas were assessed in cm² using midsagittal T2 images in a previously described planimetry procedure (Habel L et al., J Neurol 2005, 252, Suppl 2, II62 - 63)

Neuropsychological measures of general intelligence, verbal memory, and attention were compared to a MS specific cognitive screening battery (MUSIC: multiple sclerosis inventory cognition). Pearson’s correlations were calculated for midsagittal CC area and neuropsychological parameters. Results: Mean CC area was reduced to 5.08 cm² (SD 1.52 cm²) compared to a normal mean of 6.9 ± 1 cm². While there was no relationship between CC measures and general intelligence or short term memory, significant correlations were found between attention, verbal memory and most of the subtests of our MUSIC test-battery. Conclusions: Relevant correlations between midsagittal CC area and distinct cognitive domains were demonstrable, while variation of callosal area did not correlate to general intellectual ability. The MUSIC screening test, which has been specifically developed to develop the cognitive core deficits in MS, was most sensitive for this purpose. These results may indicate that working memory and flexibility-dependent cognitive operations require bilateral cerebral processing, thus needing intense interhemispheric information exchange. Since this functional mechanism is compromised by MS-related brain lesions CC pathology might result in serious cognitive deficits.

PS08

Cognitive impairment in multiple sclerosis: results of a multicentre study in Argentina
S. Vanotti, R. Benedict, F. Caceres, W. Vanon; Inaba (Buenos Aires, RA); State University of New York at Buffalo (Buffalo, USA)

Background: Cognitive impairment occurs in 40% to 60% of all patients with multiple sclerosis (MS). Impaired cognition greatly impacts the conduct of activities of daily living, leading to poor quality of life. In Argentina, information regarding the prevalence and management of cognitive impairment in patients with MS is currently unavailable. Objectives: To assess the prevalence of cognitive impairment in patients with MS in Argentina and to validate the predictive values of cognitive function tests and their correlations with both disease and disability status. Methods: We are continuing to enroll MS patients and demographically matched healthy controls from 20 multiple centers in Argentina. Cognitive performance is evaluated using the Brief Repeatable Battery-Neuropsychology. The MS Neuropsychological Questionnaire (MSNQ) was translated into Argentinean Spanish and is being used to assess patient and informant-reported perceptions of cognitive capacity. Analyses are planned that will investigate the validity of the MSNQ in Argentina. The Expanded Disability Status Scale (EDSS) and the MS Functional Composite (MSFC) are used to measure neurological disability and depression is evaluated using the Beck Depression Inventory (BDI).

Results: At the time of this writing, we have investigated 111 MS patients and 222 healthy controls. The frequency of cognitive impairment is 43.2%. A significant difference in all BRB-N test scores is found between MS patients and healthy controls (p < 0.01); verbal abilities, attention span, and executive function are more frequently affected. Cognitive impairment is more commonly observed in patients with primary and secondary progressive MS than in patients with the relapsing-remitting form of the disease (87.5% vs. 60.0% vs. 37.6%, respectively). In addition, the mean EDSS score is higher in MS patients with cognitive impairment than in those without impairment (3.79 vs. 2.73; p < 0.01). Additional results on MSNQ, MSFC, and BDI are forthcoming. Conclusions: The BRB-N is a sensitive and valid instrument for measuring cognitive impairment in a population of patients with MS in Argentina.
evaluated in our study, we achieved a higher level of validity. These tests include a neuropsychological assessment that has not been considered in previous studies.

PS10

Longitudinal cognitive changes and disease progression in multiple sclerosis
B. Duque, J. Sepulcre, J. Gállego, J. Gotli, P. Villoslada; University of Navarra (Pamplona, E)

Background: Cognitive impairment is a well known phenomenon in multiple sclerosis (MS) and could be found in approximately half of the patients. However, little and controversial information exist about its longitudinal changes and relations with disease progression.

Aims: To assess the longitudinal changes of cognitive impairment in MS, and its relations with disease progression. Methods: We study a cohort of 46 MS patients (15M/31F; age: 36.3 ± 9.7 years; education: 15 ± 5.3 years; EDSS: 2.0 (0 – 7.0); MSFC: 0.64 ± 0.68; 15 CIS; 23 RRMS; 4 SPMS; 4 PPMs). Patients were clinically followed every 3 months for 2 years and a neuropsychological assessment was done at baseline and at endpoint of the study. We used the Brief Repeatable Battery-Neuropsychology for neuropsychological assessment. To assess disease progression, we analyse the relapse-free condition, number of relapses, the onset of immunomodulatory therapy, CIS conversion to RRMS and the change of disability (measured with EDSS and MSFC and confirmed at six months). Statistical analyses were performed using parametric test or non-parametric test when appropriated (level of significance was set at p < 0.05; SPSS 13.0 software). Results: Six patients were lost in the follow-up. Relapse rate during the follow-up was 1.15, and 21 patients were relapse-free. The increase in the EDSS by the end of the study was 0.43 ± 0.8 and 11 patients had confirmed progression (one point increase in the EDSS confirmed at six months). Nine CIS patients converted to RRMS. Twenty nine patients had cognitive impairment at baseline defined as < 2SD in at least one test. We found significant differences between baseline and two-year endpoint in the three subtest of verbal memory (storage, retrieval and delayed recall) (p < 0.05 in all cases). These longitudinal changes remain significant in relapse-free and non relapse-free, progressive and non-progressive, cognitive and non-cognitive impaired groups. CIS converted patients presented a trend to decrease of verbal memory scores compared to non-converted. Symbol digit modality test (SDMT) was the only one that changed overtime in patients with confirmed progression (p = 0.04) and correlated with the EDSS change (r = −0.459; p = 0.003). Conclusions: Verbal memory is the cognitive function more sensitive to be impaired overtime, independently of relapses or physical disability progression. SDMT is a sensitive test that correlates with disease progression.

PS11

Computer-based working memory training in patients with multiple sclerosis – Design of a randomised study
A. Vogt, L. Kappos, K. Opwis, E.W. Kuida, I.K. Fenner; University of Basel (Basel, CH); University Hospital Basel (Basel, CH)

Background: The prevalence of cognitive impairment in patients with multiple sclerosis (MS) is estimated at 45% to 65%. In particular working memory is already affected at early stages. According to Baddeley (2000), working memory refers to the ability to store, actively maintain and retrieve information. Although there is evidence that dysfunction of the working memory system has a high impact on patients’ everyday lives, little has been done until now to improve this function in MS patients. Objective: The aim of the present study, for which patient recruitment is currently ongoing, is to evaluate the efficacy of a newly developed working memory training tool for MS patients (BrainStim, Fenner et al., 2006). As there is currently no consensus with regard to the optimal dosage of cognitive intervention, the present study compares a short, high intensity training to a longer and distributed training procedure. Methods: 30 outpatients with MS and 30 age-matched healthy controls will be randomised to two subgroups for the training procedures and one control subgroup without training. Participants in the high intensity training program will receive a 45-minute training session 4 times per week for four weeks. Participants in the distributed training program will receive a 45-minute training session 2 times per week for 8 weeks. All participants will be instructed to complete 12 of the 16 training sessions at home and will receive 4 additional training sessions for survey reasons under controlled conditions at the Institute of Psychology. Participants will receive a personal training booklet to coordinate the training procedure and log files will be checked once a week. Baseline testing for all groups consists of a neuropsychological test battery and self-report measures. The neuropsychological test battery will be performed twice to control for possible learning effects. The retet will be done directly after the final training session. It will consist of the same neuropsychological tests and self-report measures as at baseline and will provide primary outcome measures. Perspective: The newly developed training tool used in this study aims to train specific aspects of working memory such as visual-spatial, verbal and cognitive load features. By providing data on the effect of intensity and duration of training on treatment efficacy, this study will help to develop more precise guidelines for cognitive rehabilita-

Multiple Sclerosis 2006; 12: 51–528 www.sagepub.co.uk
PS13

Predictive value of MR parameters on cognitive function at early stages of relapsing-remitting multiple sclerosis: a 3-year study
E. Salort-Campaña, M. Bonnet, M. Deloire, K.G. Petry, V. Dousset, B. Brochet; University V. Segalen (Bordeaux, F)

Background: Cognitive performances may be altered at early stages of MS. Little is known about the predictive value of MR abnormalities on change of cognitive performances at early stages of relapsing-remitting MS (RRMS). Objective: To determine MRI predictors on short-term cognitive outcome in a 3-year longitudinal study. Methods: 57 RRMS patients, diagnosed in the previous six months, entered a longitudinal 3-year study of clinical, cognitive and magnetic resonance imaging (MRI) parameters. Each patient underwent yearly cognitive assessment using the Brief Repeatable Battery (BRB) and Boston naming test (BNT), Ruff Figural test (RFF), Wais-R similarities subtest, Go no go and Stroop test. 43 patients were individually paired with 43 healthy controls for age, sex and education. 22 controls were assessed twice one year apart to study practice effect. MR parameters included lesion load (LL), brain parenchyma fraction (BPF), ventricular fraction (VF), mean magnetisation transfer ratio (MTR) on lesion and normal appearing brain tissue (NABT) masks. Correlation between MR and cognitive metrics were assessed using Spearman’s test. Results: Patients performed worse than controls at baseline for short term verbal memory and recall (Selective Reminding Test, SRT), recall for visuospatial learning (Spatial Recall Test), attention (Symbol Digit Modalities Test (SDMT), PASAT 3s and 2s), inhibition (Stroop) and conceptualisation (similarities) and at follow-up for SDMT, PASAT 2s, similarities and Stroop. Some baseline MR parameters correlated with some cognitive score changes throughout follow-up: LL with SRT (r = −0.31; p = 0.03) and Stroop (r = 0.32; p = 0.04), BPF with SDMT (r = 0.43; p = 0.002). Conclusion: Baseline LL correlated mildly with verbal memory and inhibition changes during follow-up while atrophy was more strongly associated with a test of information processing speed and attention. This work was supported by grants of ARSEP and Schering France SA.

PS14

Compensatory cortical activations and cognitive load in early relapsing-remitting multiple sclerosis patients
M. Bonnet, M. Deloire, B. Dilharreguy, V. Dousset, M. Allard, K.G. Petry, B. Brochet; University V. Segalen (Bordeaux, F)

Background: Extended cognitive deficiencies have been reported in early MS, affecting attention, information processing speed and inhibition capacities. Cognitive compensatory mechanisms have been suggested by using functional MRI (fMRI). Objective: To explore cognitive compensatory phenomena during an attentional and inhibition task with increasing difficulty in early relapsing-remitting (RR) MS. Methods: Patients with early RRMS (mean age: 36.0 years, disease duration < 4 years) and healthy controls (mean age: 32.4 years) entered an fMRI study for which they completed four successive conditions of the go-no-go test with increasing difficulty: tonic alertness (one target), initial condition and its reversal condition (one target and one distractor) and complex go-no-go (two targets and five distractors). Subjects were instructed to respond to target presentation. Response times were recorded in scanner. Mann-Whitney analysis and two-sample t-test were used for comparisons of response times and cerebral activations between patients and controls, respectively. Results: For both patients and controls, response times increased significantly during the four conditions. Patients presented response times significantly longer than controls only for the fourth condition. Whatever the condition, patients always exhibited supplementary activated areas. For the first three conditions, these new additional areas included bilateral circular, dorsolateral prefrontal, insular, inferior parietal, middle occipital gyrus and cerebellum. In opposite, during the fourth condition, only insular and superior temporal gyrus were recruited. Conclusion: With this increasingly complicated four conditions go-no-go paradigm, it is possible to explore, by using fMRI, cerebral activations among controls and MS patients during a task requiring variable attentional and inhibition resources. For the first three conditions, performances of patients and controls were not different but patients required broader cerebral activation. In contrast, MS patients failed to reach the same level of performance than controls for the most complex condition whereas fMRI suggests that recruitment failed in this case. High cognitive requirement seems to cause failure of compensatory cerebral recruitment, leading to a cognitive breakdown. This process should be considered in compensatory phenomena studies and in therapeutic attempts in MS. This work is supported by fellowships of LFSEP and ARSEP.

PS15

A longitudinal cognitive study at early stages of relapsing-remitting multiple sclerosis
M. Bonnet, E. Salort-Campaña, M. Deloire, K.G Petry, B. Brochet; University V. Segalen (Bordeaux, F)

Background: 40–60% of MS patients exhibited cognitive deficits. Few studies examined longitudinal evolution of cognitive function in early relapsing-remitting multiple sclerosis (RRMS) patients. Practice effect may be a methodological limitation for serial cognitive assessment. Objective: To study the evolution of cognitive performance during three years at early stages of RRMS accounting for practice effect. Methods: 57 RRMS patients, diagnosed in the previous six months, entered a longitudinal 3-year study of clinical, cognitive and magnetic resonance imaging (MRI) parameters. Each patient underwent yearly cognitive assessment using the Brief Repeatable Battery (BRB) and Boston naming test (BNT), Ruff Figural test (RFF), Wais-R similarities subtest, Go no go and Stroop test. 43 patients were individually paired with 43 healthy controls for age, sex and education. 22 controls were assessed twice one year apart to study practice effect. Results: At baseline, patients performed worse than controls for short term verbal memory and recall (Selective Reminding Test, SRT), recall for visuospatial learning (Spatial Recall Test, SPART), attention (Symbol Digit Modalities Test (SDMT), PASAT 3s and 2s), inhibition (Stroop) and conceptualisation (similarities). After 3 years, cognitive performances of RRMS patients improved for all tests except for verbal fluency (Word List Generation). However patients performed worse than controls for several tests: SDMT, PASAT 2s, similarities and Stroop at follow-up. Practice effect was observed in healthy subjects at one year for SRT, SPART, RFF and Stroop. Conclusion: Despite of some cognitive performances improvement observed in MS patients, possibly related to test-retest practice effect, attention and information processing speed deficits remained detectable 3 years later. This suggests a consistent cognitive impairment since the beginning of the disease. This work is supported by grants of ARSEP and Schering France SA.

PS16

Ecological assessment in multiple sclerosis: comparison between Wisconsin cards sorting test and behavioural assessment of the disexecutive syndrome
L. Perfislerici, L. Bacci, C. Morici, L. Cattena, B. Viti, M. Silvestrini, A. Morgantini; Villa Adria Santo Stefano (Ancona, I)

Background: Executive functioning is frequently investigated in people with Multiple Sclerosis (MS). Although using the WCST we can examine problem solving abilities, mental flexibility, executive control and abstract reasoning, we haven’t information regarding predicting everyday problems arising from executive dysfunction in MS patients. Some studies demonstrated that a test ecological battery, BADS and most of its subtests, correlate with the standard executive tests administered to traumatic brain injury patients.

www.sagepub.co.uk

Multiple Sclerosis 2006; 12: 51–5228
S144  Abstracts

Goals of the Study: The aim of this study is to investigate the ecological utility of the BADS in patients with MS. We also analyse a self-report of executive ability (DEX) that supplements the primary BADS, versus performances examined (WCST and BADS). In addition, we intend to explore the relationship between self-awareness and executive abilities. Methods: Thirty patients who met the criteria for clinically definite MS were recruited at an out- and in-patients rehabilitation centre. Subjects were tested on WCST, BADS. DEX was completed by family members and the patient to evaluate the discrepancy score to indicate self-awareness of their executive functions. Variables such as age, education, sex, disease duration, degree of disability and different subtypes of MS were analysed. Results: Correlations were calculated using Pearson Test. Significant correlation was found between the WCST perseverative errors and BADS (r = 0.412; p < 0.05). A significant relationship was also noted between the DEX family and patient forms (r = 0.590; p < 0.001). In contrast the DEX self-reporting didn’t correlate with WCST and BADS (respectively r = −0.03, p > 0.05; r = −0.24, p > 0.05). Results showed that self-awareness was significantly correlated with BADS (r = 0.394; p = 0.05). Conclusions: The results of current study supports the clinical utility of the BADS and suggests that it is a good tool for measuring subjects’ executive abilities that might occur in everyday life and it is appropriate as an outcome measure after cognitive rehabilitation. Concordance between patients and family, reported on DEX, indicates the same subjective level of executive problems, even if it doesn’t correspond with the real patient’s executive abilities. Moreover, executive abilities tested on ecological instrument is related to the level of self-awareness. Thus, a clinician who have to create a neuropsychological evaluation and treatment plan should consider the patient’s awareness predicting his ecological executive performances.

P517
Cognitive disorders and disturbances of interhemispheric transfer in multiple sclerosis
B. Lenne, J. Naudrin, A. Mackowiak, L. Pezard, G. Forzy, P. Hautecoeur, P. Gallois; Hospital St Philibert (Lomme, F); University Charles de Gaulles (Villeneuve d’Ascq, F); Hôpital de la Salpêtrire (Paris, F)

Background: The physiopathology of the cognitive disturbances in the multiple sclerosis (MS) remains mysterious, in spite of their frequency and their precocity. The assumption of a bond between these disorders and the presence of callos lesions are discussed (Pelletier et al., 2004). Aim and Method: This study tries to support the assumption of the participation of a deficit of the transfer interhemispheric in the genesis and/or the importance of the cognitive deficit in the remittent forms of MS. A methodology based on the analysis of neurophysiological indices resulting from the no-linear analysis of the electroencephalogram (index of coherence (IC) (Kraskov and al., 2005) and indices of Shannon (Sh and Delta of Sh.) (Ekmann and al., 1987) was developed in order to appreciate the complexity of cerebral dynamic and the interhemispheric transfer near 54 patients with MS of remittent form. These indices were correlated with a neuropsychological evaluation specific (BCcogSEP (Dujardin et al., 2004)), according to the duration of evolution of the disease and the degree of handicap. Results: The cognitive evaluation found a deterioration at nearly 20% of the patients with mainly a memory and attentional disorders and an effect of the duration of evolution and degree of handicap. Anomalies of the neurophysiological indices were observed at more than 20% of the patients, with an increase in some interhemispheric connections (C3-4, T3-4) correlated positively with the progression of the handicap (in and negatively with the cognitive performances. Discussion/Conclusion: This study underlines the relevance of the no-linear analysis of the EEG in the exploration of the physiopathology of the cognitive disorders in MS. It highlights the possibility of a participation of a deficit of electrophysiological plasticity and transfer interhemispheric in the constitution or aggravation of these (in and negatively with the memory, attentionals and executives). The results of this preliminary study seem to indicate the relevance of the index resulting from the analysis no-lineaire from signal EEG and its complementarity of the morphological imagery in the interhemispheric study of the disturbances of the transfer and the cognitive disorders in MS.

P518
One-year cognitive follow-up in patients with multiple sclerosis
M. López-Gongora, C. García-Sánchez, A. Campolongo, A. Escartin; Hospital de la Santa Creu i Sant Pau (Barcelona, E)

Background: Although several studies have been carried out regarding cognition in multiple sclerosis (MS), few deal with cognitive changes over time, and conclusive data are lacking. Objective: The aim of this study was to evaluate cognitive changes in patients with MS at one-year follow-up. Method: The study included 90 patients (59 females and 31 males); their mean age was 36.3 years (SD = 10.3). Forty-eight of these patients were relapsing remitting (RR) and 10 had a progressive form of MS. Mean Expanded Disability Status Scale (EDSS) measure was 2.71 SD = 2.03, and the mean time from onset of symptoms until the present was 112 months (SD = 96). All patients underwent a complete neuropsychological evaluation that included measures of memory, abstract reasoning, speed processing, and visuospatial and frontal functions. The same evaluation with parallel forms of the real patient’s performed one year later. Neurological evaluation was also performed at the same time of both neuropsychological evaluations in order to obtain the EDSS score. Results: A two-way anova was used to evaluate changes in cognitive performance after one year. Verbal memory showed a significant worsening, with a P value of 0.005 for short-term verbal memory and 0.021 for long-term verbal memory. Verbal fluency also showed a significant change p < 0.001. No significant changes were observed in the other cognitive functions evaluated. Conclusions: The results from the present study sustain the hypothesis regarding deterioration in cognition over time in MS patients. Two cognitive domains (verbal memory and verbal fluency) showed a significant change at one year. No differences were observed between the two evaluations for the other variables. A longer follow-up would be useful to assess the progression of the changes observed and the possible decline of other mental functions.

P519
Predictors of longitudinal cognitive decline in paediatric multiple sclerosis
W. MacAllister, M. Milazzo, C. Christodoulou, N. McLinskey, A. Belman, L. Krupp; SUNY Stony Brook (Stony Brook, USA)

Background and Goals: Children and adolescents with multiple sclerosis (MS) may be particularly susceptible to cognitive dysfunction as the pathological processes of MS co-occur with brain development. Though prior work has documented that these children may have cognitive deficits, there is little information on factors that predict decline. The goal of this study is to assess predictors of cognitive decline in children with MS. Methods: 12 clinically definite MS patients, age 16 years or younger, were recruited and evaluated on two separate occasions, roughly one year apart. Data collected included number of relapses, age of onset, disease duration, and Expanded Disability Status Scale (EDSS). The participants received a neuropsychological evaluation assessing attention, memory, visual spatial skills, and language at both visits; a composite z-score was generated to summarize overall cognition. Correlational analyses assessed relations between clinical variables and changes in cognition. Results: Overall, five patients demonstrated cognitive decline using a criterion of more tests impaired at reevaluation than at baseline; 7 demonstrated z-score composite score declines. A significant association between baseline EDSS and the number of tests impaired (baseline – reevaluation) was found (r = −0.58). Though the correlation between baseline EDSS and z-score difference failed to reach statistical significance, the association (r = 0.49) would be considered a medium-
to large effect size. Likewise, the correlations between number of interitem relapses and change scores were not statistically significant, but would be considered medium effect sizes ($r = -0.43$ and $0.36$ for interitem relapses and change in number of tests impaired and z-scores, respectively). A medium effect was observed between age of onset and z-score difference as well ($r = -0.31$), suggesting a somewhat greater decline for younger patients. Other clinical variables, such as number of relapses at baseline and total disease length were not associated with cognitive decline. Conclusions: The current study indicates that cognitive impairment can occur in pediatric MS and that cognitive decline can be seen over a relatively short period. Neurological impairment early in the course of the disease is predictive of cognitive decline over time. Likewise, continued relapses may also predict decline. It is suggested that cognitive assessment be included in the clinical care of children and adolescents with MS.

PS20
Learning and memory measures in multiple sclerosis: reliability of parallel forms for serial assessment
T. Olivarres, J. Barroso, A. Nieto, G. Ramírez, M. Hernández; University of La Laguna (La Laguna, E); Hosp Univ NS Candelaria (La Laguna, E)

Background: The Brief Repeatable Battery of Neuropsychological Tests (BRB-N) for Multiple Sclerosis (MS) was designed as a screening tool for evaluating short-term changes in cognitive function in patients with MS. As it is a sensitive measure and easy to administer, it is a very useful tool in clinical trials. In order to reduce training effects with serial assessment, two parallel versions were constructed for all subtests. However, divergent data about equivalence and stability of the tests for repeated assessment are reported. Objectives: Two parallel versions of learning and memory tests adapted from the original battery were studied to evaluate interform equivalence and temporal stability for serial assessment. Method: 56 healthy subjects (22 male, 34 female) were evaluated in two sessions (T1-T2) with a six months interval. Two versions of the Selective Reminding Test (SRT) (Campos et al., 2000); verbal learning/memory; 10/36 Spatial Recall Test (SPART); visuospatial learning/memory, were administered. The order of test presentation was counterbalanced such that 29 participants (11 male, 18 female) with a mean age of 21.9 (sd: 5.22) were administered Version A first, and 27 participants (11 male, 16 female) with a mean age of 22.8 (sd: 4.43) received Version B first. Results: There were no significant differences between the two versions of the learning and memory tests. Form effect: Total SRT (F(1,52) = 2.14; p = 0.14); Long-term SRT (F(1,52) = 0.06; p = 0.80); Total SPART (F(1,52) = 0.05; p = 0.83); Long-term SPART (F(1,52) = 1.13; p = 0.29); A significant effect of the moment of assessment was found with a better performance for the second evaluation (T2); Total SRT (F(1,52) = 35.07; p = 0.0001); Long-term SRT (F(1,52) = 10.34; p = 0.002); Total SPART (F(1,52) = 4.11; p = 0.04); Long-term SPART (F(1,52) = 6.82; p = 0.01); Version by Order interactions in all measures were not statistically significant; Total SRT (F(1,52) = 1.88; p = 0.17); Long-term SRT (F(1,52) = 0.31; p = 0.58); Total SPART (F(1,52) = 1.94; p = 0.17); Long-term SPART (F(1,52) = 0.70; p = 0.40). Conclusions: Our results indicate the equivalence between the two forms but a significant practice effect in the use of alternate versions. This should be taken into account when used in longitudinal testing in clinical trials.

PS21
Preserved subliminal processing and impaired conscious access in patients with early relapsing-remitting multiple sclerosis
B. Audoin, F. Reuter, A. Delcu, I. Matliova, M. Au duong, P. Cozzo, S. Dehaene, J. Pelletier, J. Ranjeva; Timone hospital (Marseille, F); CRBM UMR CNRS 6612 (Marseille, F); inssem U562 (Paris, F)

Objectives: In visual backward masking, the visibility of a briefly presented stimulus is reduced by a mask presented very shortly after the stimulus. In a first stage corresponding to non-conscious visual processing, the visual stimulus is processed by a series of brain areas activated in a bottom-up manner. In a second stage, top-down feedback from higher areas to lower-level sensory regions establishes a self-amplified reverberant neuronal assembly which connects together distant brain areas, and is associated with conscious reportability. During masking, a stimulus can fail to reach consciousness if the mask replaces the stimulus before this recurrent activity has become stable. In patients with early multiple sclerosis, breakdown of large-scale cortical integrative processes, occurring as a consequence of diffuse white matter damage, may induce backward masking deficit that corresponds to a deficit in the late stages of conscious perception, whereas early bottom-up subliminal processing of masked stimuli is preserved. Method: To test this hypothesis we performed a backward masking study in 22 patients with early MS and 22 normal matched healthy controls. We used Arabic digits as stimuli and varied quasi-continuously the interval with a subsequent mask, thus allowing us to progressively “unmask” the stimuli. This manipulation allowed us to study the subliminal priming effect caused by these variably masked numbers. We also quantified their degree of visibility using both objective and subjective measures to evaluate the threshold duration for access to consciousness. Results: The threshold delay between digit and mask necessary for the conscious perception of the masked stimulus was longer in patients compared to control subjects (ANOVA $p < 0.009$). This higher consciousness threshold in patients was confirmed by an objective and a subjective measure, and both measures were highly correlated for patients (corrected $Rho = 0.8$, $p = 0.001$) as well as for controls (corrected $Rho = 0.93$, $p < 0.001$). However, subliminal priming of masked numbers was effective (ANOVA $p < 0.001$) and identical in patients compared to controls (ANOVA $p = 0.135$). Interpretation: Breakdown of large-scale cortical integrative processes related to diffuse demyelinating processes may constitute the anatomical substrate of the elevated threshold for access to consciousness in patients with early MS.

PS22
Cognitive functioning in 6 cases with childhood/juvenile multiple sclerosis
B. Goretti, S. Lori, V. Zipoli, E. Portaccio, S. Centorrino, M.P. Amato; University of Florence (Florence, I); Meyer Hospital (Florence, I)

Background: Prevalence of cognitive dysfunction in multiple sclerosis (MS) patients is estimated between 40 and 65%. Approximately 5% of patients with MS present childhood or adolescent onset. The literature on neuropsychological functioning in this age range is however limited. Only two studies so far have been published on this topic, providing preliminary evidence that MS can have a dramatic impact on school and everyday activities. Objective: To describe the impact of MS on cognitive and daily living functioning in 6 patients with childhood/juvenile MS. Material and Methods: Six MS subjects aged between 9 and 17 years were assessed through an extensive neuropsychological test battery. Their performance was compared with that of 21 demographically matched healthy controls (HC). The neuropsychological battery included global measures of cognitive function (IQ), as well as tests of verbal and visuo-spatial memory, attention, language and executive functions. Performance was considered impaired when scores fell 2 SD or more below the mean of HC performance. Finally an interview on daily living and school activities was administered to patients' parents. Results: We evaluated 6 MS patients (4 females; mean age 14.59 ± 9.01 years; mean EDSS 1.05 ± 2.12) and 21 HC. Two patients presented a mild mental retardation (IQ 59; 69) and one patient had a moderate retardation (IQ 41). Only 2 out of 6 patients had a cognitive performance comparable to that of HC, whereas the remaining 4 patients failed from 3 to 15 tests. Immediate and recall performance was impaired in 2 patients; immediate and recall visual memory in 1 patient, and attention in 4 patients. Moreover, 4 patients failed linguistic tasks. Cognitive impairment was associated with high functional impact on daily living and school activities.
Conclusions: Cognitive deficits occur in childhood/juvenile MS and have a high functional impact. The pattern of cognitive dysfunction, in comparison with that reported in adult-onset MS, seems to be characterized by a more frequent involvement of linguistic abilities. Cognitive difficulties should be taken into account for comprehensive treatment planning in childhood and juvenile MS.

A short version of the Rao's Brief Repeatable Battery can detect mild cognitive impairment in multiple sclerosis
E. Portaccio, B. Goretti, V. Zipoli, G. Sincuca, M.P. Amato; University of Florence (Florence, I)

Background: cognitive impairment is a core feature of multiple sclerosis (MS), affecting 40–65% of patients in all stages of the disease. To date the Rao's Brief Repeatable Battery (BRB) is the most widely used instrument for cognitive evaluation in MS patients. It requires on average 30 minutes for administration, whereas no quicker well validated screening tool is currently available. Objective: to assess a short version of the BRB for its ability to detect mild cognitive impairment in MS patients. Methods: the BRB version A and the Stoop Test (ST) were administered to 116 relapsing-remitting (RR) subjects (81 females, mean age 43.1 ± 9.1 years, mean education 11.8 ± 3.7 years, mean disease duration 15.9 ± 9.3 years, mean disability on the Expanded Disability Status Scale 1.7 ± 1.2). Failure of a test was assessed applying the available normative data with age, gender and education corrections for the Italian population (Amato et al., 2006 in press). Mild cognitive impairment was defined as the failure of at least 2 tests. In the study sample we selected those tests which best predicted the presence of cognitive impairment. Sensitivity, specificity, positive (PPV) and negative predictive values (NPV) for these selected tests were calculated. Results: in our sample the parallel administration of 2 tests, the Selective Reminding Test (SRT) and the Paced Auditory Serial Addition Test-3 seconds (PASAT-3) was able to detect mild cognitive impairment with a sensitivity of 83%, specificity of 92%, PPV of 90% and NPV of 87%. Adding the results of the third test, the Symbol Digit Modalities Test (SDMT), sensitivity increased to 94% with an 84% specificity, 83% PPV and 95% NPV. Conclusions: the administration of a short version of the BRB consisting in 3 out of the 10 original tests of the battery, requiring approximately 15 minutes, may represent a highly sensitive and rapid tool to detect MS-associated cognitive impairment in everyday clinical practice.

Cognitive deficits within 2 years after multiple sclerosis diagnosis
T.A.M. Nieman, A.C.J.W. Janssen, I. de Koning, J.B. Boringa, R.O. Hintzen; MS Centre Euroms (Rotterdam, NL); Euroms MC (Rotterdam, NL); Meander MC (Amersfoort, NL)

Objectives: 1) Whether cognitive deficits are detectable shortly after MS diagnosis. 2) The influence of depression, anxiety and disability status on early cognitive deficits. Methods: 101 patients within 2 years of MS diagnosis were included (70% females, mean age 37.5 years, mean time since diagnosis 7.8 months (range 0.7–23.6 months), median EDSS 2.5 (range 0.0–7.0)), as well as 117 healthy controls (54% females, mean age 41.8 years). Neuropsychological and clinical assessment included Rao's Brief Repeatable Battery (BRB): Selective Reminding Test (SRT), 10/36 Spatial Recall Test (SPART), Symbol Digit Modalities Test (SDMT), Paced Auditory Serial Addition Test (PASAT) and Word List Generation Test (WLGT); EDSS, Hospital Anxiety and Depression scale (HADS). Regression analysis was used, adjusted for age, sex and education level. Next, influence of anxiety, depression, EDSS and time since diagnosis was explored. Results: Lower scores compared to healthy controls were found on PASAT3 (p = 0.04), PASAT2 (p = 0.001) and WLGT (p = 0.04) after adjustment for age, sex and education level. The subgroup with MS diagnosis less than 6 months ago (n = 57) scored lower on PASAT2 (p = 0.01) and WLGT (p = 0.05). Of all patients clinically relevant levels of anxiety were noted in 34% and depression in 10%. Depressive patients scored relatively lower on SDMT (p = 0.05) and PASAT3 (p = 0.03). However, after adjustment for time since disease onset and EDSS, neither depression nor anxiety had a significant influence on cognitive functions. Scores on SDMT (p = 0.001), PASAT3 (p = 0.005) and PASAT2 (p < 0.001) all showed an association with EDSS; patients with EDSS ≥3.0 (n = 37) had significant lower scores, persisting after correction for depression. Only SRT scores were related to time since diagnosis. Patients with a diagnosis of MS > 6 months (n = 41) had significantly more difficulties with SRT, than patients with a diagnosis < = 6 months. Conclusions: Cognitive deficits were observed within 2 years after diagnosis of MS. These deficits were not significantly related to depression or anxiety.

Demographic and psychopathological data of patients with multiple sclerosis that attended the mental health center of north-western district of Thessaloniki
A. Adamopoulou, M. Paschalidou, I. Drenos, K. Katsigianopoulos, P. Papazisis, M. Gelagoti, T. Aftantou, A. Angelou, M. Krommyda, G. Garifallos, I. Milonas; Mental Health Care Center (Thessaloniki, GR); Aristotelian University of Thessaloniki (Thessaloniki, GR)

Introduction: Multiple Sclerosis (MS) is the most well-known demyelinating disease, of unknown origin. The hypothesis of a predisposing stress factor preceding a relapse of MS remains quite attractive. MS patients develop very often various mental disorders. The objective of the present study is the determination of the demographic and psychopathological data of the MS patients that attended the mental health center of north-western district of Thessaloniki. Method: 40 patients that attended the specific mental health center were included in the study. Record of their demographic data was kept and after a psychiatric interview a diagnosis was made according to the DSM-IV criteria. Results: From the total number of 40 patients, 29 (72.5%) were female and 11 (27.5%) were male, with a mean age of 36 yrs. Their majority was married (57.5%), while most of them (52.5%) had received an education of 7–12 yrs. 31 (77.5%) were diagnosed on axis I, especially with depressive disorders (40%) and basically major depression (35%), while the anxiety disorders appeared at a smaller percentage (20%). 55% received a diagnosis on axis II (personality disorder), as dramatic (30%), borderline (20%), narcissistic (12.5%) and dependent (12.5). In a significant percentage of patients a stress factor had preceded the relapse. In 10 patients (25%) this stress factor was the death of a close relative or friend. Reversely in the percentage of 22.5% MS was the stress factor for the development of psychological problems. The functionality level of the patients according to the GAF scale was satisfactory (66.4). Conclusions: The average background of the MS patient that attends a mental health center is about 35-years old, married and of higher education. As far as the psychopathological elements are concerned, depression and personality disorder of cluster B, mainly dramatic, are most commonly observed. Stress factors play an important role in the development of relapses, while the disease itself is a stress factor for the appearance of mental disorders. When dealing with these psychological problems, the quality of life of these patients can significantly improve on all aspects.

Influence of depression on cognitive impairment in patients with early-onset relapsing-remitting multiple sclerosis: results of an Italian multicentre study (COGIMUS)
F. Patti, M.P. Amato, M.R. Tola, M. Trojano, P. Ferrazza, O. Piccioni, S. Bastianello on behalf of the COGIMUS study group

Objective: To assess the association between depression, cognitive impairment and structural brain anormalities in patients with early-
onset relapsing-remitting multiple sclerosis patients. Methods: A cohort of 430 Italian patients (282 women and 148 men) with early onset RRMS were analyzed with the Hamilton scale for depression to distinguish depressed from non depressed RRMS patients, and the Italian validated version of the Rao’s neuropsychological battery for the cognitive profile. All patients underwent brain MRI investigation: T1, T2, gadolinium enhancing lesions load were considered for the analysis. Results: We found 134 RRMS patients (86 women and 48 men) who resulted depressed and cognitively impaired (group A); 188 patients were only depressed (group B); 44 patients were only cognitively impaired (group C); and the remaining 64 patients were neither depressed nor cognitively impaired (group D). Patients of the group A showed significantly higher T2 (p = 0.008) than patients in group D. Group A patients have also higher, but not statistically significantly values of T2 lesion load than patients in group C (p = 0.095). Furthermore patients in group A showed significantly higher values of T1 lesion load than patients in group C (p = 0.04) and patients in group D (p = 0.003). Conclusion: These results show that depression and cognitive impairment in MS may be associated and may recognize a common pathological substrate.

PS27

Alternatives to the PASAT for measuring speed of information processing in multiple sclerosis patients: the Adjusting-PASAT and the computerized tests of information processing

T.N. Tombarth, J.I. Reicker, L. Walker, M.S. Freedman; Carleton University (Ottawa, CAN); The Ottawa Hospital, General Campus (Ottawa, CAN)

Background: The most common measure used to clinically assess information processing speed (IPS) in patients with Multiple Sclerosis (MS) is the Paced Auditory Serial Addition Test (PASAT). However, the PASAT is prone to several shortcomings such as the elicitation of undue anxiety and frustration and susceptibility to the effects of practice, age, education and math ability. Objective: To assess and compare the utility of two newly developed tests as alternatives to the PASAT for detecting IPS deficits in patients with MS. Methods: The Adjusting-PASAT (A-PASAT) is a computerized modification of the original PASAT in which the duration of time between digits depends on the correctness of a response. This systematic variation of the interval between numbers permits the calculation of a temporal threshold representing the fastest speed of digit presentation at which an individual is able to respond correctly. The temporal threshold measure offers a more precise way of determining when information processing breaks down than is achieved with the standard PASAT, which records only the number of correct responses. The second test, the Computerized Tests of Information Processing (CTIP), uses a completely different paradigm based on research showing the ability of reaction time (RT) procedures to detect deficits in IPS is related to the complexity of the task. Consequently, the CTIP consists of three RT tests (simple, choice, and semantic search) which progressively increase in task complexity. Both the A-PASAT and the CTIP were administered to 35 patients with relapsing-remitting MS and 35 healthy controls (HC). Results: There were no statistically significant differences in threshold scores between MS and HC groups on the A-PASAT. The mean threshold achieved by the MS group was 1.8-seconds, which is substantially shorter than the 3.0-second interval commonly used in the standard PASAT when assessing IPS in MS patients. Analyses of the CTIP data revealed the MS group obtained significantly longer RT scores on each test. The difference between groups progressively increased as a function of task complexity with the smallest difference occurring for simple RT and the largest for semantic search RT. Conclusion: Overall, the current results suggest that, although the A-PASAT does offer advantages over the standard version, the CTIP is better able to detect deficits in IPS and offers a viable alternative for the assessment of IPS in patients with MS.

PS28

Reaction time: a viable alternative for assessing processing speed in multiple sclerosis

T.I. Reicker, T.N. Tombarth, M.S. Freedman, L. Walker; Carleton University (Ottawa, CAN); The Ottawa Hospital, General Campus (Ottawa, CAN)

Background: Cognitive dysfunction is a major problem complicating the evolution of Multiple Sclerosis (MS), particularly affecting the speed at which information is processed. Most neuropsychological tests are lengthy and, although a few have been used clinically to assess information processing speed (IPS), practice effects have been observed and patients often find the experience stressful. Therefore, a need exists for alternative tests of IPS. Objective: To introduce a newly developed series of reaction time (RT) tests called the Computerized Tests of Information Processing (CTIP) and to assess the clinical utility of this measure in assessing IPS in patients with MS. Method: The CTIP is composed of three RT tests (simple, choice, and semantic search) which progressively increase in task complexity. Participants respond as quickly as possible to stimuli presented on a computer screen. In the simple task a key is pressed every time an ’X’ appears, for choice one of two words appears and the appropriate key is pressed, and for semantic search one of two keys is pressed indicating whether a word (e.g., “chair” or “duck”) represents a member of a semantic category (e.g., “furniture”). Shown to be sensitive to IPS deficits in traumatic brain injury, the CTIP is not susceptible to practice effects, making it ideal for serial administrations. 60 MS patients and 60 age-matched healthy controls were assessed using the CTIP. Results: Significantly longer RTs on all tests (p < 0.001) were observed for MS patients compared with controls, with a progressive separation between the two groups occurring as the cognitive load increased. To control for generalized effects of motor dysfunction, percent-change (%-change) scores for the choice and semantic search tasks were calculated using simple RT scores as the baseline measure. Analyses based on these %-changes yielded similar results. The clinical utility of the CTIP was evaluated by comparing the performances of MS patients to indexed age-appropriate scores from a normative sample consisting of 330 individuals. 40– 50% of MS patients scored at the 10th percentile while 25–40% scored at the 5th percentile. The ability of the CTIP to detect IPS deficits increased as a function of task complexity. Conclusions: These data suggest that the CTIP is an effective technique for assessing IPS in patients with MS and offers a viable alternative possessing several advantages to the more traditional neuropsychological tests.

PS29

Psychiatric illness in long-term multiple sclerosis cohort

J. Tham, A. Traboulsi, J. Oger; University of British Columbia (Vancouver, CAN)

Background: Psychiatric illness has been documented over the years in patients with MS. Although reasons for development of such illnesses are poorly understood, previous studies have suggested central nervous system pathology as a major contributor. Furthermore, psychiatric illnesses are a treatable major contributor to patient-described measures of quality of life. Objective: An observational study to describe the psychiatry profile of a consecutive cohort of MS patients. Method: Patients were recruited from a single center (University of British Columbia) participating in the 16 year longterm follow-up study of beta interferon 1b (Betaseron) in relapsing-remitting MS. Patients underwent detailed clinical interviews, Structured Clinical Interview for DSM-IV TR (SCID-I) diagnostic interview along with the Geriatric Depression Scale Long Form
Background: Approximately half of persons with MS experience cognitive impairment that can negatively impact occupational and social functioning. It is not clear how well patients are able to perceive their cognitive abilities as compared to their actual performance. Persons with multiple sclerosis accurately perceive their cognitive abilities when asked about specific performances (C. Christodoulou, P. Melville, W. Scherl, W. MacAllister, R. Abensur, J. James, N. McLinskey, L. Krupp; SUNY Stony Brook (Stony Brook, USA))

Background and Goals: The following MR data were obtained from the Brief Repeatable Battery (BRB), which includes measures of cognitive performance. At 7-year follow-up, the cognitive domains of IQ, IQ decline, memory, speed of information processing and executive function were assessed. IQ decrease of 15 points or more from premorbid levels denoted IQ decline. Otherwise, performance at or below the 5th percentile of age-standardised normative data was considered ‘impaired’. Impaired performance in 4 or more tests denoted general CI. Results: Patients were impaired significantly more often than would be expected from a healthy population in the PASAT 3, PASAT 2, SDMT (Binomial single proportion exact tests all p < 0.001) and Brixton Spatial Anticipation Test (p < 0.01). Low premorbid IQ, high anxiety and high atrophy over the first year from baseline predicted patients falling PASAT 3 (logistic regression, Omnibus Test p = 0.008) and PASAT 2 (p < 0.001). High anxiety, high atrophy and increasing numbers of T1-weighted lesions predicted Brixton Test failure (p = 0.018). Premorbid IQ, self-reported anxiety and increasing numbers of T2-weighted lesions predicted general CI (p = 0.003). Conclusions: The data suggest that those patients with CIS most at risk of developing CI are those with high atrophy, T1- and T2-weighted lesions, lower premorbid IQ and higher anxiety. In particular, early rapid atrophy predicts the later presence of a slowed speed of information processing and poor spatial reasoning in CIS patients.

Objective: To examine speeded information processing (SIP) in MS patients using a measure that distinguishes this cognitive ability from motor deficits involving nystagmus and dysarthria. Nystagmus, dysarthria, and speeded information processing in multiple sclerosis

A. Bodling, D. Denney, S. Lynch; University of Kansas (Lawrence, USA); University of Kansas Medical Center (Kansas City, USA)

Objective: To examine speeded information processing (SIP) in MS patients using a measure that distinguishes this cognitive ability from motor deficits involving nystagmus and dysarthria. Background: Previous studies using measures that require rapid processing of a series of stimuli (e.g., the Stroop Test) have led us to conclude that the core cognitive problem in MS patients is likely an impairment in SIP. These results have been challenged because the measures used to assess this impairment could be affected by ancillary motor problems such as nystagmus and dysarthria. To address these concerns, we devised a measure, the Picture Naming Test (PNT), that allowed us to vary the challenge posed by the task with respect to nystagmus and dysarthria. Design/Methods: 63 MS patients and 59 healthy controls completed the three trials typically composing the Stroop Test (word reading, color naming, and color-word naming) and the four trials devised for the PNT. In the PNT, subjects named simple line drawings of common objects presented sequentially on a computer screen, a new picture appearing each time the subject responded. The trials varied with respect to the location and novelty of the pictures. Each picture was presented either in the center (fixed) or in one of nine locations (distributed) on the screen, the latter condition augmenting the difficulty of the trial with respect to nystagmus. Novelty was varied by using only four different pictures with each picture repeated many times throughout the trial (low novelty) or by showing unique pictures with no repetitions (high novelty), the latter condition augmenting the difficulty of the trial with respect to dysarthria. The number of stimuli completed during each of the three 60-sec trials of the Stroop and the four 60-sec trials of the PNT was recorded. Results: Relative to controls, patients had significantly lower scores on all trials of the Stroop and all trials of the PNT. A Brinley plot of the mean scores for the controls and the patients on each of the seven trials indicated that the common factor differentiating patients’ performance from that of controls is a decline in SIP and that neither nystagmus nor dysarthria played a substantial role in this outcome. Conclusion: A decline in speeded information processing is a central feature of the cognitive impairment seen in conjunction with MS and can be demonstrated independent of ancillary motor problems involving nystagmus or dysarthria.
and verbal fluency. Perceived cognitive abilities were assessed with two types of measures: 1) general self-report measures of cognitive abilities (i.e., Perceived Deficits Questionnaire, the Multiple Sclerosis Neuropsychological Questionnaire, the Brief Assessment of Memory and Attention), 2) specific self-report measures of perceived performance on individual cognitive tasks from the BRB. The neuropsychological performance of the MS subjects was compared to an age matched group (n = 22) of healthy persons. Results: The MS sample displayed a range of overall cognitive abilities on neuropsychological testing (Z score mean on the BRB = −0.82 Z, SD = 0.85, ranging from −2.66 Z to +0.58 Z). There were moderate to strong correlations between patients’ performances on specific tasks and their self-report on how they had done on those specific tasks, for example, the Selective Reminding Test (r = 0.546, p < 0.001) and the Paced Auditory Serial Addition Test (r = 0.546, p < 0.001). The general self-report measures did not correlate significantly with any of the neuropsychological measures. Conclusions: MS subjects without depression were able to gauge their level of cognitive function when the cognitive activity in question was specific and well-defined. General self-report measures performed less well. Possible explanations for this pattern of results will be discussed.

PS33
Cognition in the early stage of multiple sclerosis
I. Fedotova; Kharkov Medical University (Kharkov, UKR)

Objective: Cognitive dysfunctions may contribute to limitation of everyday activities of patients with multiple sclerosis (MS). Recent studies have demonstrated that 45 to 65% of MS-patients are cognitively impaired. The profile of MS-related cognitive dysfunctions varies greatly. It includes memory and learning deficits, attention deficits, executive dysfunctions and visual-spatial deficits. Most studies of cognition in MS examined patients in later stages, often including MS-patients with marked physical disabilities. Studies of cognitive dysfunctions in the early stage of the disease are rare. This study specifically aimed at evaluating and characterizing cognitive impairments in the early stage of MS, and determining specific patterns of cognitive dysfunction. Methods: 42 MS patients, experiencing their first neurological symptoms not more than two years previously, and 40 healthy controls were compared. A comprehensive neuropsychological test-battery was used to evaluate MS-related cognition. The battery consisted of memory and learning tests, executive functioning tests and a visual spatial functioning test. A computerized attention test-battery was also included, which assess accuracy and speed of test responses. In addition depression and intellectual capabilities were assessed. Results: Compared with healthy controls, MS-patients in the early stage of the disease performed significantly lower on each neuropsychological assessment, except for verbal short-term memory. In particular, MS-patients showed a lengthened reaction time for simple and focused attention (17 – 37%), impaired non-verbal memory function and a planning deficit (28%). Associations between information processing speed and disease course and the employment situation were additionally found. However, patients did not have clinically relevant depression rates on the ADS-L and visuo spatial abilities remain preserved. Conclusion: Our findings revealed discrete cognitive dysfunction in MS-patients within the early stage of the disease.

PS35
An interhemispheric cooperation and clinical function in multiple sclerosis
K. Rasova, J. Krusensky, J. Tintera, H. Kalistova, M. Zalisova, P. Martinкова, E. Havrdova, K. Rasova, P. Žemána, J. Žeman; Charles University (Prague, CZ)

Background: In our previous project, we have noticed the stronger dependence of amplitude’s signal (fMRI) between left and right hemispheres in healthy than in MS patients, and hypothesised that it could be caused by a dysfunction of the interhemispheric cooperation. Purpose: The aim of the study was to find out whether a dysfunction of interhemispheric cooperation could play role in the limitation of clinical functions in multiple sclerosis. Method: 28 clinically stabilized outpatients with multiple sclerosis were studied. Upper and lower extremities and cognitive functions were evaluated using Multiple Sclerosis Functional Composite (The Timed 25-Foot Walk-T25FW, The 9-hole peg test-9HPT, Paced Auditory Serial Addition Test-PASAT3). Interhemispheric cooperation was assessed by the dependence between the right and left hemisphere of the amplitude of signal in four anatomical areas on functional magnetic resonance imaging. Results: The patients with better values of some clinical functions achieve higher dependency of amplitude’s signal between the right and left hemisphere in some brain areas. The function of right upper extremity (9HPT(right)) correlates (p < 0.001) with the dependency of amplitude’s signal between right and left hemispheres in both cerebellums. The function of left upper extremity (9HPT(left)) correlates with the dependency of amplitude’s signal between right and left hemispheres in primary sensory-motor cortex during performance of the paradigm by right hand (p < 0.001) and cerebellum during performance of the paradigm by right hand (p < 0.001). The function of lower extremities (T25FW) correlates (p < 0.001) with the dependency of amplitude’s signal between right and left hemispheres in putamen during performance of the paradigm by left hand. Cognitive functions (PASAT3) correlate (p < 0.001) with the dependency of amplitude’s signal between right and left hemispheres in supplementary motor cortex during performance of the paradigm by right hand and putamen during performance of the paradigm by left hand. Conclusions: Interhemispheric cooperation could play an important role in the manifestation of clinical functions in multiple sclerosis, but requires verification in an additional study. Ministry of Health, Czech Republic (1A/8628-S) supported the clinical part of the study. The statistical part of the study was supported by the Institutional Research Plan AV0Z10300504.
Functional implications of multiple sclerosis: profile analysis with the repeatable battery for the assessment of neuropsychological status

A. Davis, A. Gupta, R. Williams; Ball State University (Muncie, USA); Fort Wayne Neurological Center (Fort Wayne, USA)

Objective: Multiple Sclerosis (MS) is a debilitating demyelinating disorder which has been linked to a wide variety of functional cortical and subcortical neurobehavioral deficits. Although MS is principally linked with white matter degradation, many patients with MS experience impairment in higher-order cortical processes such as executive functions and attentional control, which are primarily associated with anterior cortical functioning. The purpose of the current study was to evaluate the neurofunctional profile of patients with MS on the composite indexes of the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS, Randolph, 1998) across a wide range of abilities with an administration time. Participants: This sample consisted of individuals diagnosed with Multiple Sclerosis (mean age 45.70 years, standard deviation = 9.17 years). The average time since diagnosis was 9.57 years with a standard deviation of 8.95 years. Each participant received the RBANS as part of a comprehensive neuropsychological battery. Results: Paired Sample t-tests were conducted on the five composite indices of the RBANS by comparing the mean scores for the group of patients with MS to the normative mean (100). All five of the composites were statistically significantly different from the normative mean at the 0.01 level. However, the Attention (mean difference = 17.92), Immediate Memory (mean difference = 14.33), and the Delayed Memory (mean difference = 14.12) composites all were above or approached a full standard deviation from the normative mean. The Visual/Spatial Construction (mean difference = 7.16) and the Language (mean difference = 7.47) composites were also significantly different from the normative mean, but the group means still fell in the average range of functioning. Conclusion: In this study, patients with MS demonstrated a significant level of impairment in a wide variety of neurobehavioral functions. Broad-based measures of left hemisphere and right hemisphere functioning were statistically different from the normative mean but still fell in the average range. The findings in this study are consistent with other recent findings in the literature which demonstrates that white matter anomalies are associated with deficits in long term memory as well as anterior cortical deficits. The implications will be discussed in regards to practitioners and researchers.

Neuropsychological investigations of brain atrophy with cognitive dysfunction in multiple sclerosis

Reyhaneh Akbarzadeh, Reza Akbarzadeh, M. Akbarzadeh; Russian University of Economics & Culture (Moscow, RUS); Mashhad University of Medical Sciences (Mashhad, IR)

Introduction: Multiple sclerosis (MS) is a relatively common, chronic progressive neurological illness affecting individuals primarily in the third and fourth decades of life. Cognitive impairment (CI) may develop at any time during the course of the disease in the presence or absence of neurological disability. Objectives: In this review we summarize distinctive features of cognitive and psychopathological impairments of multiple sclerosis. Results: Neuropsychological impairment is a common feature of multiple sclerosis. Affected patients often have deficits in information-processing speed and memory and exhibit psychopathological states such as depression. Cognitive disorders are observed in 40 to 65% of the cases at any period of the disease. It is a major contributing factor to unemployment, accidents, impairment of daily functioning, and loss of social activity in those affected by MS. Structural brain imaging studies show a positive correlation between the extent of brain atrophy and cognitive dysfunction. However, measures of tissue atrophy including whole-brain and central atrophy are especially well correlated with and predictive of cognitive impairment. The degree and pattern of cognitive dysfunction is highly correlated with the amount and location of white-matter disease within the cerebral hemispheres. Conclusion: Recent studies have shown that conventional measures of brain atrophy explain more variance in neuropsychological dysfunction than do measures of lesion burden. In particular, neuropsychological outcomes correspond highly with linear measures of subcortical atrophy such as ventricle enlargement. Continuing research focuses on the possible relationship between measures of regional brain atrophy and cognitive and emotional impairment.

Attentional impairment in the benign and relapsing-remitting forms of multiple sclerosis evaluated with psychophysiological techniques

J.I. Gonzalez-Rosa, M. Vazquez-Marrufo, P. Duque, E. Vaquero, M.A. Gamero, M. Borges, C.M. Gomez, G. Izquierdo; University of Seville (Seville, E); Neuroinvest Association Virgen Macarena Hospital (Seville, E)

Psychophysiological techniques such as event-related potentials (ERPs) can help in evaluating cognitive impairment, a common feature in multiple sclerosis (MS). The main aim of the study was to observe the differences in the performance (behavioural variables) and EEG signals of the ERP components among these groups, and to check if the benign form is so benign in the evolution of the cognitive impairment of the subject. Three experimental groups were included in the study: 1) a relapsing-remitting group (RRMS), 2) a benign multiple sclerosis group (BMS) and 3) a Control group. The paradigm employed was a visuo-spatial attention task with cues (Posner experiment). Behavioural measures were reactions times (RTs), percent of correct responses (CRs), and errors. EEG signal was recorded during the experiment and latency and amplitude of diverse components from ERPs were obtained for all stimuli presented in the Posner paradigm (ERPs to the cue, ERPs to the readiness period, and ERPs to the standard and target stimuli). The Control group obtained faster reaction times than both MS groups (p < 0.001), a better percentage of hits than both MS groups (p < 0.003). In the latency analysis for ERPs there was a statistical difference between control and MS groups. Control group obtained a shorter latency for the P2 component in the cue stimuli (p < 0.001), for the P3 component in the standard stimuli (p < 0.001), and for the P2 (p < 0.001), N2 (p < 0.001) and P3 components in the target stimuli (p < 0.000). The highest delay of P3 latency was presented by the BMS group for the standard stimuli. The latency of the posterior N1 component for the standard stimuli was statistically different between Control and BMS group (p < 0.001) but failed between RRMS group and control subjects. On the other hand, the measure of the amplitude showed a higher individual variability and was statistical significant between Control and MS groups in the posterior N1 component for the standard stimuli (p < 0.003), and between Control and BMS group in the early negativity of the CNV component (p < 0.031). Our data indicate that both groups of MS showed poorer development in the attentional task. Moreover, the deficit in the BMS group, indexed by behavioural and psychophysiological variables, was more pronounced compared to the RRMS group. These results suggest a silent deterioration of cognitive skills for the BMS that is not usually treated with pharmacological or neuropsychological therapy.

An interim analysis of fatigue and cognition in relapsing-remitting multiple sclerosis patients receiving interferon-beta-1a (Rebif®)

M. Namaka, C. Gramlich, M. Doupe, L. Wong, K. Bergen, M. Melanson; University of Manitoba (Winnipeg, CAN); Manitoba Centre for Health Policy (Winnipeg, CAN); Health Sciences Centre (Winnipeg, CAN)

Purpose: Fatigue and cognitive dysfunction are the most frequently encountered disease-induced symptoms suffered by multiple sclerosis
(MS) patients. Although interferon-beta (IFNβ) slows disease progression, the extent by which it influences fatigue and cognition is not well known. **Aim:** To investigate the effect of IFNβ treatment on fatigue & cognition. **Methods:** 108 RRMS patients are scheduled to complete a 15 month, single centre, open-label fatigue/cognition study. Sample size was based on a 1% type I error and 20% type II error. The current interim analysis was conducted in 17 of the 108 patients that completed the study. Disease progression, fatigue and cognition were measured at each of the 4 scheduled visits (months 0, 3, 9 & 15). Baseline values obtained at 0 & 3 months were comparatively assessed to post-IFNβ treatment values obtained at 9 & 15 months. The primary study outcome was the change in fatigue/cognitive scores from pre to post-IFNβ treatment. Fatigue was assessed by a Modified Fatigue Impact Scale (MFIS). Cognition was assessed using a Brief Repeatability Battery (BRB) of neuropsychological tests which include: Buschke Selective Reminding Test (BRST), the 10/36 Spatial Recall Test (SPART), the Symbol Digit Modalities Test (SDMT), Paced Auditory Serial Addition Task (PASAT) and Word List Generation Test (WLG). The treating neurologist was blinded to all fatigue/cognitive assessments. Treatment allocation to Rebif, Avonex, or Betaseron was decided by the neurologist and patient based on factors such as tolerability, disease severity and clinical diversity of disease. **Results:** Treatment distribution for the 17 patients were as follows: Rebif (n=12); Avonex (n=2); Betaseron (n=3). The interim analysis was only conducted in the Rebif group due to limited enrollment in the other 2 treatment arms (Avonex, Betaseron). Rebif therapy was associated with an overall improvement in cognition as reported in all BRB cognitive domains, with the achievement of statistical significance in two sub-categories: SPART delayed and PASAT. A statistically significant improvement in the Kurtzke Expanded Disability Status Scale (EDSS) (t=1.1, NS) and MFIS (t=-3.6, p<0.004) was also identified. **Conclusion:** The interim results support the secondary benefits of Rebif treatment for MS-induced fatigue and cognitive deficits. Comparative analysis amongst IFNβ treatments at study closure may contribute to initial drug selection in accordance to the patient’s fatigue/cognitive status.

**PS40**

**Lower amplitude of the auditory amplitude modulation-following responses in multiple sclerosis patients with fatigue**


**Background:** Steady-state potentials are oscillatory responses due to the rhythmic stimulation of a neural pathway. In the auditory pathway, these responses can be obtained with clicks or amplitude-modulated tones (AM-following responses, AMFR), and are maximal at stimulation rates around 40 Hz and between 80 and 120 Hz. Recent studies have shown that phase synchronization mechanisms contribute substantially to the generation of AMFR. Phase synchronization implies a stable transmission of the sensory inputs together with a synchronous processing of the information. Axonal damage and widespread demyelization in multiple sclerosis (MS) might impair phase synchronization. **Aim:** The aim of our work was to study the AMFR in a wide frequency range in a group of patients with MS, in order to investigate the origin of these responses and to advance in our knowledge on the pathophysiology of the disease. **Methods:** Twenty three MS patients were included in the study (SM/15F, age: 43.5±10.8 years; EDSS: 4.5 (1.5-8.0); MSFC: 0.79±1.02; 11RR/7SP/5PP). AMFR were studied by means of 2000 Hz tones modulated in amplitude at increasing rates between 1 and 120 Hz (chirp-modulated tones). By this way, a wide range of stimulation rates was explored in a single test. Peak energy levels and maximal frequencies of response around 40 Hz and in the 80-120 Hz range were measured by means of time-frequency transforms. We analyzed different clinical variables, such as lesion topography at disease onset, physical disability, cognitive impair-

**PS41**

**Central fatigue in patients affected by multiple sclerosis: an electrophysiological study by means of transcranial magnetic stimulation**

V. Rizzo, V. Dattola, D. Crupi, M. Russo, F. Morgante, C. Mastromeni, P. Girlanda, C. Messina, A. Quartarone, University of Messina (Messina, I)

**Objective:** Fatigue is a common and disabling symptom of multiple sclerosis (MS). Aim of the present study was to ascertain, by means of TMS, if central fatigue in MS relies on corticospinal pathway abnormalities or on a dysfunction in cortical areas upstream pyramidal tracts. **Methods:** Fifteen patients with multiple sclerosis with and without fatigue and fifteen age-matched controls were included in the present study. Fatigue was assessed and scored using the Fatigue Severity Scale (FSS). Central conduction time, intracortical inhibition and facilitation, the first peak of I wave summation, a rTMS protocol (5 Hz, 10 stimuli at suprathreshold intensity) and the pre-movement facilitation associated at a reaction time paradigm in comparison with MS patients without fatigue. rTMS did not induce an increase in MEP size in both groups of MS patients. **Conclusions:** The lack of pre-movement facilitation in a reaction time paradigm in comparison with MS patients without fatigue. rTMS did not induce an increase in MEP size in both groups of MS patients.

**PS42**

**The FSMC (Fatigue Scale for Motor and Cognitive functions): first results from a multicentre validation study on a new patient reported outcome measure for cognitive and motor fatigue in multiple sclerosis**

I.K. Penner, C. Raselli, M. Stöcklin, K. Opwis, L. Kappos and the FSMC Study Group; University and University Hospital, Basel, CH

**Background:** The high impact of fatigue on social and occupational functioning of MS patients requires reliable and early diagnosis. Instruments available today do not differentiate sufficiently between cognitive and physical aspects of fatigue and show methodological limitations regarding scale construction and validation. **Objective:** To develop a new patient reported outcome measure for clinical routine that provides reliable diagnosis for patients and relatives. **Methods:** The FSMC is a 20-item scale that can be administered in 5 minutes. It consists of a cognitive (FSMCc) and a motor subscale (FSMCm), each comprising 10 items. In a multi-centre design including centres from Switzerland and Germany, the scale is tested against several external criteria such as rating by neurologist, two common fatigue scales (FSS, MFIS), cognitive testing (BRB-N, FST, MSNQ), depression (BDI), physical functionality (MSFC), personality traits (NEO-FFI), motiva-

www.sagepub.co.uk

*Multiple Sclerosis* 2006; 12: S1–S228
tion (HAKEMP-90) and quality of life (SF-36, FAMS). Data collection is currently ongoing. The following analyses included 107 MS patients and 81 controls. Results: An independent sample t-test revealed a maximal differentiation for all 20 items between controls and patients (p < 0.01). In patients, the internal consistency was Alpha ≥ 0.90 and in controls Alpha ≥ 0.85, for both subscales. The FSMCc was significantly correlated with MFI5, the FSMCm with FSS, and both showed the weakest correlation with the ESS (r ≥ 0.435) (p < 0.001). The relationship between fatigue and other clinical features of MS was statistically significant (r ≥ 0.487) (p < 0.001). The final statistical analysis is ongoing, and will be presented. This study will allow to better define the role of sleep disorder in MS fatigue.

P544

Relationship between fatigue and physical disability with depression in multiple sclerosis

A Nourian, H. Madayeni awal, H. Keshooy, M. Shakeri, M. Saeedian; Isla-

nic Azad Medical University ( Mashad, IR); College of Nursing (Mashad, IR)

Introduction: Multiple sclerosis (MS) is a neurological illness char-

acterized by various somatic and emotional symptoms, such as physical disability, fatigue, and depression. Depression is a particularly common form of psychopathology in MS patients. Objective: The aim of the present study was to investigate the effect of fatigue and physical disability on depression in patients with multiple sclerosis. Methods: We studied 105 out-patients that they completed the following measures: The Beck Depression Inventory scale (BDI), The fatigue Severity Scale (FSS) and The Krutzke Expanded Disability Status Scale (EDSS). Results: 105 MS patients (73 women and 32 men, mean age 31.03 years, SD 8.19) with clinically definite with multiple sclerosis (Relapsing – remitting 86.7%, Primary progressive 2.9% and Second-

ary progressive 10.5%). Mean FSS score of the subjects was 4.78, SD 1.5, mean of the beck depression Inventory was 16.83, SD 7 and mean of the expanded disability status scale was 2.54, SD 1.31 There was significant correlation between fatigue (FSS) and depression (BDI) (t = 0.617, p < 0.001) and noted lower correlation between Physical Disabil-

ity (EDSS) and depression (BDI) (t = 0.499, p < 0.01). Conclusions: The present study sought to examine and clarify the relationship between fatigue, and physical disability with depression in a sample of MS patients. We conclude that depression is very high correlated to fatigue and its correlation to Physical Disability was less than fatigue.

P545

The relation between fatigue and other clinical features of multiple sclerosis

R.J. Mills, C.A. Young; Walton Centre for Neurology and Neurosurgery

(Liverpool, UK)

Background: Fatigue may be defined as reversible, motor and cognitive impairment with reduced motivation and desire to rest, brought on separately by either mental or physical activity, humidity, acute infection, relapse, food ingestion or it may occur spontaneously. It is relieved by sleep or rest without sleep and while it may occur at any time it is usually worse in the afternoon. On the basis of this clinical description, a self report measurement scale has been devised. Objective To observe the levels of fatigue and other symptoms in a cross section of an MS population. Method: A questionnaire pack, containing the fatigue scale, multiple sclerosis impact scale (MSIS), an MS self efficacy (SE) scale, the Hospital anxiety and depression scale and the Epworth Sleepiness scale, was mailed to a large sample of an MS population based in Liverpool, UK. Data on age, disease type, disease duration, EDSS, and average length of sleep (night and day) were also collected. Results: Analysis was based on 630 respondents (n = 220 for MSIS and SE data). All raw scale scores were converted to interval level data using the Rasch measurement model. Fatigue correlated strongly with the MSIS motor measure (Pearson r 0.763) and this relation was linear (linear regression R square 0.58). It also correlated with the MSIS psyche measure (rho 0.748, linear regression R square 0.56) and SE, in a negative direction (rho -0.598, linear regression R square 0.36). There was moderate correlation with sleepiness (rho 0.435) and anxiety and depression (rho 0.524). There was borderline correlation with hours of daytime sleep (rho 0.36). Fatigue did not correlate with hours of nocturnal sleep, duration of MS or patient age. Fatigue worsened with increasing EDSS with a clear step down from fully ambulatory patients (EDSS 0–4) to those with EDSS 4.5 or greater (mean difference 1.47 logits, p < 0.001), there was no significant difference in fatigue between higher EDSS groups. Females had more fatigue than males (mean difference 0.39 logits, p = 0.008, eta square 0.11). Those with progressive disease had more fatigue than relapsing remitting (mean difference 0.48 logits, p = 0.004, eta square 0.21) Conclusions: Fatigue plays a large part in...
determining the impact of MS. Fatigue is worse in those with progressive disease and clearly worsens once ambulation is affected, however, it is not simply related to neurological deficit. Fatigue is not related to disease duration or patient age.

MS variants

P546

Marburg type of multiple sclerosis in an eighteen-year-old woman: therapy with ultra-high dose methylprednisolone and cyclophosphamide

M. Harzheim, J. Feucht, D. Paullet, U. Kallweit, D. Pöhlaus; Kamillus-Klinik (Asbach, D); Radiologische Gemeinschaftspraxis (Bad Honnef, D)

The Marburg type is a malignant variant of Multiple Sclerosis (MS) often leading to death only weeks or months after the onset of neurological symptoms. Treatment options rarely reported in the literature include plasma exchange, immunosuppressive therapy with mitoxantrone or cyclophosphamide, and administration of corticosteroids. Here we report about an 18-year-old woman who developed severe multifocal neurological symptoms including somnolence, tetraparesis, ataxia of the trunk and extremities as well as bladder and bowel incontinence within a few weeks. Clinical features, investigation of the cerebrospinal fluid yielding positive oligoclonal bands by isoelectric focusing, and cranial magnetic resonance tomography (cMRI) findings led to the diagnosis of Marburg’s type of MS and intravenous treatment with ultra-high dose methylprednisolone (2000 mg per day for 5 days, tapered) and cyclophosphamide was immediately initiated. The patient clinically improved paralleled by a marked reduction of inflammatory lesions in cMRI. The follow up four weeks later revealed only mild neurological signs without any functional impairment. This good clinical condition was contrasted by a large amount of new, partly gadolinium-enhancing lesions. In addition we provide the data of a follow up 44 weeks after the last control did not reveal any new lesions in T2-weighted cMRI but no neurological impairment.

P547

A case of Balo sclerosis with autonomic dysfunction

G. Vorobeychik, A. Kruissiok, J. Spring, J. Nelson, N. Beaurogard, C. Bozek; Fraser Health MS Clinic (Burnaby, CAN); University of British Columbia (Vancouver, CAN)

Background: Balo sclerosis is a rare demyelinating disease. Little information is published on the autonomic dysfunction of patients with Balo sclerosis. Method: Case presentation included a formal autonomic function assessment including medical history, neurological assessment, baseline blood pressure (BP) and heart rate (HR), orthostatic function assessment, Holter monitoring for 24 hours and autonomic function assessment including medical history, neurologic examination, orthostatic challenge test, isoelectric focussing, and cranial magnetic resonance tomography (cMRI) findings led to the diagnosis of Balo’s type of multiple sclerosis. Combined, these observations suggest that Balo’s type of multiple sclerosis may represent a subtype of multiple sclerosis. Conclusion: This is the first reported case of patient with Balo sclerosis who has autonomic function assessment. The mechanism of autonomic dysfunction during relapse in patients with Balo’s sclerosis is poorly understood and rarely assessed in spite of possible significant impact on a patient’s quality of life. Routine inclusion of these tests should be considered in the management of these patients.

P548

Concentric sclerosis: clinical and radiological features of seven Chinese cases

X. Wu, K. Zhang, C. Wang, G. Huang; Jiangxi Provincial Hospital (Nanchang-Jiangxi, RC)

Balo’s Concentric Sclerosis (BCS) is a rare demyelinating disorder characterized pathologically by alternating lamellae of demyelinated and myelinated white matter frequently organized into concentric configurations. The pathogenesis of the disease remains unclear. In this report we summarize the clinical and radiological features of 7 Chinese cases recently admitted to our hospital, attempting to understand its relation to multiple sclerosis. The diagnosis of BCS was reached according to the clinical course, CSF and characteristic MR imaging findings. The onset of symptoms was subacute or chronic and the age ranged from 20 to 50 years (mean 43 years) old. In contrast to the domination of manifestations of cognitive and behavioral dysfunction in the reported cases, 6 out of 7 cases in the present group displayed hemiparesis and 1 aphasia and apathy. In addition, 1 patient complained headache and 2 experienced vomiting. In all cases, MR imaging revealed multifocal lesions in a pattern of typical concentric rings or whorled appearance surrounded by long T1, long T2-weighted adena. Ring-enhancement in the peripheral of the lesion was detected in 2 cases by contrast-enhanced MRI. Follow-up MRI after treatment with steroid in one case showed a consistent but reduced focal but vanished perifocal adema. Contrasting the fulminant and fatal course of the most reported cases, these cases displayed effective responses to treatment and a relatively benign process. Clinical improvement was observed to some degree in all the cases after the administration of corticosteroid. During the follow-up, only 1 case has experienced relapse while the 6 others have not. Interestingly, one patient manifested initially in the form of multiple sclerosis and relapsed as concentric sclerosis, while another patient displayed symptoms of concentric sclerosis and followed by a relapse in the form of multiple sclerosis. Combined, these observations suggest that there are differences of the clinical and radiological features between the Oriental and Western concentric sclerosis patients. We propose that concentric sclerosis may represent a subtype of multiple sclerosis. Concentric sclerosis and multiple sclerosis may be different manifestations of the same disease range and, therefore, be convertible to each other under certain conditions.

P549

Relapsing neuromyelitis optica is a prevalent disease in Cuba. A clinical and epidemiological study


tion Center (Havana, CUB); Latin-American Medical School (Havana, CUB); Calixto Garcia General Hospital (Havana, CUB); Cira Garcia Clinic (Havana, CUB); CIMEQ (Havana, CUB); Institute of Neurology and Neurosurgery (Havana, CUB); Gustavo Aldereguia Hospital (Cienfuegos, CUB); Medical School (Cienfuegos, CUB); Manuel Asuncion Hospital (Camagay, CUB); Division Clinical Trials (Camagay, CUB); Guantanamo University Hospital (Guantanamo, CUB); Saturnino Lora University Hospital (Santiago de Cuba, CUB); Hayano University Hospital (Baracoa, CUB); Sancti Spiritus Children Hospital (Sancti Spiritus, CUB); Faustino Perez Rehabilitation Hospital (Sancti Spiritus, CUB); CIGB (Havana, CUB)

Abstracts S153

Multiple Sclerosis 2006; 12: S1 – S228

www.sagepub.co.uk

Downloaded from msj.sagepub.com by Shula Edelkind on October 1, 2010
Background: Cases of NMO have been reported worldwide but the prevalence is still unknown. Objective: To estimate the prevalence of R-NMO in the Cuban population (11. 122, 308). Design/methods: The island was surveyed from October 2003- November 2004. Neurologists of the Cuban National Cooperative Study on NMO reported 93 cases with possible NMO. Neurologists trained to diagnose NMO, reviewed each patient and strictly selected the cases with Wingerchuck et al. criteria (absolute criteria and one major supportive and two of three minor criteria). Cases were considered to be prevalent if alive and resident in Cuba on November 30th, 2004. Results: Fifty-four patients were identified and prevalent, in November 30th 2004, was 0.49/105 (95% CI 0.4; 0.6 Classic and Bayesian). Females (94.4%) with an onset age 32.35 ± 8.96, Caucasians 62.9% and mulattoes-negroes 37%, triggering factors were observed in 77.7% of cases. Index events were isolated in 82% with transverse myelitis 23 (46%) and optic neuritis 18 (36%). The duration was 10.95 ± 7.68 years, number of relapses 4.2 ± 2.2 and EDSS 5.21 ± 2.86. The most abnormal tests were spinal cord MRI (81.4%), somatosensory (SSP) (77.4%) and visual evoked potentials (VEP) (74.2%). Brain MRI showed abnormalities, non compatible with MS in 65% of cases and neuropsychological tests in 78.5%. The clinical form was relapsing-remitting 1b and a benign form was observed in 12 (22.2%). Conclusion: R-NMO is a prevalent disease in Cuban middle-aged women, with a frequency of ethnicity depending on the predominant population, triggering factors at onset, isolated index events, presence of benign forms and moderate-severe disability. The value of VEP and SSP; In the diagnosis of NMO with isolated index events and brain MRI abnormalities deserve further studies.

P550
Benign relapsing neuromyelitis optica
J.A. Cabrera-Gomez, A. Gonzalez-Quevedo, Yanely Real-Gonzalez, A.Y. Cabrera-Nuñez, Yiselle Real-Gonzalez; CIREN (Havana, CUB); INN (Havana, CUB); School of Medicine (Cienfuegos, CUB); School of Medicine (Santa Clara, CUB)

Background: The epidemiological study of relapsing-NMO (R-NMO) in Cuba showed a prevalence of 0.49/105 (95% CI 0.4; 0.6). Evaluation of patients showed cases with R-NMO of long duration and slight to moderate physical and visual disability. Objective: To present cases considered as benign forms of R-NMO. Methods: Data were obtained from 26 patients with R-NMO (Wingerchuck et al.) with 5 or more years of evolution and physical disability according to the EDSS of 4.5 or less and a degree of impairment 3 or less in the Visual Functions. These patients were considered as having a benign form of R-NMO. The demographic, clinical and laboratory data were compared with patients having the same physical disability (EDSS 4.5 or less), but with a Visual FS of 4 or more, and it was considered as a clinical form with predominant visual impairment. Demographic, clinical and laboratory data were compared between groups. Statistical analysis was carried out employing the Mann-Whitney nonparametric test and the Chi square test. Results: No significant differences between the benign form (B-RNMO) and that with predominant visual impairment (V-RNMO) was demonstrated according to: age of onset (34.1 ± 6.8 years in B-RNMO and 29.7 ± 8.9 years in V-RNMO (p= 0.193)); number of relapses (B-NMO 4.0 ± 2.8 and VNM 3.4 ± 1.6), (p= 0.117); duration of disease, (9.6 ± 4.3 B-NMO versus 13.8 ± 7.2 years, (p= 0.117). Never-theless, when time between the first and second relapse was analyzed, it was shorter in B-NMO than in the V-NMO (1.4 ± 3 ± 3 and 3.4 ± 4.4 years respectively) with a trend toward significance (p= 0.06). With respect to the first sentinel event, in B-NMO the transverse myelitis (TM) clinical form predominated with 9 cases (60%) as compared to only 2 cases in the V-NMO form (18.2%). However, the opposite occurred regarding optic neuritis (ON) as the initial event, predominating ON in 7 of the cases of the V-NMO form (63.5%), while in the benign form it was only observed in 4 (26.7%). These differences were statistically significant (p = 0.03). There were no differences between groups with respect to the simultaneous appearance of TM + ON as the initial neurological symptom. Conclusions: The possibility of a benign form of R-NMO after 5 or more years after onset, considering a low physical and visual disability is associated with the presence of TM as the initial neurological event and with a shorter time for the second relapse.

P551
Clinical course of optic neuritis in patients with recurrent neuromyelitis optica: ethnic comparison
R.M. Alharenga, S. Carellos, L.C. Santos Thuler; UNI-RIO (Rio de Janeiro, BR)

Objective: To describe the clinical characteristics, course and prognosis of optic neuritis in patients with neuromyelitis optica. Methods: Sixty unselected cases of optic neuritis occurring in patients with recurrent neuromyelitis optica (Mayo Clinics criteria, 1999) were evaluated. All patients were seen at Hospital da Lagoa-Rio de Janeiro – Brasil, between 1985 and 2004. Descriptive statistics and life-table analyses were performed using SPSS for Windows. Results: Optic neuritis was the presenting feature in 51.6% of patients; visual impairment was unilateral in 33.0% of cases, being severe in 61.7% of patients; a high remission rate was achieved and 18% of patients experienced severe visual impairment. The time between the index events ranged from 1 day to 20 years (median =6 months). In a nine months median period, 30% of patients developed loss of visual acuity and 45% were blind in at least one eye. In a median disease duration of 8 years (6 months to 30 years) we registered 152 visual and 228 medular events. After 9 months (median time), 30% of patients had severe visual impairment and 45% were blind in at least one eye. At last follow up, 53% of patients had bilateral visual impairment and 63% were blind in at least one eye. The high mortality rate (23%) was due to cervical myelitis causing tetraplegia and respiratory failure. Mortality rate were significantly higher in the Afro-Brazilian patients, who represents the majority of the subjects studied (55%). Conclusion: Optic Neuritis in Recurrent Neuromyelitis Optica had a severe and acute onset, with predominant unilateral lesion and spontaneous remission. Disease has severe and bilateral visual impairment in a long-term course. Mortality rate were higher mainly in the Afro-Brazilian patients.

P552
Benign neuromyelitis optica over 18 years: relapsing para-proteinemic central demyelination
L. Costelloe, M. Hutchinson; St. Vincents’s University Hospital (Dublin, IRL)

Background: Neuromyelitis optica (NMO) is a disabling demyelinating disease associated with antibodies to aquaporin-4 in 70% of patients. We describe a patient with a benign NMO for 18 years associated with an IgM kappa paraproteinemia; disabling relapses responded well to steroids. Case History: A 42- year old man developed a left retrobulbar neuritis in 1988; acuity has remained reduced to counting fingers despite steroid treatment. MRI brain was normal. Over the next 18 years he had six further neurological episodes consistent with NMO; four of these were in the spinal cord with typical longitudinal central cord swelling extending up to five segments responding well to steroid therapy. In addition he had two episodes of extensive, steroid responsive brain stem demyelination, one in the pons and the other in the midbrain. Repeated MRI brain has revealed no cerebral hemisphere involvement. His present deficit consists of a blind left eye, mild gait ataxia, a neurogenic bladder, and erectile dysfunction. He can play 18 holes of golf. In 2000, a monoclonal IgM kappa band was detected in the serum. Urine immunofixation, b2-microglobulin, and skeletal survey were normal. Bone marrow aspirate showed 7% plasma cells. Serum NMO IgG was negative on two occasions. CSF examination between relapses showed no white cells with normal protein and glucose. IgGolobular bands were negative and IgG index was normal. Discussion: Our patient
fits the clinical phenotype of antibody-negative NMO. Unusually, he has had a very benign course with minimal residual deficit. He has demonstrated exquisite steroid responsiveness even to severe relapses. Interestingly, there is a persistent comitant monoclonal gammopathy of undetermined significance in the serum. We postulate that this monoclonal band may be producing an antibody to the aquaporin-4 water channel. This may in fact be an example of central demyelination analogous to peripheral paraproteinemic CIDP which is well described.

P553

The rate of conversion to a secondary progressive course is lower in neuromyelitis optica than multiple sclerosis

D. Wingerchuk, S. Pittcock, V. Lemon, C.F. Lucchinetti, B. Weinshenker; Mayo Clinic College of Medicine (Scottsdale, USA); Mayo Clinic College of Medicine (Rochester, USA)

Objective: To compare the probabilities of secondary progressive disease in neuromyelitis optica (NMO) and multiple sclerosis (MS).

Background: Whereas relapsing-remitting MS patients accrue most of their disability during the secondary progressive (SP) phase of the disease, NMO patients are disabled early in the disease course by severe attack residua. We have observed that a SP disease course is uncommon in NMO. Methods: We evaluated the clinical course of 96 NMO patients. We included patients with evaluable function in at least 2 limbs and 1 eye. We defined a SP course as continuous, objective deterioration without remission over > 12 months and preceded by one or more attacks. We used actuarial life table analysis and the Mantel-Cox chi-squared test to evaluate the probability of SP conversion, compare the results with those derived from published population-based MS natural history data (London, Canada), and derive an overall relative risk for SP conversion.

Results: Ninety-five per cent (91 of 96) patients met inclusion criteria. Median values were: age of onset = 38.3 y (IQR = 26.0 – 48.3); follow-up = 5.3 y (3.0 – 9.5); relapses = 5 (3 – 8); EDSS = 6 (4 – 8). We expected that 20 NMO patients would convert to an SP course but observed only two cases (2.1%; p = 0.00003; overall relative risk = 0.095). In both cases, the SP course manifested as a progressive myelopathy; latency from NMO onset to SP onset was 3 y (after one attack) and 13 y (10 attacks). Conclusions: NMO patients were older at disease onset and experienced a high rate of early, severe attacks. Despite the presence of these characteristics, which are associated with greater risk of SPMS, the risk of SP conversion was low in NMO patients. NMO-related disability is almost exclusively attack-related. These data contribute to mounting evidence that illustrates the dissociation between relapses and progression in CNS demyelinating diseases.

P554

The pathology of brain involvement in neuromyelitis optica spectrum disorder

D. Jacobs, S. Roumey, B. Weinshenker, S. Pittcock, D. Wingerchuk, V. Lemon, C.F. Lucchinetti; University of Pennsylvania (Philadelphia, USA); Mayo Clinic College of Medicine (Rochester, USA); Mayo Clinic College of Medicine (Scottsdale, USA)

Introduction: The serum autoantibody NMO-IgG is both a sensitive and specific marker for an emerging “NMO spectrum” of CNS demyelinating disorders that include recurrent longitudinally extensive myelitis (rLETM). These disorders are clinically, radiologically and pathologically distinct from classical MS. Contrary to the traditional concept of brain sparing, recent MRI studies demonstrate brain lesions in 60% of NMO patients. The pathological substrate of these brain lesions is unknown. We report an NMO-IgG seropositive patient whose initial manifestations of NMO were restricted to the brain but who subsequently developed a typical NMO spectrum disorder characterized by rLETM. Case report: A 48-year-old woman presented with tongue numbness, hiccups, vomiting, diplopia, and diffuse numbness. MRI revealed lesions in the dorsal medulla, basal ganglia, and a necrotic, enhancing lesion in the right temporal lobe. Cervical MRI was normal. Symptoms resolved spontaneously. Two years later she presented with a LETM which improved after corticosteroid therapy, but recurred and enlarged to involve the entire cervical cord, from the cervicomедиulitary junction to T1, with a new lesion at T3. There was extensive edema and enhancement in the cord parenchyma and along the pial surface. The patient received plasmapheresis and cyclophosphamide. One year later, she developed a recurrent episode of LETM with acute quadriplegia. MRI revealed recurrent enhancement of the right temporal lobe lesion and several new enhancing lesions in the brain. A biopsy of the temporal lobe lesion revealed a dense inflammatory infiltrate of lymphocytes and macrophages. Eosinophilia and perivascular C9 neoantigen deposition were marked with profound destruction of both myelin and axons. The lesion was similar to pathology of spinal cord lesions of NMO. NMO IgG was detected in the serum. There was improvement after rituximab therapy; the patient regained some arm and leg function enabling her to operate a motorized wheelchair.

Conclusion: This case demonstrates that lesions pathologically typical of NMO do occur within the brain in patients with NMO spectrum disorders. Whether this pathology is representative of the more typical brain lesions in periventricular or diencephalic regions in NMO is unknown. Further revision of NMO diagnostic criteria allowing for the existence of symptomatic brain lesions, and incorporating the novel serum autoantibody marker NMO-IgG, is warranted.

P555

Anti-aquaporin 4 antibody in Japanese opticospinal multiple sclerosis

K. Tanaka, T. Tani, M. Tanaka, T. Saita, J. Idzuka, K. Sakimura, M. Nishizawa; Brain Research Institute, Nigata University (Nigata, JP); Utano National Hospital (Kyoito, JP); Ojiya Sakura Hospital (Ojiya, JP)

Neuromyelitica optica (NMO-IgG), whose target molecule is probably an aquaporin 4 (AQP4), is reported to be a specific marker for NMO and will be a good tool to distinguish NMO from multiple sclerosis (MS). Opticospinal form of MS (OSMS) which has been categorized in a subtype of MS is prevalent in Asian countries. The differences between OSMS and NMO have long been under discussion. To clarify this argument, we established the immunofluorescence detection system of AQP4 antibody (AQP4-Ab) using AQP4-transfected HEK 293 cells. We examined this antibody in the sera of OSMS with long spinal cord lesions (LSCl) with or without cerebral lesions, OSMS without LSCl, conventional form of MS (CMS) and healthy controls. AQP4 was positive in 62% of OSMS with LSCl but was negative in any other forms of MS. Among OSMS with LSCl, the AQP4-positive group was all women with severe visual disturbance, however other features such as patients of mean age, severity of limb and truncal disabilities, existence of cerebral lesions were not different from AQP4-negative OSMS with LSCl. It is not yet clear that AQP4-Ab is directly related to the lesion development, however, there accumulated many data to support that OSMS with LSCl is antibody-mediated.

P556

Neuromyelitis optica autoantibody (NMO-IgG) in patients with suspected NMO or limited forms of NMO

L. Zuliani, Y. Blanco, B. Tavolato, B. Gionetto, F. Graus, A. Saiz; Hospital Clinic (Barcelona, E); S. Antonio Hospital (Padua, I); Ca’ Foncello Hospital (Treviso, I)

Background: NMO-IgG is considered a specific marker autoantibody of NMO, although it is also found in limited forms of NMO as

Abstracts S155

www.sagepub.co.uk

Multiple Sclerosis 2006; 12: 51 – S228
recurrent transverse myelitis (RTM) and recurrent optic neuritis (RON).

**Objective:** To analyze the frequency of NMO-IgG in serum samples sent to our laboratory because of suspected NMO, RTM and RON.

**Methods:** The presence of NMO-IgG in the serum from 26 patients was analyzed, by immunohistochemistry (dilution 1/500) using an avidin-biotin technique on paraffin-embedded frozen sections of cerebellum and hippocampus of rat and indirect immunofluorescence (Zuliani L, et al. Neurology 2006; 51). Bland to the clinical diagnosis. Patients were classified based on accepted criteria (Lennon VA, et al. Lancet 2004; 364: 2106) by referring physicians, and the diagnosis reviewed without knowledge of the serological results.

**Results:** Seven samples were positive. Both immunohistochemistry methods yielded the same results. Five out of 10 (50%) NMO, 2/2 (100%) RTM, 0/4 RON, 0/8 multiple sclerosis, and 0/2 other neurological diseases. One seropositive NMO patient had a primary Sjögren’s syndrome and one seronegative a systemic lupus erythematosus. No other relevant differences were found between seropositive or seronegative patients.

**Conclusions:** Our study confirms NMO-IgG as a highly specific marker of NMO and related disorders. The avidin-biotin technique is as sensitive as the indirect immunofluorescence in this unselected series of suspected NMO patients.

**Acknowledgement:** We thank all neurologists who referred the samples and the clinical information.

---

**P557**

An audit of the diagnostic usefulness of the NMO-IgG assay for neuromyelitis optica

E.T. Littleton, A. Jacob, M. Bogild, J. Palace; Radcliffe Inffirmary (Oxford, UK); The Walton Centre for Neurology and Neurosurgery (Liverpool, UK)

**Background:** The ability to reliably distinguish neuromyelitis optica (NMO) or Devic’s disease from multiple sclerosis (MS) with predominant optico-spinal involvement has important treatment implications. Recently a diagnostic assay for NMO has become available from the Mayo Medical Laboratories and has started to be used in clinical practice. The test is currently expensive (500 US dollars) and our ability to request this test has been limited by our hospital. We therefore audited the results of this assay performed upon all patient sera sent from Oxford to assess the potential usefulness of the test in confirming or excluding a diagnosis of NMO.

**Methods:** All sera which had been sent for NMO-IgG diagnostic testing from Oxford were included. Clinical details were retrospectively collected on these patients and were assessed to see which of the patients would meet the 1999 and the 2006 Mayo clinic diagnostic criteria for NMO.

**Results:**

- **Assays were requested on 25 patients.**
  - 0% of typical MS patients had positive results (0/10).
  - 50% NMO patients had positive results (4/8 using the 1999 Mayo diagnostic criteria and 5/10 using the updated 2006 criteria).
  - Of the remaining 3 NMO-like “high risk” patients, the two who had myelitis but no optic nerve involvement both tested positive, whereas the one who had repeated alternating optic neuritis without spinal cord disease tested negative.
  - 2 further patients, one of whom had Sjögren’s Syndrome with both myelitis and optic neuritis and one who had an inflammatory encephalitis, both tested negative.
- 8 of the 13 NMO or NMO-like “high risk” patients had been followed up and treated in Oxford, and all 8 (5 NMO, 3 NMO-like) had responded to chronic immunosuppressive treatment (corticosteroids with or without azathioprine) and had relapsed upon its withdrawal. These 8 treatment-responsive patients included 5 who had tested positive for NMO-IgG and 3 who had tested negative.

**Conclusions:** Whilst a positive result makes MS unlikely, a negative result is common in clinically definite NMO. Thus the test appears to have high specificity in excluding MS but not good diagnostic sensitivity for NMO. Both NMO-IgG positive and NMO-IgG negative patients who meet the clinical criteria for NMO or NMO-like “high risk” conditions were responsive to long-term immunosuppression. This small and independent audit is in accord with previously published data on the diagnostic usefulness of this new assay.

---

**P558**

Early relapse of Devic’s disease after rituximab B-cell depletion

M. Capobianco, S. Malucchi, R. Bottero, A. Di Sapo, F. Gilli, F. Marnetto, M. Caldano, P. Valentino, A. Sala, C. Doriguzzi Bozzo, A. Bertolotto; Regional Multiple Sclerosis Centre (Orbassano, I); Neurological Unit (Pinerolo, I)

Neuromyelitis optica (Devic’s disease) is an aggressive inflammatory disease involving optical nerves and spinal cord with a large rate of immunosuppressive treatment non-responders. The pathogenesis is probably due to inflammation caused by the presence of anti-aquaporin-4 antibodies. In order to achieve treatment response, rituximab (a monoclonal antibody anti-B cell directed to CD-20) was used in two patients who did not respond to traditional immunomodulant/immunosuppressive therapy.

**Clinical case 1:** A 20-year-old girl was diagnosed in September 2005 after she experienced 2 optical relapses in June 2004 and September 2005. She was early treated with Rebif-44 but without any response as defined by the presence of 2 subsequent spinal cord relapses (November 2005 and January 2006) with EDSS progression to 3.0. After high dose intravenous methylprednisolone for 10 days, rituximab was administered at the dose of 375 mg/m2 every week for 4 weeks with a complete recovery of the neurological examination. The dosage of B-cell precursors (CD19+ cells) showed B-cell depletion and an MRI performed 1 months later showed no new or enhancing lesions.

**Clinical case 2:** A 30-year-old woman was diagnosed after 2 spinal cord relapses in November 2005 with EDSS score of 6.0. She was treated with intravenous high dose methylprednisolone and then with 5 courses of plasmaexchange with little recovery. Then she was treated with 2 monthly bolus of intravenous cyclophosphamide, but she experienced new relapses in January and March 2006 reaching EDSS score of 7.5. She was re-treated with 5 courses of plasmaexchange and soon after with rituximab at 375 mg/m2 every week for 4 weeks with reduction of EDSS score to 6.0. Even if CD19+ cells were undetectable, after 1 month she experienced a new spinal cord relapse (EDSS 7.5) with a new concomitant enhancing lesion in the dorsal medulla. Even if prompt plasmaexchange was done, she has not yet recovered. Even if rituximab seems to be the best pathogenetic treatment for Devic’s disease, there is the possibility of early unresponsiveness. To better evaluate the impact of rituximab treatment, a large randomised trial is needed in patients unresponsive to traditional immunomodulant/immunosuppressive agents.
(March-October 2005) and mitoxantrone 12 mg/m² (December 2005- April 2006), the patient became paraplegic. The thoracic radiological examination showed a pulmonary mass that was not seen at the beginning of the disease. There were no other radiological or biological abnormalities, except CSF pleocytosis of > 50 WBC/mm³. The particularities of the case are the long period of evolution, the clinical and radiological characteristics fulfilling diagnostic criteria for NMO, who appears in this stage as a paraneoplastic condition. We suggest that patients with NMO should have a long-term follow-up, with regularly check-up for underlying neoplasia.

NMO-IgG and opticospinal multiple sclerosis and idiopathic recurrent myelitis
K.K. Kim; Asan Medical Center (Seoul, KOR)

To find the sensitivity and specificity of NMO-IgG in Opticospinal form multiple sclerosis (OSMS) and idiopathic recurrent transverse myelitis (IRM) in Korea and to review the clinical and MRI findings in patients of serum NMO-IgG (+) MS. Indirect immunofluorescence with a composite substrate of mouse tissues identified a characteristic NMO-IgG staining pattern of Lennon's report. We tested masked serum samples from 27 Korean patients with OSMS and 39 patients of IRMT. Fifty-three control patients had conventional form of MS (CMS), acute myelitis of unknown etiology, or optic neuritis. The clinical and MRI findings were analyzed in reference to NMO-IgG. Sensitivity and specificity were 19% (95% CI 10–24%) and 96% (95% CI 92–99%) for OSMS and 3% (95% CI 1–6%) and 96% (95% CI 95–99%) for IRMT. NMO-IgG was detected in 9 patients, 5 with OSMS, all of those positive patients also fulfilled diagnostic criteria of Neuro- myelitis Optica (NMO), and one with IRMT (3%) and 2 with CMS (8%). In the 9 NMO-IgG-positive patients, longitudinally extensive (> 3 vertebral segments) spinal cord lesions (78% vs. 46%) and permanent, complete blindness (no light perception) of at least one eye (67% vz. 35%) were the significant features as compared with NMO-IgG-negative OSMS. One case of CMS with NMO-IgG had unusual brain lesions, but in other respects had features suggesting OSMS. One positive NMO-IgG patient of recurrent myelitis had bilateral prolonged latency of P100 in visual evoked potentials. The low sensitivity of NMO-IgG in Korean OSMS and IRMT patients suggest that the novel NMO-IgG antibody may not be a specific marker of OSMS and IRMT. But high incidence of NMO-IgG positivity is significantly associated with severe optic nerves and spinal cord lesions of OSMS or atypical fulminant white matter lesion in MS. These findings suggest that NMO-IgG may be an autoantibody produced in repeated optic-spinal common specific antigen stimulation in severe optico-spinal or rarely white matter lesions, not cause the OSMs lesions.

Probable NMO IgG in Turkish patients with Devic’s disease, and multiple sclerosis
G. Akman-Denir, M. Mutlu, S. Icoz, M. Kurtuncu, N. Yesilot, M. Eraksoy; Istanbul University (Istanbul, TR)

Neuromyelitis optica (NMO), or Devic’s disease is a disorder of the demyelinating disease spectrum which involves mainly the optic nerves and the spinal cord within the central nervous system (CNS). Recently, abnormalities of the humoral immune system have been shown in cases with NMO, with an antibody against aquaporin-4. Here we aimed to look for NMO IgG in Turkish cases with Devic’s disease in comparison to cases with classical multiple sclerosis (MS) and healthy controls (HC). Serum samples from 14 patients with Devic’s disease, 14 cases with MS and 15 HC were assessed using an indirect immunofluorescence kit containing monkey cerebrum, cerebellum, intestineum and HEp2 cells. The sections were assessed blinded to the clinical diagnosis. Eight patients with Devic’s disease showed staining around the vessels and pial surfaces in cerebrum and cerebellum sections. On the other hand none of the MS cases or HC showed such a staining. Among the 8 patients with Devic’s disease that were positive, 5 showed strong binding, and among these 4 had severe clinical involvement. On the other hand, among the cases with Devic’s disease that were negative, 2 had severe disease while 4 had milder forms of the disease. However this difference did not reach statistical significance. This relatively small cohort of patients suggest that the presence of NMO IgG might have an association with the disease course and severity. Such a relationship should be further assessed in larger series.
clinical outcome in Brazilian NMO patients with abnormal brain MRI. **Methods:** We retrospectively reviewed the records of six patients followed at the Neuroimmunology Clinic of the Federal University of Sao Paulo between 2000 and 2006. **Results:** The mean age of onset was 24.2 (17–43) years, there were 4 Caucasians patients, one African-Brazilian and one Asian. The mean EDSS score on first evaluation was 4.7 (4.0–6.0), 2 of them developed severe disability with EDSS of 9.5 and one died during follow-up. The mean relapse rate and progression index were 1.45 (1–2.5) and 1.6 (1–3.2) respectively. Four of our patients had symptoms other than ON and myelitis: dysphonia, vomiting, ocular deviation, headache and nystagmus. All the patients were treated with disease modifying drugs, such as: betainterferons, cyclophosphamide, azathioprine, immunoglobulin and corticosteroids. During the follow up brain MRI disclosed brainstem, diencephalic and calosal abnormalities, and 3 of them had unusual encephalic lesions similar to other NMO patients recently reported. None of them had a brain MRI with typical MS lesions. **Conclusion:** Recently, a normal brain MRI has been considered a distinctive feature of NMO. However, some patients may have brain MRI lesions which are not typical of MS. In our series of NMO patients with brain abnormalities all seemed to have a more aggressive disease with a progression index higher than one; if the evidence of brain abnormalities in NMO patients has clinical and therapeutic implications is yet unknown and demand further studies.

**PS65**

**Multiphasic disseminated encephalomyelitis: report of 3 cases**

B.C. Suh, S.M. Kim, H.D. Kim, H.Y. Shim, D.S. Shim, J.H. Cho, I.N. Sunwoo; Yonsei University College of Medicine (Seoul, KOR); National Health Insurance Corporation Ilsan Hospital (Ilsan, KOR)

Multiple sclerosis (MS) and acute disseminated encephalomyelitis (ADEM) are the most important acquired inflammatory demyelinating diseases of central nervous system. Classically, ADEM is monophasic and this feature is frequently used to differentiate ADEM from MS. Recently, a few case reports are published about recurrence of ADEM. Here, we present 3 cases of recurrent ADEM known as multiphasic disseminated encephalomyelitis (MDEM). The age of onset was 4 to 13 years old and they presented with fever, headache, neck stiffness, abdominal pain, seizure and decreased mentality as well as focal neurologic deficits. Number of attacks in each patient was 3 or more and the interval of the 1st episode to the 2nd one was 4 to 10 months. Brain magnetic resonance image (MRI) revealed variable sized, multifocal, subcortical white matter lesions with gray matter involvement and each lesion had obscure boundary. Locations of these lesions which showed relapsing and remitting features, were different at each attack. The cerebrospinal fluid (CSF) study showed mild pleocytosis and normal IgG index with negative oligoclonal band. All of these cases showed relatively good responses to steroid treatment and sometimes even dramatic, but the response to beta interferon which tried to one patient was poor. Recovery from each attack was good considering severe symptoms and abnormality of brain MRIs and the long-term prognosis was thought to be relatively good. We diagnosed these 3 cases as MDEM instead of MS on the basis of following reasons; young age of onset, prominent meningeal irritation sign with fever which is not usual symptoms of MS, normal IgG index with negative CSF oligoclonal band and brain MRI findings which favored ADEM. And considering good steroid response but poor beta interferon response to these cases, the therapeutic approach may be differentiated from MDEM to MS although further study needed.

**PS66**

**Atypical recurrent demyelinating diseases: clinical features with MRI correlation in a series of patients**

C.I. da Silva, A.J. da Rocha, M.F. Mendes, A.C.M Maia Jr., F.T. Braga, N.L. Cabral, C.P. Tilbery; Santa Casa de Misericodia de Sao Paulo (Sao Paulo, BR); Laboratorio Fleury (Sao Paulo, BR); Hospital Sao Jose Joinville (Joinville, BR)

**Introduction:** The McDonald criteria (2001, 2005) based mostly on the features of Caucasian patients, describes the spinal lesion should not be longer than two vertebral length, despite the fact that such lesions were seen in 2–3% of MS. Long spinal cord lesion (LCL) extending more than three vertebral segments is described to be the most important characteristic of neuromyelitis optica (NMO). Although the absence of brain symptoms is the core finding of NMO, brain involvement has been documented as an exception. We report here the clinical features of Japanese MS patients with LCL (LCL-MS) often accompanied by brain involvement. **Methods:** We found a total of 79 consecutive patients with LCL among MS patients examined at Utano National Hospital MS Center after 2000 and analyzed their clinical and laboratory features. **Results:** The site of lesions at disease onset was optic nerve 23%, spinal cord 49%, or both 7.5%. LCL appeared at a very early stage, in 84% within 1.5 years and on average 1.6 years after the onset. At the time of the first myelitis episode, 64% already developed LCL. Although cerebral symptoms are rare (2.5%) at clinical onset, 44% of LCL-MS later (on average 5.4 years) developed clinically manifest cerebral lesions. Brainstem lesions were seen in 57% of LCL-MS patients, 15% of whom required artificial ventilator. **Conclusions:** Although LCL-MS shares many features common with NMO, brain involvement is frequent and not exceptional, at least in Japanese population. Since the absence of brain symptoms is implied by the name NMO, we propose LCL-MS as a more adequate term for this MS variant.
P567

Course and prognosis of paediatric multiple sclerosis are different according to age at diagnosis: a regional study in Basse-Normandie (France)

R. Giurca, C. Creveuil, K. Droulon, G. Defer; Neurology Clinic (Cluj Napoca, RO); CHU (Caen, F); Réseau Bas-Normand pour la SEP (RBN-SEP) (Caen, F)

Background: The early onset forms of multiple sclerosis (Pediatric MS, P-MS) have a low frequency, occurring in 3% to 10% of all MS cases. Age at diagnosis is a key feature for progression and prognosis in adult MS. Objective: Evaluate the clinical features and course of MS in patients having the disease onset before age of 18 years according to age at diagnosis. Methods: All patients with MS onset before age of 18 (n=41) identified from Basse-Normandie MS regional registry between 1961 and June 2005 were included in the study. The clinical and course data were verified and validated by two independent examiners with systematic access to the medical records. Patients were divided in two groups depending on age at MS diagnosis (MS diagnosis established according to Poser or McDonald criteria depending on the date of diagnosis): group A (n=20) including all patients having MS diagnosis set before age of 18 years and group B (n=21) including all patients older than 18 years at MS diagnosis. Data related to clinical features and disease course were evaluated and compared between the two groups and also with some clinical data of 605 adult MS patients included in the same regional database. Results: We found significant differences between the two study groups. Patients from group A had a more rapid progression to mild handicap (EDSS 3.0) than patients from group B (time to reach EDSS 3.0 being 6.34 vs. 14.36 years; p=0.02) and they reached this level of disability at a younger age (21.17 vs. 29.79 years; p=0.002). The duration of first remission was significantly shorter in group A (1.44 vs. 3.14 years; p<0.001) and the evolution to secondary-progressive (SP) form was faster in these patients when compared to group B (14.97 vs. 27.57 years; p=0.01). Patients from group A had a greater risk (Kaplan-Meier survival curves) to reach EDSS 3.0 (p=0.01) or EDSS 6.0 (p=0.007) as well as a greater probability to reach SP form (p<0.001) than patients from group B. Progression index (PI) was not significantly different between the two groups (group A-PI=0.37 and group B-PI=0.21) but mean PI for the entire paediatric group was significantly lower than mean PI in the adult group (n=605) (0.29 vs. 0.45; p=0.003). Conclusion: P-MS forms show clinical peculiarities especially those with the age of diagnosis less than 18 years. This raises the question of early use of disease-modifying treatment owing to the particular clinical course in these patients.

P568

Encephalopathy as an associated manifestation during the first event of CNS inflammatory demyelination in a paediatric population

E.A. Yeh, P.K. Dauffner, B. Weinstock-Guttman; SUNY (Buffalo, USA)

Objective: To evaluate the clinical utility of recent operational definitions of pediatric ADEM, with attention to the presence or absence of encephalopathy in the clinical presentation of the disease. Background/Methods: ADEM is usually a monophasic illness of presumed post-infectious and auto-immune etiology. Recent operational definitions of ADEM require the presence of a polysymptomatic encephalopathy. This definition may aid the clinician in distinguishing monophasic illness from multiple sclerosis. We reviewed the clinical presentation of 22 children who were found to have white matter lesions on MRI suggestive of inflammatory/post-infectious changes. Results: Age of presentation was 19 months to 17 years, with a mean age of 9.25 years. The presentation included seizure in 6/22, ataxia in 4/22, speech difficulties in 3/22, optic neuritis in 4/22, headache in 2/22, INO in 2/22, urinary retention in 4/22, rigidity and apnea in 1/22 and sensory level in 5/22. Encephalopathy was seen in only 5/22 patients (23%). All of these patients were under 7 years of age. The presence of encephalopathy did not predict recurrence. Demyelination recurred in 8/22 (36%) patients, two of whom presented initially with encephalopathy. Conclusions: This study reinforces the view that although younger children with presumed ADEM may present with frank encephalopathy, older children who have a monophasic illness compatible with ADEM are less likely to present with encephalopathy, children who present with MRI white matter lesions on MRI. Better and more sensitive tools to evaluate the subtle neurocognitive/behavioral changes that may occur in this population are required.

P569

Familial autoimmune disorders increase the risk for recurrent events in paediatric patients with acquired demyelination

E.A. Yeh, B. Weinstock-Guttman; SUNY (Buffalo, USA)

Objective/Methods: We evaluated a cohort of 28 children with first onset of demyelinating disease for risk factors for recurrence or persistence of white matter lesions, with special attention to family history of autoimmune disease. Results: Twenty eight children were included in the analysis. The age at onset of first episode of demyelination was 1.5 years to 17 years. Average age at onset was 9.13, with two peaks, ≤7 and ≥11 years of age. Of these children, 12, or 43%, had recurrence or persistence of white matter lesions. Twelve children had a positive family history of autoimmune disease. Of these 12 children, 8, or 67%, had recurrent disease. Of the sixteen children with no family history of autoimmune disease, only 4, or 25%, had recurrent or persistent disease. Children with residual deficits were numerous. Nineteen of 28 children (68%) had residual problems, including residual MRI changes, seizures, and/or cognitive and motor deficits. Ten of 12 children (83%) with family histories of autoimmune disease had residual problems, whereas nine of 16 children (56%) without family histories had residual deficits. Conclusions: Little information on risk of recurrence in children with demyelinating disease is available. Our study suggests that recurrence of white matter lesions or persistence of lesions is far more common in children with family histories of autoimmune disease than in those without such family histories. Family history may be predictive of poor outcome. This suggests that disease in these children may represent a specific subgroup of autoimmune mediated white matter disease with an earlier onset and, possibly, a more aggressive course. Further prospective studies of risk factors for early onset demyelinating disease are needed.

P570

Rigorous definitions for “benign” and “mild” multiple sclerosis

J. Herbert; NYU Hospital for Joint Diseases (New York, USA)

Background: The term “benign multiple sclerosis” (BMS) implies long-term predictability; however, follow-up of “benign” cohorts reveals significant disease progression, suggesting definitions of BMS may be too permissive. Objective: To establish uniform, quantitative definitions for BMS and mild MS (MMS), with predictive power. Method: The following disability/duration (D/D) coordinates were chosen as endpoints: (1) For BMS, Expanded Disability Status Scale (EDSS) <2 at 30 years disease duration (yrs dd); this specifies abnormal signs in Kurtzke functional systems (KFS) but no minimal
disability; (2) For MMS, EDSS < 4 at 30 yrs dd; this specifies mild to moderate disability on KFS but no limitation of mobility. These coordinates were applied to the Multiple Sclerosis Severity Score (MSSS) published by Roxburgh et al. (Neurology, 2005). This algorithm ranks patient cross-sectional EDSS scores on a decile scale compared to the distribution of disability in a large reference cohort with comparable dd. MSSS scores assigned after one year’s dd, based on a single cross-sectional EDSS assessment, are representative of overall disease severity over time. Multiplying MSSS scores by ten yields the predicted prevalence of that subpopulation within the entire cohort. Results: (1) For BMS, D/D coordinates proposed here yielded an MSSS score <0.45, which predicts a prevalence of 4.5% within the reference population. MSSS .45 corresponds to an EDSS of 1 at 15 yrs, which is consistent with the International Consensus definition of “fully functional in all neurological systems 15 years after disease onset” (Lublin and Reingold, Neurology, 1996). (2) For MMS, D/D coordinates proposed here yielded an MSSS score <1.7, which predicts a prevalence of 17% within the reference population. MSSS 1.7 corresponded to approximate milestones: EDSS 1 at 3 yrs dd, EDSS 1.5/9 yrs, EDSS 2/14 yrs, EDSS 2.5/17 yrs, EDSS 3/20 yrs, and EDSS 3.5/30 yrs. The proposed definition for MMS of MSSS 1.7 coincided most closely with previous definitions for “benign MS” by Kurtzke (Ann NY Acad Sci, 1984) and Pittock et al. (Ann Neurol, 2004), after correcting their stated dd for mean delay to diagnosis of 5 yrs. (EDSS ≤2 at 13 yrs dd, yielding MSSS <1.65). Conclusion: BMS may be defined as MSSS <0.045 and MMS may be defined as MSSS <1.7. These definitions present the advantage of predictive value, since MSSS may be assigned based on a single-point EDSS assessment after only one year’s disease duration.

P571

Benign multiple sclerosis: possible clinical predictors of outcome

N. Yucyav, Z. Tanriverdi, B. Cetin, O. Ekmekei, A. Kocaman; Ege University Medical School (Izmir, TR)

A small fraction of multiple sclerosis (MS) patients can have a benign course of disease and can live many years without restriction of daily living and work. In considering immunomodulatory treatment, early identification of these patients is important. Therefore many studies have been dealt with factors influencing clinical course and survival. In this study, 20 MS patients (diagnosed either by Poser or Mc Donald criteria) having at least 10 years of disease duration and EDSS of ≤2 were evaluated. Demographic and clinical features such as gender, age at onset, initial course, symptoms at onset, interval between attacks, attack frequency have been extensively evaluated to define the possible predictors of outcome. The mean age at onset was 28.5 yrs with female predominancy (63% of all patients). Ninety five percentage of patients presented with monoregional and monosymptomatic symptoms and signs. The mean time between the first attacks was 3.8 years. The cumulative number of attacks at the end of 5 years was 2.38. These clinical features were compared with the ones of 72 MS patients having at least 10 years of disease duration before immunomodulating therapy and having EDSS of >3. Female gender, sensory symptom at onset, monoregional and monosymptomatic onset, less frequent cumulative attacks at first and fifth years of disease and having no signs and symptoms related to spinal and cerebellar system have been significantly related with a more favorable outcome. In contrast to other studies, younger onset, optic neuritis at onset and long interval between the first attacks have not significantly associated with benign MS in our study. Benign MS is a retrospective diagnosis and seems to be a temporary condition. Although some possible clinical predictors have been suggested, may be due to differences in study design, the conclusions from these studies have somewhat varied. These patients, who are fully ambulatory, may still be disabled by other symptoms such as fatigue, depression and cognitive dysfunction. We also aimed to evaluate our benign MS patients from this point of view.

P572

Early-onset multiple sclerosis in the sanitary departments of the city of Valencia, Spain

A. Brocalero, A. Cervello, I. Bosca, F. Coret, B. Casanova, A. Pascual-Lozano, L. Landete on behalf of the GITEM Study Group

Introduction and objective: The early-onset multiple sclerosis (EOMS) supposes less than 5% in different series. For this reason the different studies collect few patients. We have retrospectively reviewed the cases of four hospitals of Valencia. Patients and methods: We review the data base that share all four departments and analyze, of all the patients, those with presentation of symptoms before the 16 yrs. In our study we describe their main characteristics: age, sex, form of onset and clinical course, analysis of the cerebrospinal fluid (CSF), neurophysiological/ visual evoked potentials (VEP) and neuroimaging/magnetic resonance (RM) tests, diagnostic category according to the criteria of Poser and the evaluation across the scale of disability of Kurtzke, Expanded Disability Status Scale (EDSS). Results: We reviewed the clinical protocols of 38 patients between 9,82 and 16,89 years at the onset of the disorder, 63,2% women. The median time to diagnosis was 7,85 ± 9,97 yrs, with a median of 3,77. The most frequent form of onset was monosymptomatic; optic neuritis 18,4%, followed by hemiparesis at 13,2%; close to polineural, sensorimotor also in 13,2%; and thirdly brainstem disorders with 10,5%. The clinical course was relapsing-remitting in 79% and secondary progressive in 10,5%. There were oligoclonal bands in the CSF in 23,7%. The VEP were abnormal in 34,2%. The RM was compatible in 65,8%. The current EDSS score is 2,19 ± 1,85, after a mean disease duration of 12,83 ± 11,06 yrs. Conclusions: These results are similar to previous studies, the EOMS was more frequent in women, the clinical course was predominantly relapsing-remitting whereas no patient had the primary progressive course in our series. The patients who reached secondary progressive course were associated with growth disability after long-term disease (more than 18 years in this serie).

P573

The presymptomatic phase of multiple sclerosis: preliminary results of a nationwide survey

C. Bensa, C. Giannesini, O. HeinzelF, E. Roulet on behalf of the French PreMS Study Group

Background: Up to 70% of patients with first symptoms suggestive of MS have an abnormal cranial MRI, frequently suggesting long lasting previous pathological activity. To our knowledge this presymptomatic phase of MS has not been evaluated systematically. However, a better characterization of this phase would help to explain differences in prognosis and treatment response in early MS.

Patients and Methods: The French PreMS study is a nationwide retro- and prospective study aiming to characterize the unknown time period which precedes the so-called “clinical onset” of MS. Information is collected upon patients who have a cranial MRI suggestive of MS, but no symptoms typical of MS (group A), and from MS patients who had MRI performed before their first symptoms, for any reason (group B). The content of this phase is explored via structured interviews in different MS and preMS patients and controls. MRI are analyzed blindly according to established diagnostic criteria and to a specially designed “back-time” scale. Results: During early 2006, 530 French neurologists (including 13 MS centres) who had joined a previous study (HeinzelF O et al.). Criteria for non response to IFN b in RR MS patients, ECTRIMS 2005) were asked to participate. A region-based, Paris area, panel of 190 neurologists was also assessed. At the time of submission, the responses from one MS centre (6 neurologists) have been analyzed. In the preceding 5 years, 10 patients had been recruited (group A: 8, group B: 2, male: 1, age: 18 – 41, mean 33). Reasons for performing MRI were headache (n = 6) and hyperprolac-tinemia, viral meningitis, nasal polyposis or trauma (1 each). Bar- kho’s criteria for dissemination in space were fulfilled in 4 patients.
During follow-up (8 pts, range 6–36 months), 3 patients had a first event suggestive of MS; MRI was unchanged in 4 and worse in 4. At unblinded interview, neurological symptoms before the first MRI were found in 3 patients, defining an earlier onset of MS according to McDonald’s criteria in one patient. **Discussion and conclusion:** A literature search found only three case-reports of pre-symptomatic MS patients. Our results indicate that a large number of such patients may have been diagnosed and managed by neurologists, and that the attitudes towards diagnosis and follow-up are variable. Some of these patients record neurological symptoms before the "official" clinical onset of MS. Updated results will be presented.

**P574**

**Is a 10-year follow-up enough to predict to subsequent disability in multiple sclerosis?**
M. Mutlu, H. Ceylan, G. Akman-Demir, M. Kurtanca, M. Erokasy; Istanbul University (Istanbul, TR)

Long term disability in patients with multiple sclerosis (MS) is widely variable. Patients with slow disability rates are defined as having “benign MS”. However, the concept of “benign MS” is still controversial. Up to now there is no consensus on which patients should be called to have “benign MS”. In this study our aim was to assess whether the disability score at 10 years can be predictive of subsequent disability. We evaluated patients who had been examined at our MS unit between January 2005 and April 2006. We compared the disability scores (EDSS score) at the 10th year and 15th year, and evaluated the time to reach an EDSS of 6.0. Within this period 328 patients with clinically definite MS were seen. Among these 97 (29%) had a disease duration of 10 years or more. Mean age at last visit was 43.11 ± 9.08 years, mean disease duration was 17.12 ± 6.70 years. The mean EDSS score was 3.40 ± 1.65 at the 10th year (range 0–7.0). Patients were divided into four groups according to the 10th year EDSS score: 0–2.0 (group A), 2.5–3.0 (group B), 3.5 (group C) and >4 (group D). Disease duration was 17.33 ± 8.49 years in group A, 15.21 ± 6.53 years in group B, 20.63 ± 7.23 years in group C, and 17.45 ± 4.91 years in group D (not significant). 15th year EDSS score was 2.58 ± 1.10 in group A, 3.60 ± 1.13 in group B, 4.42 ± 1.16 in group C, and 5.96 ± 0.76 in group D. (p = 0.000). The mean time to reach EDSS 6 was 28.67 ± 7.57 years in group A, 20.75 ± 5.44 years in group B, 16.75 ± 5.60 years in group C, and 10.93 ± 3.47 years in group D (p = 0.000). There was no significant difference between groups in terms of the attack number within first 5 years, and duration of the first attack interval. In conclusion, 10th year disability score seems to be a useful parameter predictive of at least 15th year disability score. Early course, first five year attack number and first attack interval, do not seem to be useful predictors of subsequent disability.

**P575**

**Active inflammation is common in very late onset multiple sclerosis: characteristics of 111 patients**
R.A. Bermel, R.J. Fox; Cleveland Clinic Foundation (Cleveland, USA)

**Background:** MS presenting after the sixth decade is a recognized phenomenon, although atypical and poorly characterized. These patients are frequently excluded from accurate diagnosis and treatment on the basis of age alone. **Design/Methods:** Using an electronic medical record search, patients identified who were diagnosed with MS at or after age 60 and evaluated at a tertiary referral center over the last five years. Each chart was reviewed to confirm the diagnosis of MS and extract clinical, laboratory, and imaging characteristics. **Results:** 111 cases were identified, with a mean age at diagnosis of 64 yrs (range 60–76; 15 were over 70 yrs), mean duration of symptoms prior to diagnosis was 9.8 yrs (age at symptom onset 8–71; 47 developed initial symptoms at or over 60 yrs). 67% were female; 90% were Caucasian. The most common disease course at diagnosis was relapsing-remitting (RRMS, n = 37), followed by primary progressive (PPMS, n = 35), secondary progressive (SPMS, n = 26), clinically isolated syndrome (CIS, n = 9), and progressive relapsing (PRMS, n = 4). Two patients with RRMS had biopsy-proven MS. Where imaging was reviewed by an MS specialist, 86% of brain MRIs showed typical changes of MS, as did 80% of spine MRIs. Where gadolinium was given, 46% of patients with RRMS or CIS demonstrated gadolinium enhancement, and 75% of all patients had oligoclonal bands or elevated IgG index. At the time of diagnosis, 39% were mildly disabled (EDSS ≤ 3), and 34% needed an assistive device for walking or were non-ambulatory (EDSS ≥ 6.0). **Conclusions:** MS in older adults may be under-recognized and accurate diagnosis is often delayed by many years. Some patients have symptom onset at more typical ages, but a sizable proportion have onset after age 60. Nearly half of relapsing patients (RRMS and CIS) presented with inflammation on MRI, which suggests that the disease course is dependent upon the inflammatory component of MS and not just age.

**P576**

**Risk factors for secondary progressive multiple sclerosis**
E. Gruzska, A. Pokryszko-Dragan, M. Dubik-Jezierzanska, M. Bilinska; Wroclaw Medical University (Wroclaw, PL)

The aim of study was to define parameters of clinical course of secondary progressive multiple sclerosis (SPMS), with a special regard to early phase of the disease and its potential prognostic value. The study comprised 71 patients (39 women, 32 men, aged 34–71, mean 52.1) diagnosed with SPMS, followed-up in an outpatient MS clinic at Neurological Department of Wroclaw Medical University. On the basis of their medical documentation, the following data were defined: age at beginning of the disease, first clinical symptoms, duration of initial relapsing-remitting phase and annual exacerbation rate (AER) within this period, annual progression of disability (APD) – estimated by means of Expanded Disability Status Scale (EDSS) – during relapsing-remitting and progressive phase. Relationships between these data were statistically analysed. Mean age at the beginning of the disease was 31.7 years. First clinical symptoms in 40 patients were motor deficits, in 20 – retrobulbar optic neuritis, in 8 – symptoms of brainstem involvement, in 6 – cerebellar symptoms, in 1 – sensory deficits (in 4 – multi-system involvement was noted). Mean AER was 0.53. Progressive phase started after 1–29 years (mean:10.7). Mean APD in relapsing-remitting phase was 0.7 and in progressive phase – 0.28, which was significantly lower (<0.05). No other significant differences or correlations were found. In patients with SPMS most frequent first clinical symptoms are motor deficits. Other parameters of early phase of MS do not show significant relationships with further progressive course of the disease. Increase in disability is significantly slower during progressive than relapsing-remitting phase.

**P577**

**Descriptive study of a primary-progressive multiple sclerosis population**
L. Landete Pascual; Hospital Dr. Peset (Valencia, E)

**Introduction:** Primary-progressive multiple sclerosis (PPMS) is a type of multiple sclerosis (MS) characterized by lack of clinical relapses, not much inflammatory activity in MRI, progressive myelopathy with clinical of pyramidal and cerebellous damage and more males and older than in other forms of MS. That type of MS is perhaps the worst known from a clinical ang pathogenic point of view. **Objective:** To describe patients with diagnosis of PPMS of our clinical serie, from a clinical point of view. **Patients and Methods:** A database compound of 839 consecutive patients from 3 hospitals in Valencia (SPAIN). We selected patients with diagnosis of PPMS according to Schumacher criteria. We calculated male/female proportion, actual mean age, evolution time, age at onset, annual progression index measured at 3 years, and mean time to different values of EDSS, comparatively with another forms of MS (relapsing-remitting and secondary-progressive). **Results:** PPMS patients are 6.5% of the whole database (55 patients). 24/31 male/female proportion. Mean age
Clinical aspects: diagnosis and differential diagnosis – Part II

P578
Proteasome autoantibodies to human proteasomes predict multiple sclerosis in monosymptomatic optic neuritis

J. Milthers, N.H. Beyer, G. Houen, J.L. Frederiksen; University Hospital of Copenhagen (Glostrup, DK); Statens Serum Institut (Copenhagen, DK)

Introduction: Mayo et al. [1] suggested the human proteasomes as a major autoantigen in MS. We have previously shown that proteasome autoantibodies (PAB) in serum to human purified proteasomes were present in 28% of optic neuritis (ON) patients and 47% of multiple sclerosis (MS) patients and 8% of a control group using ELISA, based on AMON data. Objective: We wanted to confirm that our ELISA method of PAB detection is valid. Additionally, we wanted to investigate the presence of serum PAB in acute monosymptomatic ON (AMON) predictive to MS. Method: All patient samples were collected at the time of ON and samples were taken and 79 patient samples (33 AMON and 46 MS patients) were included. Objectives criteria’s and information on MS development after AMON was sought in all available patient files. A quantitative immunoassay, ELISA, was used for the detection of autoantibodies against human proteasome in all patient sera using a pool of purified human proteasome for patient sample investigation. Results: 6 out of 33 (18.2%) AMON patients were PAB positive and all 6 patient developed MS, confirmed by patient files. From the 26 PAB negative AMON patients, 11 developed MS and 13 did not. No information was available on the remaining 3 patients. The distribution is significant using chi-square testing (p ≤ 0.05). These findings corroborate with our previous results using ELISA for the determination of proteasome autoantibodies in MS-patients. Discussion: We have used population based data prospectively and patient material from a well described cohort of ON and MS. We can confirm that serum PABs are predictive in AMON for development of MS. Our ELISA method for serum PAB detection was reproducible and validated. Our numbers of PAB positive AMON patients may be too small and more detailed prospective sample studies are necessary.

P579
Relationship of pupillary dynamics to retinal nerve fibre layer thickness in multiple sclerosis

E. Frohman, A. Conger, A. Salter, A. Shah, O. Stuve, T. Frohman; University of Texas Western (Dallas, USA)

Objective: To characterize the relationship between pupillary measures, low contrast letter acuity, and axonal degeneration within the retinal nerve fiber layer (RNFL) of MS patients. Background: The retinal nerve fiber layer contains ganglion cell axons in the absence of myelin, and transmits information within the optic nerve to the brain for visual processing, sleep wake cycles (via the retinohypothalamic tract), and pupillary light reflexes. Previous work has demonstrated a predictable relationship between low contrast letter acuity and RNFL thickness, as revealed by bi-annual optical coherence tomography (OCT). We utilized quantitative infrared pupilometry to study the relationship between vision, RNFL, and the dynamics of pupillary light reflexes.

Methods: We recorded direct and consensual pupillary light reflexes utilizing an infrared pupilometry device (NeuroOptics, Irvine, California). Stimuli consisted of consecutive light flashes of 10 microwatt intensity and 0.026 sec duration. Eight flashes were utilized for both direct and consensual responses to generate average values for reflex latency, percent change in pupillary diameter, and maximum constriction velocity. MS patients with and without a history of optic neuritis were compared to normal subjects with respect to RNFL thickness, low contrast letter acuity (Sloan Chart 1.25%), and pupillary dynamics. Results: Our investigations demonstrated a direct relationship between low contrast letter acuity, RNFL thickness, and parameters of pupillary dynamics. MS patients with a history of optic neuritis that exhibited reductions in letter acuity and RNFL thickness, also showed reductions in the percent change in pupillary diameter, prolongation in response latency, and a reduction in maximum constriction velocity. Patients with a history of unilateral optic neuritis revealed the most compelling left-right differences in these measures. Conclusions: The unique structural architecture of the retina (containing no myelin) makes it an ideal tissue for modeling the processes of neurodegeneration, neuroprotection, and potentially even neurorestoration. We provide confirmatory evidence to support the close relationship between vision, RNFL thickness, and objective measures of the pupillary light reflex. Our observations suggest that both structural and physiologic measures of the anterior visual system can be utilized as a biomarker for neuroprotection clinical trials.

P580
Clinical significance of the 30 days cut-off for demonstrating dissemination in time with magnetic resonance imaging


Background: In the recent revisions to diagnostic criteria for multiple sclerosis (MS), a new T2 lesion appeared at least 30 days after a clinically isolated syndrome (CIS) qualifies for dissemination in time (DIT). Therefore, scans performed earlier than 30 days after symptoms onset cannot be used as reference for this purpose. Objectives: To compare the increase in risk of relapse conferred by new T2 lesions between patients first scanned at least 30 days after a CIS and patients scanned earlier. Methods: Consecutive CIS patients examined within three months of symptoms onset and followed for at least three years were included. The presence of new T2 lesions was assessed on a MRI scan performed one year after the CIS. We analyzed the interaction between the timing when first scan was performed (less than 30 days vs. at least 30 days after symptoms onset) and the effect of the presence of new T2 lesions in time to first relapse. Results: The study group comprised 218 patients, 152 women and 66 men, with a mean age (95% Confidence Interval) of 29.2 (28.1–30.4) years who were followed by a mean of 77.7 (74.8–80.6) months. Eighty-six (39.4%) patients had optic neuritis, 55 (25.2%) brainstem syndromes, 59 (27.1%) myelitis, and 18 (8.3%) other syndromes. At least one T2 lesion was seen on baseline MRI in 152 (70%) patients and 104 (47.7%) had new lesions on follow-up scan. Ninety-seven (44.5%) patients had a relapse. The median time to relapse was 101 (78–124) months. There were no statistical differences in any of these variables between the 159 (73%) patients whose first scan was performed earlier than 30 days (mean [95% CI] 13.7 [12.5–15] days) and those 59 (27%) who were scanned afterwards (49.4 [45.2–53.6] days). Timing of first scan did not modify the effect of the presence of new T2 lesions in time to first relapse (Hazard Ratio to relapse when there is a new T2 lesion [95%CI] 5.86 [3.62–9.47], regardless the time to first scan; HR if this scan is performed before vs. after than 30 days: 5.29 [2.88–9.71] vs. 5.88 [3.64–9.51]). Conclusions: It is common practice in patients with CIS to obtain a brain MRI before 30 days after symptoms onset. According to this yearly scanning protocol and based on the similar clinical significance of new lesions regardless of time to first scan, there is no reason for discarding these scans as reference for demonstrating DIT.
PS81

Importance of oligoclonal banding in multiple sclerosis: relation with demographic, clinical features and genetic background
E. Idman, S. Özkab, Y. Dogan, G. Kösehasanogulları; Dokuz Eylül University (Izmir, TR)

Intratheically produced IgG is found in more than 95% of clinically definite Multiple Sclerosis (MS) patients in western countries. The aim of the present study was to compare the clinical and demographic features and Human Leucocyt Antigen (HLA) profile in Oligoclonal Band (OB)-positive and OB-negative MS patients. We investigated the serum and Cerebrospinal Fluid (CSF) from 210 clinically definite MS patients. Demographic and clinical features were recorded. HLA profiles were also recorded in 79 patients. OBs were detected with isoelectric focusing. 178 healthy people were disposed as the control group when comparing HLA profiles. OB was positive in 149 patients (70.9%) in the first testing. In 34 out of 61 patients OB determination was repeated, and 21 (61.7%) were found to have positive OB. Total percentage of OB-positive patients was 80.9 after second lumbar puncture OB testing was repeated in 11 of OB-negative patients, and 3 of them (27.3%) were found to have OB. Total OB-positive patients was 82.3% after the third testing. Attack severity was higher in OB-negative patients. HLA DR-15 antigen was more frequent in OB-positive group than both OB-negative and control group. The data obtained from this study ascertained that the second OB testing is sufficient, when it was negative in the first determination, and our MS population was closer to western populations in terms of OB existence. Our findings also showed that OB existence might be a good prognostic factor in patients with clinically definite MS.

PS82

Active TTV infection in serum and cerebrospinal fluid samples of multiple sclerosis patients
M. Bartolome, R. Alvarez-Lafuente, M. Garcia-Montojo, V. De las Heras, R. Arroyo; Hospital Clínico San Carlos (Madrid, E)

Background: The TT virus (TTV) is a newly discovered pathogen that may belong to the Circoviridae family; latest studies describe TTV prevalences of up to 90%, and it is suspected to be associated with several diseases, although no definitive evidences have been found. Recently, it has been proposed that TTV could be involved in the pathogenesis of multiple sclerosis (MS) as a potential trigger of the immune system through the mechanism of molecular mimicry.

Objectives: The aim of the present study was to determine the frequencies of TTV infection in serum and cerebrospinal fluid (CSF) samples of MS patients in comparison with patients with other neurological diseases and healthy blood donors (HBD), to evaluate the possible involvement of this virus in the pathogenesis of MS.

Methods: Serum samples of 46 MS patients and 22 HBD, and CSF samples from 48 MS patients, 18 patients with other inflammatory neurological diseases (OIND), and 18 patients with other non-inflammatory neurological diseases (ONIND) were analyzed. Total DNA was extracted from 200 microlitres of centrifuged serum and CSF samples; blank reactions were interspersed in order to evaluate a possible cross-reaction. Finally, a sensitive and specific quantitative real-time polymerase chain reaction (PCR) assay with specific sets of primers and dual-labelled probes. Each specimen was measured in duplicate.

Results: The results were as follows: i) For TTV genomes (viral load, 16.6 copies/ml of CSF), and among controls, 5/18 (27.8%) OIND patients (17.2 copies/ml of CSF), and 3/18 (16.7%) ONIND patients (12.2 copies/ml of CSF) were positive too; we did not find any statistical significant difference, although seems to be a trend, when DNA prevalences of TTV were compared between patients with inflammatory diseases (MS and OIND) and patients with ONIND. Conclusions: TTV is a highly prevalent virus in human population, and blood and serum samples appears to be worthless in the study of MS. Regarding CSF, TTV infection seems to be widespread in patients with inflammatory diseases; this virus should be studied deeper to analyze its possible role.

PS83

Detection of human herpesvirus 6 genomes, and human endogenous retroviral H and W sequences in cerebrospinal fluid
R. Alvarez-Lafuente, M. Garcia-Montojo, V. De las Heras, M. Bartolome, R. Arroyo; Hospital Clínico San Carlos (Madrid, E)

Background: Human herpesvirus 6 (HHV-6), a member of the Herpesviridae family, and human endogenous retroviral sequences (HERVs) H and W, have been recently associated with multiple sclerosis (MS), and different mechanisms have been proposed for them in the pathogenesis of the disease. HERVs consist of up to 8% of the genome, and recently, it has been described that combinations of the endogenous retrovirus HERV-H and HHV-6 antigens result in highly increased cellular immune responses among MS patients.

Objectives: 1) To analyze the prevalence and viral loads of HHV-6, HERV-H and HERV-W in cerebrospinal fluid (CSF) of MS patients and controls. 2) To establish the prevalence of possible co-infections. 3) To evaluate the role of the HHV-6 infection as a possible trans-activator of the HERV sequences. Methods: A total of 92 CSF samples were analyzed: 46 from MS patients, 23 from patients with other inflammatory neurological diseases (OIND: 3 systemic lupus erythematosus with CNS involvement, 4 meningitis, 7 myelitis, 5 optic neuritis, 2 encephalitis, and 2 Guillain-Barre syndrome), and 21 from patients with other non-inflammatory neurological diseases (ONIND: 6 headaches, 7 normal pressure hydrocephalia, and 8 benign intracranial hydrocephalia). All the CSF samples were obtained by sterile lumbar puncture and they were immediately aliquoted and stored at −80°C. Total DNA and RNA were extracted from 200 microlitres of centrifuged CSF, respectively. A reverse transcription (RT) was carried out, and then, HHV-6 genomes from extracted DNA, and HERV-H, and HERV-W sequences from cDNA were searched by a sensitive quantitative real-time polymerase chain reaction (PCR) assay with specific sets of primers and dual-labelled probes. Each specimen was measured in duplicate. Results: The results were as follows: i) For HHV-6: 5/48 (10.4%) MS patients had HHV-6 genomes in their CSF (128.1 copies/ml of CSF), but no positives were found among OIND and ONIND patients. ii) Regarding HERVs prevalences, any positive sample was found among MS, OIND, and ONIND patients for none of the genome, and recently, it has been described that combinations of the endogenous retrovirus HERV-H and HHV-6 antigens result in highly increased cellular immune responses among MS patients. iii) Regarding co-infections, any positive sample was found among MS, OIND, and ONIND patients for none of the genome, and recently, it has been described that combinations of the endogenous retrovirus HERV-H and HHV-6 antigens result in highly increased cellular immune responses among MS patients. iv) Regarding HHV-6 infections, any positive sample was found among MS, OIND, and ONIND patients for none of the genome.

Conclusions: HHV-6 was found in a subset of MS patients, but not in patients with other neurological diseases, suggesting a possible involvement in the disease, in spite of the low rate of infection. We did not find any sequence of the HERVs included in the study, even though five MS patients suffered from active HHV-6 infection.

PS84

Immunocompetent cells in blood of non-exacerbated paediatric patients with remitting-relapsing multiple sclerosis
L.M. Vysotskaya, V.M. Stadenkin, N.A. Torubanova, O.V. Bykova; Scientific Center of Child Health (Moscow, RUS)

Multiple sclerosis (MS) with onset under 18 yr constitutes 7–10%. Autoimmune considerations of MS pathogenesis imply a complex of immunological factors involved (HLA, various interleukins, T- and B-lineage cells etc). Cerebrospinal fluid is the traditional object for MS neuromyelitis studies, in childhood lumbar puncture it as excessively invasive. Goal: Evaluation of immunocompetent subsets percentage in blood of non-exacerbated children with RMS.
Methods: 43 non-exacerbated children, aged 6-17 yr, with RRMS were observed; 26 of them receiving DMT, 17 cases investigated before DMT, maximum EDSS score was 2.5. MS diagnosis was verified according to McDonald criteria. Identification of immunocompetent subsets in blood was performed via computerized immunoflowcytometry (FACStrak, Becton Dickinson, USA) with monoclonal antibody panel of the same manufacturer: CD3, CD4, CD8, CD19, HLA-DR, CD16/56, CD3/DR, CD3/CD16/SD56, CD9/DR, CD57/CD8, N2D5, CD122, SD95. Results: The percentage of basic T-cell subset was not abnormal, while immunocompetent cells with membrane expression of some markers, rare in normal children, was encountered. CD16/56 natural killers were determined in 26 MS patients, reaching 11–41%. Normally this index rarely exceeds 7%. Activation antigens, reflecting presence of active immune process, were also revealed (2 molecules of IL-2 receptor expression: CD25, CD122). CD122 had been revealed more frequently (18 cases -41.8%). Special attention should be attributed to CD95 receptor, susceptible to action of pro-apoptotic factors, initiating cell death. Percentage of CD95+ cells varied from 4% to 34%. This marker is not encountered on blood lymphocytes in normal individuals. Supposedly, apoptosis activation results in reactive T-cell elimination, which limits demyelination. There is no way to elaborate, if this expression is a feature of cell death in spontaneous MS course, or the result of DMT. Conclusion: Non-exacerbated RRMS pediatric patients demonstrate the basic features of immunocompetent cells’ activation: CD25 and CD122 expression, high percentage of CD16/56, CD57/8 and CD95. Latter antigen is the main marker for lymphocytes, entering apoptosis. Increased CD95 expression seems characteristic for RRMS patients and should be viewed as prognostically favorable.

PS85

Correlation of IgG index and oligoclonal bands in CSF of patients with multiple sclerosis and other neurology diseases

J. Mares, R. Herzig, K. Urbanek, V. Sladkova, P. Hlustik, J. Skinarova, V. Bekarek, P. Schneidtora, J. Zapletalova, P. Kanovsky; University Hospital (Olomouc, CZ)

Objective: The aim of this study is to compare the correlation between IgG index values and the number of oligoclonal IgG bands in the CSF of patients with multiple sclerosis (MS) and patients with other neurology diseases (ND). Material and Methods: We compared both of groups of MS patients (N = 150) consisted 41 males and 109 females (aged 18-68, mean 36,6±10.1 years) and group of ND patients (N = 417) consisted 197 males and 220 females (aged 2-82, mean 42,8±14,71 years). The CSF collected by a lumbar puncture was examined evaluating intrathecal synthesis using the IgG index and determining oligoclonal immunoglobulins. The number of alkaline OCB in the CSF was assessed by the method of isoelectric focusing. Pearson’s correlation analysis and chi-square test of homogeneity were used to evaluate the statistical significance of the results. Paired-sample t-test (parametric) and Wilcoxon signed-ranks test (nonparametric) were applied when assessing statistical significance. Results: We didn’t find any positive correlation between the IgG index and the number of oligoclonal IgG bands by MS patient group. The Mann-Whitney test didn’t demonstrate significantly (p = 0.939) differences between IgG index values by patient with the number of oligoclonal IgG bands ≥ 2 and patients with number of oligoclonal IgG bands < 2. In the ND patient group we find significantly increased values of IgG index (p = 0.001) by patients with the number of oligoclonal IgG bands < 2. Conclusion: We can’t confirm the hypothesis of positive correlation between both of parameters of intrathecal synthesis but we can confirm the tendency to the higher IgG index values by ND patients with the low number of OCB. This result can support the significance of OCB assessment for the diagnosis of MS, because the higher IgG index is not regulatory finding in MS patients.

PS86

Visualising the effectiveness of brain compensatory processes in early multiple sclerosis during a working memory task. A one-year fMRI follow-up study

M. Au Duong, B. Audoin, F. Reuter, I. Malkova, S. Comfort-Gouny, P. Cozzone, J. Pelletier, J. Ranjeva; CRBBM UMR CNRS 6612 (Marseilles, F); Timone Hospital (Marseille, F)

Objectives: Cortical reorganization involving mainly the prefrontal cortices (PFC) has been widely demonstrated in multiple sclerosis (MS) patients performing working memory (WM) tasks. However, the effectiveness of these compensatory processes are not well understood. In order to investigate brain activation profiles susceptible to be the most effective to compensate for MS related brain injury, the relative changes in cortical activations have been studied in two groups of early MS patients showing or not showing maintenance of WM capacity over a one-year period of time. Methods: Thirteen early MS patients underwent two fMRI explorations (baseline and M12) using paced auditory serial addition test (PASAT) as paradigm. Twenty one controls matched for age, sex and educational level were also evaluated at a single time point. Relative to baseline, PASAT scores recorded during fMRI at M12 were decreased in 5 and increased in 8 patients. fMRI data were analysed using the SPM2 software. Random effect analyses have been performed to compare activations of each group of patients relative to controls, at baseline and at M12. Results: The group of patients with increased PASAT scores (between M0 and M12) has shown at M12 larger brain activation inside the prefrontal cortex relative to controls (p < 0.001, corrected for extent threshold). In this group, activation inside the PFC was not different at M12 relative to baseline (p < 0.001, corrected for extent threshold). In contrast, MS patients with reduced PASAT scores between M0 and M12 did not show at M12 larger activation inside the PFC, but an increase in activation inside the temporal and the occipital regions relative to controls (p < 0.001, corrected for extent threshold). Moreover, relative to baseline, the PFC was less activated at M12 (p < 0.001, corrected for extent threshold). Interpretation: Various studies in healthy subjects have suggested that working memory score is strictly related to activation inside the PFC that mediate executive processes. In patients with maintenance of WM capacity, the role of the PFC has appeared predominant and could be related to the efficiency of the compensatory processes. In contrast, in patients with decreased WM capacity, less specialized regions have appeared to be highly recruited at the expense of the PFC recruitment, that could potentially explain the degradation of effectiveness in adaptive cerebral plasticity.

PS87

Clinical validation of MSdiagTM test for predicting multiple sclerosis activity

P. Villolosada, J. Sepulcre, B. Duque, J. Goti, B. Fernandez, C. Lynnais, M. Geffard; University of Navarra (Pamplona, E); Gencalho SA (Cenon, F)

Background: The MSdiagTM test assess a set of 34 circulating antibodies directed against fatty acids, acetylcholine-like, azelaic acid, malondialdehyde residue, NO-modified amino-acids and enterobacterial antigens by ELISA (M Geffard, J Neuroimmunol 1996). These set of antibodies have been described to be differentially expressed in different MS disease course. Objective: To assess the association between MSdiagTM test and disease activity in a cohort of MS patients. Methods: We study a cohort of 61 MS patients with early to medium disease duration (22 CIS, 28 RRMS, 5 SPMS, 6 PPMS), low-medium disability (EDSS: 2.0 (0–7.0); MSSS: 4.6; MSFC: 0.64+1.68) and a relapse rate in the 2 previous years of 1.43. Patients were followed every 3 months for 2 years. The MSdiag test was performed every 3 months in a blind manner. A 3D MRI with gadolinium study was performed at baseline. Results: Two patients were lost for follow-up. Relapse rate during the follow-up was 1.23, and 26 patients were relapse-free. The increase in the EDSS by the end of the study was 0.41±0.7 and 12 patients had confirmed progression (1 point increase
in the EDSS confirmed at 6 months). Twelve CIS patients converted to RRMS and 5 RRMS patients developed SPMS. We found moderate correlation between MSdiagTM subtest directed against enterobacterial antigens and disability (EDSS and MSFC) at the end of the study: 1) the rise of antibodies 5M, 16M, 12M, 19M and 5A was associated with disease progression; 2) we found a moderate correlation between 16M and 5M antibody titers and EDSS change (confirmed at 6 months). The rise of antibody 16M was associated with the conversion from CIS to RRMS; and the increase of antibody 17A titers was associated with being non relapse-free by the end of the study. None of the tests correlated with the number of relapses in the follow-up, disease subtype at baseline, response to immunomodulatory therapy or gadolinium enhancing lesions. **Conclusions:** The assessment of several antibodies in the MSdiagTM test might be useful to identify patients with active disease.

**PS88**

**MRI in Leber’s optic neuropathy with multiple sclerosis (Harding’s disease)**

W. Kaker, A.F. Weir, G. Quaghebeur, J. Palace; Radcliffe Infirmary (Oxford, UK); University Department of Clinical Neurology (Oxford, UK)

Leber’s hereditary optic neuropathy (LHON) is caused by a number of mutations of mitochondrial DNA. In addition to bilateral loss of central vision, multi-focal neurological symptoms indistinguishable from multiple sclerosis may occur (LHON-MS). In such cases MRI changes similar to those found in MS are often seen. We report two patients with mitochondrial gene mutations typical of LHON (11778 and 3460 mutations) who otherwise fulfill criteria for multiple sclerosis. The imaging appearances in both cases consisted of mainly periventricular T2-lesions which were however distinct from typical MS plaques in size, morphology and signal intensity. Absence of T1 hypodensities, juxta-cortical lesions, gadolinium enhancement and corpus callosum involvement may be further distinguishing features. Further work with larger numbers of patients may establish imaging features suggestive of LHON-MS.

**PS89**

**Using Virtual Reality to assess memory abilities of patients with multiple sclerosis**

A. Miller, A. Dinerman, P.L.T Weiss, R. Kizony, A. Rizzo, N. Josman; Carmel Medical Center (Haifa, IL); University of Haifa (Haifa, IL); University of Southern California (California, USA)

Among the cognitive disabilities recently described in patients with Multiple Sclerosis (MS) are decline in memory, attention and visual spatial abilities, which may appear separately or together. Virtual Reality (VR) has been used as an assessment and intervention tool in cognitive rehabilitation with patients who have traumatic brain injury and who have left lateral spatial neglect due to stroke. The objectives of this study were (1) to investigate the potential of using a virtual environment, the Virtual Office (VO), to characterize short and long memory test in participants who have different types of MS and (2) to demonstrate the flexibility of using the VO to test conditions such as relevant versus non-relevant stimuli. Twenty-six patients with MS (21 females and 5 males) with a mean age of 42.6 years (SD = 13.6) were tested. The mean time since disease onset was 9.4 years (SD = 6.5). Twenty-six healthy participants (8 females and 18 males), with a mean age of 26.6 years (SD = 3.24) were also tested. The Virtual Office, developed by A.A. Rizzo (University of Southern California), was used to assess memory. It consists of a simulated office environment wherein eight objects belonging in an office (e.g., computer) and eight objects not typically found in an office (e.g., dog) are displayed. The participants wore a Head-Mounted Display (HMD) through which they observed objects within a virtual office from a first-person point-of-view for a period of 90 seconds. The HMD was then removed and the participants were asked to list the items they saw in the office (Short Term Memory). A demographic questionnaire was administered, and after 20 minutes, participants were again asked to recall the observed items (Long Term Memory). Significant differences were found between memory performance of MS (M = 9.76) and healthy (M = 14.44) participants in the Virtual Office test (t = 5.96). Participants significantly remembered more items which are not relevant to the office than those that are typically found in an office, both for STM (t = 6.29) and for LTM (t = 6.97). This study revealed differences in memory abilities between MS patients and healthy people when tested via the VO. The flexibility of including a variety of different stimuli and in administering the VO under different test conditions points to the potential of this technology for evaluation of cognitive function of people with MS.

**PS90**

**Functional handwriting abilities and Activity of Daily Living performance among multiple sclerosis patients**

S. Rosenblum, A. Miller, P.L.T. Weiss; University of Haifa (Haifa, IL); Carmel Medical Center (Haifa, IL)

**Background:** This study addresses the ongoing challenge of developing sensitive and objective performance-based measures for people with Multiple Sclerosis (MS). Most tools used for this purpose are questionnaires, based on individuals’ reports of their own abilities. One of the most common activities for daily living (ADL) performed by adults in a variety of settings is handwriting. **Objectives:** To evaluate functional handwriting performance through the use of integrated spatial, temporal and pressure measures, and to identify the relationships between measures of handwriting and ADL performance. **Method:** Forty outpatients with clinically defined and laboratory supported MS, according to the Poser criteria, aged 18–65 years, and 20 age and gender matched healthy subjects participated in the study. For the MS group, inclusion criteria included a disability score ranging from 0–5 on the Expanded Disability Status Scale (EDSS). Participants completed the 72-item Daily Living (DL) and the Fatigue Severity Scale (FSS) Questionnaires. They then performed four functional writing tasks and copied the Rey complex figure on a WACOM Intuos II digitizer, which was a part of a computerized system (Pennmanship Objective Evaluation Tool (POET)). Spatial, temporal and pressure measures were sampled during each task and then analyzed offline. **Results:** Significant differences were found between the two groups for temporal measures when participants wrote their own names, as well as for the DL questionnaire score. More than 30% of the MS participants had difficulty in various cognitive tasks as well as in tasks require organization in space and time. Significant correlations were found between the temporal measures of handwriting performance and the total DL questionnaire score. The highest correlation was found between the total copying time of the Rey complex figure and the total DL questionnaire score (r = 0.70, p < 0.01). Moreover, significant moderate correlations were found between the total score of the DL questionnaire and both the FSS (r = 0.42, p < 0.01), and EDSS (r = 0.41, p < 0.05) scores. **Conclusion:** These results are the first step toward demonstrating the added value of using both objective handwriting analysis techniques and the DL questionnaire in order to evaluate everyday functional performance among MS out-patients. The presentation will focus on a comparison of the relative attributes of both measurements for the evaluation of disability level among patients with MS.

**PS91**

**Co-occurrence of herpes simplex virus-1 and human herpes virus-6 DNA in the cerebrospinal fluid of a CIS patient**

M.T. Ferro’, D. Franciotta, E. D’Adda, T. Riccardi, S. Sala, P. Cinque; Center of Multiple Sclerosis, “Ospedale Maggiore” (Crima, I); Neurological Institute C. Mondino (Pavia, I); San Raffaele Scientific Institute (Milan, I)

**Background and Objective:** Herpes viruses have been suggested to be implicated in the pathogenesis of multiple sclerosis (MS). Herpes
Simplex virus-1 (HSV-1) can possibly trigger MS relapses, as it has been detected in peripheral blood mononuclear cells of patients with acute disease. Human herpesvirus-6 (HHV-6) DNA has been recently amplified from brain tissue and cerebrospinal fluid (CSF) of MS patients and an immune response to HHV-6 has been demonstrated in the early MS. We describe a patient with clinically isolated syndrome (CIS), in whom high doses of HSV-1 and HHV-6 DNA have been detected in the CSF, leading to acute diplopia with paraparesis two weeks after an episode of hyperpyrexia and cutaneous herpes of right hemi-thorax (C8-T1). No cephalalgia or urinary disturbances were reported. Medical history of patient was unremarkable, except an asymptomatic Wolf-Parkinson-White syndrome. Brain and spinal-cord MRI showed multiple T2-hypointense lesions involving periventricular white-matter, corpus callosum, cervical (C1-C3) and dorsal (DS-D6) cord regions. Some of brain and both spinal cord lesions enhanced following iv gadolinium.

CSF examination revealed 4 lymphocytes/mm³, numerous oligoclonal IgG bands (OCBs) by IEF, and elevated copy numbers of HSV-1 DNA (26790 c/mL) and HHV-6 DNA (58500 c/mL) by real-time PCR. EEG and bilateral BAER were both normal. VEP showed high latency of the left P100. High-dose iv methylprednisolone and acyclovir (10 mg/Kg tid) were administered, followed by progressive clinical improvement. After three months, MRI showed lack of enhancement of brain and spinal cord lesions, and disappearance of DS-D6 alterations. Six months after the onset, the patient was completely asymptomatic, with the exception of a post-herpetic hemi-thorax neuralgia. CSF examination revealed persistence of OCBs, absence of HSV-1 DNA, and decreased levels of HHV-6 DNA (676 c/mL). Conclusions: The association of cutaneous herpes manifestations with neurological symptoms in our patient led us to search for herpes viruses in the CSF. Although the finding of HSV-1 in the CSF is usually associated with herpetic encephalitis, clinical and radiological picture was atypical in our patient and consistent with MS-related CIS. Further studies by updated bio-molecular techniques are warranted to ascertain whether detection of CSF HSV-1, with or without HHV-6, is an exceptional finding, or an MS, although rare, feature.

P592

High-resolution proton magnetic resonance spectroscopy of cerebrospinal fluid in multiple sclerosis: results of 25 patients

F. Reis, L. Figueiredo, F. Cendes, A. Marsaloti, M. Ferreira, V. Zanardi, A. Faria, C. Brandão; Unicamp-Universidade Estadual de Campinas (Campinas, BR)

The application of 1 H magnetic resonance spectroscopy in cerebrospinal fluid (CSF) analysis may detect metabolites in normal and pathological conditions. The purpose of this work was to evaluate what biochemical changes were related to multiple sclerosis (MS) and establish whether the CSF composition in MS patients reflects metabolic changes occurring in demyelinating plaques. Methods: CSF samples obtained by lumbar puncture in 25 MS patients (clinically definite), 8 male and 17 female, with mean age of 36 years; patients with idopathic polyneuropathy and meningitis, were used as controls (12 patients). The study was approved by our Ethics Comitee and all individuals gave informed consent. A portion of each CSF sample was stored until NMR analysis, at which time 0.5 ml CSF were added to 0.1 ml of 0.75 mM sodium 3-trimethyl-silylpropionate-2,2,3,3-d (TSP), chemical shift reference (0.0 ppm), in D2O. A Varian INOVA-500 (11.7 T) spectrometer, operating at 499,886 MHz, was used. All spectra were treated prior to the multivariate statistics and pattern recognition by adjusting the TPS peak to the same height and by correcting the spectrogram shift and base line. Metabolite concentra- tions in CSF from the patients with MS and controls were determined by integration of isolated peaks measured relative to TSP, correcting for the relative number of protons. Results: We found increased levels of acetooacetate; glutamine/glutamate and beta-hydroxybutyrate in MS patients, compared to controls. Conclusions: Alanine, threonine, valine, leucine and isoleucine account for 40% of the myelin proteolipid protein and for 20% of the myelin basic protein. Acetoacetate and beta-hydroxybutyrate result from the degradation of these aminoacids, which are related to the breakdown of myelin, may result of oligodendrocyte pathology. During inflammation, lymphocytes, microglia and macrophages release excessive amounts of glutamate; Astrocytes impairment of glutamate uptake may contribute to excitotoxic damage of oligodendrocytes.

P593

Pattern pulse vs. frequency doubling illusion. Sensitivities and specificities in optic neuritis patients

R. Riseckaite, T. Maddess, A.C. James; Centre for Visual Sciences (Canberra, AUS)

The goal of our study was to compare the diagnostic capabilities of Pattern Pulse (PPVEPs) and Frequency Doubling (FD) Illusion (FDVEPs) based multifocal visual evoked potentials in Normal subjects, Multiple Sclerosis (MS) and Optic Neuritis patients. 27 Normal subjects and 30 MS patients, 26 of whom had experienced Optic Neuritis (ON+) participated in our study. The patient groups were matched for length of disease and number of clinical attacks. All subjects had refraction corrected to 6/9 or better. The recordings were obtained dichoptically stimulating 8 regions/eye. Contrast threshold testing was performed using a FD technology perimeter, consisting of low spatial frequency (0.25 cpd) sinusoidal gratings that underwent rapid (25 Hz) counterphase flicker. The FDVEPs stimulus was presented at 95% contrast to each location. PPVEPs were recorded to the pattern pulses (the small chequerboards being presented transiently but infrequenly against a neutral grey background) of one video frame at 1.3 presentations/s. Compared to the responses of Normal subjects those of MS patients had significantly smaller response amplitudes, lower signal to noise ratios, more complex response waveforms and longer response delays. To estimate sensitivities and specificities for the different stimulus types we used the receiver operator characteristic plots (ROCs). Bootstrap estimates of the accuracies of the ROCs for the PPVEPs template delays indicated 92% sensitivity at a false positive rate of 0% in ON+ patients. The results were similar for patients with no history of ON. The accuracy of the classification model based upon the FDVEPs was poor in ON+ patients: the model performed at a specificity of 62% for a sensitivity of 63%. This finding suggests that PPVEPs has better diagnostic capabilities than FDVEPs.

P594

Monocular and binocular multifocal visual evoked potentials in normal and multiple sclerosis subjects

R. Riseckaite; T. Maddess, A.C. James; Centre for Visual Sciences (Canberra, AUS)

Purpose: To compare monocular and binocular Pattern Pulse multifocal visual evoked potentials (mPVEPs) in Normal subjects and Multiple Sclerosis (MS) patients. Methods: Monocular and binocular multifocal VEPs were concurrently recorded from 19 normal subjects and 50 MS patients: 26 of whom had optic neuritis (MSON) and 24 of whom had no visual symptoms (MSNON). We employed multiple regression to examine the differences between monocular and binocular viewing. We examined the first response negativities (C1), positivities (C2) their implicit times C1T and C2T and fitted delays. Results: Binocular mPVEPs waveforms predictably had larger amplitudes than monocular ones, but they were also smaller in MSNON and MSON patients. Monocular and binocular responses were both delayed in the patients, but there was no significant difference between monocular and binocular latencies. We also found, that the binocular delays were intermediate between the best and the worst Comments: mPVEPs recorded to the Pattern Pulse stimulus in binocular viewing condition have larger amplitudes, but their laten- cies do not differ from the latencies in monocular responses.
A panel of anti-glycan IgM antibodies for predicting the development of relapsing-remitting multiple sclerosis after the first neurological event

M.S. Freedman, A. Miller, M. Schwarz, O. Weissshaus, R.T. Alstock, A. Dukler, N. Dotan, C. Sindic; University of Ottawa (Ottawa, CAN); Carmel Medical Center (Haifa, IL); Glycominds Ltd. (Lod, IL) Cliniques Universitaires (Brussels, B)

Background: There is an unmet need to develop specific serum based biomarkers for the diagnosis and prognosis of Relapsing Remitting MS (RRMS). We have reported that elevated levels of serum anti-Glc(alpha1,4)Glc(alpha) (GAGA4) IgM antibodies (Ab) exist in RRMS patients in comparison to patients with other neurological diseases (OND) enabling to discern which post-CIS patients convert to RRMS vs. OND. We have further investigate whether other anti glucose based IgM Ab may improve on the RRMS prediction for CIS patients. Aim: To evaluate the predictive value of IgM Ab against Glc(alpha1,6)Glc(alpha) (GAGA6), alpha-GlcNAc (GNa), and GAGA4, for identifying patients with CIS that will evolve to RRMS or will have a more active disease. Methods: Retrospective analysis on of 88 frozen sera from CIS patients presenting for diagnostic work-up and were followed for a minimum of 4 years, Forty four patients were subsequently confirmed to have RRMS, whereas the other 44 developed OND (other inflammatory (OND), n = 23, or non-inflammatory neurological disease (ONIND), n = 21). The groups were matched for gender composition, age, and total IgM. Sera were diluted 1:1200 and levels of GAGA6, GAGA4 and GNa IgM Ab measured by enzyme immunoassay normalized to IgM levels. Results: Significantly higher levels of anti-GAGA6 IgM (p = 0.01) and anti-GAGA4 IgM (p = 0.005) Ab were observed in CIS patients who converted to RRMS as opposed to OND. Using the OND sample set and a cut-off of mean + 2SD for anti GAGA6 and GAGA4, we have found that 17/44 (39%) converting CIS patients were positive, whereas 42/44 (95%) OND patients were negative for both Ab, corresponding to a sensitivity of 39%, a specificity of 95%, PPV of 89%, and NPV of 61%. In addition, higher levels of anti-GAGA4 and anti-GNa Ab (>median) predicted a greater number of future attacks. RRMS patients with levels>median vs. patients with lower levels (<median) of anti-GAGA4 and anti-GNa IgM antibodies went on significantly (16/20 (80%) vs. 10/21 (47%), and 17/20 (85%) vs. 9/21 (43%), (t test, p = 0.025) odds ratio 4.4 (CI 95% 1.6 - 11.8), and odds ratio 7.5 (CI 95% 2.4 - 23.8), respectively to have further attacks within 2 years. Conclusion: Measuring Anti-GAGA4 together with Anti-GAGA6 IgM yields higher sensitivity (39%), specificity (95%) and PPV (89%) of CIS patients evolving to RRMS. In addition higher levels of IgM antibodies to the GAGA4 and GNa epitopes predicts at CIS which patients will have imminent attacks.

The application of transcranial magnetic stimulation in multiple sclerosis, evaluation of the relation of EDSS and motor-evoked potentials

J. Agaoglu, N. Kale, G. Onder, C. Emir, O. Tanik; Okneydani Training Hospital (Istanbul, TR)

Background: The technique of transcranial magnetic stimulation (TMS) allows the investigator to stimulate the cortex of the brain magnetically through the skull, resulting in an induced electrical discharge in the cortex.Axonal loss and damage are responsible for the persistent functional deficits found in multiple sclerosis (MS).An axonal hypothesis of disability can be tested by defining the relationship between axonal pathology and persistent neurologic dysfunctions.In this study, we tried to assess the relation of EDSS and motor-evoked potentials in patients with MS using TMS. Method: In this study, 50 patients,(37 females, 13 males) and 51 controls(35 females, 16 males) were entered the study. As for the inclusion criteria, patients who had not suffered from an attack for the last 6 months or received pulse therapy were included. The mean age of the patient group was 32, females were aged between 14–47 years, and males were aged between 24–52 years. The control group and the patient group matched in terms of age. The patient group was evaluated by routine neurological examination. Overall disability was assessed using the expanded disability status scale (EDSS).The patients were assessed for their motor evoked potentials with the application of TMS. The patients were positioned and the circular shaped coil was targeted to the coordinates of the patient’s hand area of the motor cortex.The recordings were performed from nervous medianus, Erb point, 7th cervical process and the hand area of the motor cortex.Latency and amplitudes of the evoked potentials were assessed. Results: EDSS evaluation of the patient group was between 1– 5.5 points. The mean EDSS was 3. When latency and amplitudes were evaluated, decrease in amplitudes and prolongation in latency was evaluated in SPMS patients which seemed to be in correlation with high EDSS scores.This study is still underway.

Differentiating clinical and paraclinical features of the earliest multiple sclerosis

U. Rot, A. Meser, A.I. Horvat Ledinek, S. Šega Jázbicz; Medical Centre (Ljubljana, SVN)

Effective therapy in the earliest stages of multiple sclerosis (MS) demands early correct diagnosis. The aim of our study was to find the clinical and paraclinical profile of the earliest MS by comparison of clinical, MRI, CSF and electrophysiological characteristics of patients with clinically isolated syndrome (CIS) suggestive of MS and patients with relapsing-remitting (RR) MS. Retrospective analysis included 194 patients (139 women) with a median age 36 years, median duration of the disease 2 years and median EDSS score 3. Among them 47 patients had CIS (30 isolated spinal cord syndrome, 8 isolated brainstem syndrome, 3 isolated hemispheric syndrome, 2 optic neuritis, 4 polysymptomatic presentation) and 89 RR MS at the diagnosis. The diagnosis of MS was established according to the McDonald criteria, patients with CIS had either brain MS-like MRI lesions or positive CSF oligoclonal bands. Patients with CIS were younger (p < 0.0001), had shorter duration of the disease (p < 0.0001) and lower EDSS score (p = 0.02) than patients with RR MS. They were more likely to have predominant sensory symptoms (p = 0.0045) but less likely to have predominant cerebellar (p = 0.01) and motor symptoms (p = 0.07) than patients with RR MS. Brain MRI MS-like lesions were found in 84% of CIS patients compared to 99% of RR MS patients (p = 0.002). Modified Barkhof MRI criteria fulfilled 56% of CIS patients and 85% of RR MS patients (p = 0.0003). Patients with CIS had higher CSF cell counts than patients with RR MS (p = 0.04). Oligoclonal bands were present in 79% of CIS patients and 95% of RR MS patients (p = 0.01). Prolonged latencies of visual evoked potentials (VEP) were found in 30% of CIS patients compared to 68% of RR MS patients (p = 0.0008). Patients with the earliest MS often present with less specific sensory symptoms. Brain MRI can be inconclusive in more than 40% of them and VEP are usually normal but elevated CSF cell count and positive CSF oligoclonal bands are helpful in establishing the correct diagnosis.
presenting with a CIS have come into focus due to the concerns about starting long term treatment at the first attack suggestive of MS. The aim of this study is to assess the conversion to definite MS in Turkish patients presenting with a CIS. We included all consecutive patients presenting with a CIS since 2002. Within 4 years a total of 129 patients with a CIS were evaluated. Mean follow up duration was 23 months. Of these, 44 patients presented with a brainstem syndrome, 35 with optic neuritis, 30 with a spinal attack, and 24 with hemisensory or motor syndrome. Dissemination in space was evident radiologically in 54 patients according to Barkhof criteria, and in 6 patients, >2T2 lesions and additional CSF evidence was present, while 10 patients had additional spinal lesions fulfilling the criteria for dissemination in space (Group I, 70 patients). On the other hand, in 46 patients there were abnormal findings in cranial and/or spinal MRI not fulfilling the criteria for dissemination in space (Group II). Finally in 13 patients MRI was normal (Group III). In Group I 25 of 70 patients had a second attack, whereas in Group II 8 of 46 patients, and in group III 1 of 13 patients had a second attack. 124 patients had a follow-up MRI examination; 42 patients had a new lesion on their second MRI. Among these, 24 had a second attack. On the other hand, among 77 patients who did not have a new lesion their second MRI, 6 had a second attack. Sensitivity of MRI positivity was 80%, specificity was 75.5%, positive predictive value was 51.1%, negative predictive value was 92.2%, accuracy was 76.6%. The mean time to the second attack was 15 months, and the mean time for fulfilling radiological criteria (McDMS) was 11 months. 63 patients had CSF analysis: 45 had oligoclonal bands. Among the patients with positive CSF 16 had a second attack, whereas among those that were negative 3 had a second attack. Sensitivity of CSF positivity was 88.2%, specificity was 34.1%, positive predictive value was 35.6%, negative predictive value was 83.3%, accuracy was 49.2%. Our results suggest that although MRI positivity is highly predictive for developing definite MS, CSF findings could also be supportive, and should not be completely left out of the diagnostic procedure.

**P599**

**Serum uric acid levels are decreased in patients with relapsing-remitting multiple sclerosis, in particular during relapses**

A. Tsakiri, J.L. Frederiksen; University of Copenhagen Glostrup Hospital (Glostrup, DK)

**Background:** A few studies indicate that patients with Relapsing Remitting Multiple Sclerosis (RRMS) have low serum levels of the endogenous antioxidant uric acid (UA). The object of this study was to examine if patients with RRMS have decreased serum levels of uric acid and if the value of UA changed during relapses. We correlated the value of UA to the results of CSF examinations and MRI of the brain performed at the time of diagnosis of RRMS.

**Design/Methods:** In a population-based study we included 300 consecutively examined patients (190 f, 110 m) with RRMS with a mean age of 35 years. The patients were treated with interferon-beta (260 patients) and copaxone (40 patients) and had blood examinations routinely every 6 months during treatment. In addition we measured serum UA when they presented with a relapse. The serum reference value from our laboratory was 15–35 for women and 20.0–45.5 Mm for men. Conventional methods were used for CSF and MRI examinations.

**Results:** Sixty patients (20%) (45 f, 15 m) had decreased a serum level of UA below normal values. The mean serum UA was lower in patients than in healthy controls. During relapses, (relapse rate 0.3 relapses per year) the values tended to be lower than outside relapses.**Conclusions:** The results of this large population based study of patients with RRMS support the hypothesis that serum levels of UA are decreased in this group of patients. The serum level of UA tending to be lower during relapses, indicating that UA serum may be a marker of disease activity in RRMS. The serum levels of UA are being related to the results of CSF and MRI examination.

**P600**

**Thrombophilia screening in patients with multiple sclerosis**

T. Afrantou, A. Angelou, R. Lagoudaki, E. Stavridou, M. Gelagoti, A. Halati, K. Dimitrakopoulos, A. Kazeptidou, M. Paschallidou, I. Milonas; Aristotle University of Thessaloniki (Thessaloniki, GR); 1st General Hospital Ag. Pavlos (Thessaloniki, GR)

**Introduction:** Multiple sclerosis is a chronic autoimmune inflammatory disease with unpredictable course of unknown aetiology. Various studies have evaluated the role of coagulation indices in multiple sclerosis. There is an aspect that components of haemostasis are impaired in patients with MS but it is not clear in which way. The patients are immobilized at later stage of disease and may be at higher risk for impairment of coagulation system with predisposition to hypercoagulated state. Therapies which usually are used in immune mediated neurological diseases like corticosteroids and immunoglobulins seem to be safe, having minor adverse effects. However, vein thrombosis may occur either during corticotherapy after lumbar puncture involving the cerebral veins or receiving immunoglobulins as adverse effect with deep vein thrombosis manifestation. **Objective:** The aim of this study is to establish the prevalence of thrombophilia using a screen in patients with MS. **Patients and Methods:** 44 patients with MS diagnosis according to McDonald’s criteria were examined, 6 males and 38 females, with mean EDSS 3.7. 29 patients suffered from relapsing-remitting MS and 15 patients suffered from secondary progressive MS. In our study we evaluated Protein C, Protein S, antithrombin III, Activated protein C resistance, Fibrinogen and Lupus anticoagulant. **Results:** Increased levels of Fibrinogen were detected in 12 patients (27.3%). The Lupus anticoagulant was negative in all patients. We detected protein S deficiency in 2 patients (4.5%) whereas no protein C deficiency was detected. Antithrombin III levels were decreased in 4 patients (9%). The testing for Activated protein C resistance showed normal levels for all patients. **Conclusion:** The 12 patients that had high levels of fibrinogen were either in a relapse state or at the onset of the disease, suggesting that the fibrinogen levels might serve as an inflammatory factor. Although fibrinogen has important haemostatic properties and it is a well known marker of hypercoagulation, none of our patients experienced a thrombotic event. Low levels of protein S and antithrombin III were detected in a few patients which experienced a relapse with mean EDSS 4. This deficiency could not be attributed to a common cause, such as liver diseases or peripheral vein thrombosis. On the other hand these values could be explained by the inflammatory process present during the relapse and the early stages of the disease.

**P601**

**Oxidised low density lipoprotein in serum of relapsing-remitting multiple sclerosis patients**

A. Sena, R. Pedrosa, R. Roque, M.A. Tavares, M.J. Cascais, V. Ferret-Sena, C. Araújo, M.G. Morais, R. Coudert; Faculdade de Ciências Médicas (Lisbon, P); Hospital dos Capuchos (Lisbon, P); Instituto Superior de Ciências da Saúde Egas Moniz (Lisbon, P); Hôpital Trousseau (Paris, F)

**Background:** Oxidative stress is thought to play a major role in the pathogenesis of multiple sclerosis (MS). Plasma oxidized low density lipoprotein (oxLDL) is found in activated microglia and macrophages in very early lesions of MS. Increasing evidence suggests a potential role of oxLDL in mediating inflammatory and neuronal toxicity processes underlying MS pathogenesis. **Goals:** To analyse serum oxLDL levels in relapsing-remitting (RR) MS patients and its eventual associations with clinical disease activity and immunomodulatory treatments. **Methods:** 25 patients (23 females and 2 males) with definite RR-MS (32.44±8.52 years) and 15 normal controls matched for age and sex were studied. In 17 patients, serum oxLDL was determined before and 6 months after the beginning of interferon beta 1b (Betalferon) or glatiramer acetate (Copaxone) therapy. In 8 patients, oxLDL was analysed in a remission condition and during a clinical relapse. Serum oxLDL was determined by ELISA (Mercodia)
and for statistical analysis ANOVA and Student’s test were used.

**Results:** Untreated MS patients had higher serum oxLDL levels (69.41 ± 17.75 U/L) in comparison to normal controls (39.60 ± 9.07 U/L) (p = 0.018). Serum oxLDL decreased about 20% after 6 months of therapy (p = 0.007, test for paired values). Serum oxLDL levels during relapses (61.25 ± 12.01 U/L) were higher than in remissions (46.13 ± 10.02 U/L) (p = 0.005). A trend to higher oxLDL levels in smokers was observed (p = 0.06).

**Conclusions:** This study supports a role of oxidative stress in MS pathogenesis. Our results suggest an association between serum oxLDL and disease activity that could be favourably influenced by immunomodulatory treatments. These data should be confirmed in a larger sample of patients and prospective studies.

Supported by Schering Lusitana and Sanofi-Aventis.

**P602**

**Correlation between urinary levels of 6-sulfatoximelatonin and clinical aspects of multiple sclerosis**

C. Tillbery, G. Kelam, M. Peres, R. Thomaz; Santa Casa de Misericórdia de São Paulo (São Paulo, BR); Albert Einstein Hospital (São Paulo, BR)

**Introduction:** Multiple sclerosis (MS) is more common in Caucasians individuals of the north of the Europe than in African blacks. The solar incidence in these regions is well distinct and the melatonin secretion could be involved in the pathophysiology of MS. The melatonin is a hormone with function to transform information about circadian cycle to biochemical signals which modulate the organization time-dependent of anatomical functions, neuroendocrine and behavioral. It is only produced during the absence of light and exerts effect on the immunological system. Constantinescu, in 1995, showed the possible influence of melatonin secretion in MS on autoimmune and its influence in adaptation of external factors within internal environment. The light is a strong suppressor of melatonin secretion, and also its actions in immunological system.

Until now the real influence of melatonin in MS was not established.

**Objective:** Study the nocturnal urinary levels of melatonin in MS patients and controls.

**Method:** 43 patients with diagnosis of Multiple Sclerosis according to McDonald criteria (2001), age varying between 18 to 59 years old, and 43 controls, matched by gender and age.

A nocturnal urinary sample (12 hours period) was collected and we analyzed 6-Sulfatoximelatonin (6-SM) dosage, by ELISA.

**Results:**

- The urinary levels of 6-SM in MS patients were 40.16 ± 27.29 ng/mL, and in controls 27.17 ± 21.43 ng/mL (p = 0.026).
- We detected an increase in melatonin levels during relapses (Wilcox on Signed Rank Test), varying from 10.76 to 56.8 ng/mL (median = 63.1) after relapses (p = 0.036).
- Anyhow correlation was found between melatonin levels and gender, age, MS type or disability.

**Conclusion:** We concluded that MS patients have higher urinary melatonin (6-SM) levels than controls and these levels increase during relapses.

**P603**

**Chitotriosidase cerebrospinal fluid index correlates with actual disability and disability after 3 years in secondary progressive multiple sclerosis**

P.J.H. Jongen, E.A. Notting, B. Faas, R. Claessens-Linskens, M.M. Verbeek; Multiple Sclerosis Centre Nijmegen (Nijmegen, NL); Radboud University Nijmegen Medical Centre (Nijmegen, NL)

**Introduction:** Chitotriosidase (CTTS) is produced by chronically activated macrophages and microglia. In multiple sclerosis (MS) these cells play a major role in pathogenesis. We performed an exploratory analysis of CTTS activity in serum and cerebrospinal fluid (CSF) in various types of MS.

**Patients and methods:** 196 patients were studied comparing to the group of non-inflammatory neurological disorders (NIND), secondary progressive MS (SPMS) (n = 24), primary progressive MS (PPMS) (n = 20), Possible MS (n = 43) and no MS (n = 29). Expanded Disability Status Scale (EDSS) score was determined at time of lumbar puncture (LP) and at follow up. CTTS activity was assessed by a conventional enzyme assay. CTSS index was 1) compared between groups and with controls (non-inflammatory neurological disorders, NIND), 2) per group between patients with and without CSF signs of inflammation/immune activation (mononuclear cell count [MNC], immunoglobulin G [IgG] production, and oligoclonal IgG bands [OCB]) and 3) per group correlated with EDSS scores at LP and at follow up.

**Results:** For RRMS, SPMS and PPMS mean age at LP was 38.7, 42.3 and 48.3 years (yrs), mean disease duration at LP 5.6, 12.7 and 10.5 yrs, and follow up period 2.4, 3.1 and 2.4 yrs, respectively. Mean EDSS scores at LP for these groups were 3.0, 4.7 and 4.9 yrs, at follow up 3.2, 5.2 and 5.5 yrs, respectively. CSF CTTS activity was significantly higher in patients with MS, whereas serum CTTS activity did not differ between patient with MS, possible MS, non-MS and NIND. Both in RRMS and SPMS patients the CTSD index was increased compared to controls: 0.10 vs. 0.10 ± 0.02. In RRMS-not in the SP/PPMS group-the CTSS index was significantly higher in patients with increased CSF MNC or IgG index.

In SPMS-not in RRMS or PPMS-the CTSD index was positively correlated to the EDSS score, both at time of LP (r = 0.62) and at follow up (r = 0.62).

**Conclusion:** The CTSD index is elevated in MS. RRMS patients with CSF signs of inflammation/immune activation have a higher CTSD index than those without. In SPMS patients the CTSS index shows a marked positive correlation to EDSS score, both at time of LP and at 3 yrs follow, a finding suggesting that the CTSS index is a potential marker for disease activity and prognosis in SPMS.
memory test than controls but they showed preserved circadian profile secretion and response to a cognitive task.

Imaging – Part II

P605

MRI predictors of short-term disability in early multiple sclerosis patients
M. Deloire, E. Salort-Campana, M. Bonnet, K.G. Petry, J.C. Ouallet, V. Douset, B. Brochet; University V. Segalen (Bordeaux, F)

Background: Lesion load (LL) on MRI is moderately correlated with disability outcome in MS. Predictive value of other MR parameters has still to be established. Objectives: To assess the predictive value of various MR parameters on short term disability at early stages of MS.

Methods: A population based sample of 69 consecutive patients have been recruited by the AQUISEP network less than six months after a diagnosis of MS. 68 participated to this study, 57 with RRMS and 11 with progressive MS. They had 4 yearly clinical evaluations (EDSS and MSFC). MR parameters measured at baseline, year one and two, included LL, brain parenchyma fraction (BPF), ventricular fraction (VF), mean magnetisation transfer ratio (MTR) on lesion and normal appearing brain tissue (NABT) masks.

Results: In RRMS patients: EDSS and MSFC remained stable over 3 years. On 3-year follow-up, MRI lesion parameters either deteriorated significantly (T2 LL, p < 0.001) or remained stable (mean lesion MTR). However, all MR parameters reflecting diffuse brain involvement deteriorated during the first year: BPF (p < 0.001), VF (p < 0.002), mean NABT MTR (p < 0.001) but remained stable during the second year. Mean lesion MTR at baseline correlated with EDSS change over 3 years (r = −0.31, p = 0.03) and baseline BPF with MSFC change over 3 years (r = 0.36, p = 0.01). In progressive MS: EDSS deteriorated significantly (p < 0.01). BPF (p < 0.05) and mean NABT MTR (p < 0.01) deteriorated significantly during the second year. Mean baseline NABT MTR correlated strongly with EDSS (r = −0.81, p = 0.007) and MSFC change over 3 years (r = 0.95, p < 0.001). LL at baseline correlated with EDSS change throughout 3 years (r = 0.7, p = 0.03). Conclusion: In early progressive MS, diffuse brain abnormalities may be useful predictors of short-term disability outcome. In mostly clinically stable RRMS patients, lesions severity and atrophy were modestly predictive of disease progression. This work is supported by grants of ARSEP and Schering France SA.

P606

Ring-enhancing lesions in relapsing-remitting multiple sclerosis: insight into tissue injury and repair mechanisms
O. Khun, A. Boster, M. Mackenzie, J. Perumal, C. Cuon, F. Bao, Z. Lutfi, A. Tselis; Wayne State University School of Medicine (Detroit, USA)

Objective: To investigate the mechanisms leading to disappearance of gadolinium enhancement by studying ring-enhancing lesions (REL) in relapsing-remitting MS. Background: Gadolinium enhancement (GdE) visualized on brain MRI scans represents disruption of the blood brain barrier (BBB). It has been suggested that GdE lesions in MS may represent tissue inflammation and is a commonly studied outcome in exploratory phase II studies. GdE lesions are usually homogeneous (HEL) and occasionally ring-enhancing (REL) in appearance, the latter more likely to represent irreversible axonal injury. However, histopathologic data of REL has shown significant remyelinating potential and large REL have been shown less likely to be associated with tissue destruction than small REL. We investigated the behavior of REL in RRMS patients by employing MTR and also proton MRS when possible. Methods: We studied 37 RRMS on no treatment with monthly brain MRI scans. Patients elected to remain untreated because of needle phobia or unable to tolerate available disease modifying therapies. All patients had monthly brain MRI scans for 6 months with additional scans at months 12 and 18 when ever possible. Results: A total of 261 lesions were identified in the first 6 months of which 47 were REL and 214 HEL. 14 of 47 REL were > 10 mm in diameter. 11 of 14 large REL resolved spontaneously without any residual hypointensities on T1W images. 8 of 11 large REL that resolved were examined with MTR at initial presentation and follow up scans. All 8 large REL showed MTR values similar to NAWM and improved on follow up scans. In contrast, 18 of 33 small REL showed MTR values significantly decreased compared to surrounding NAWM and 16 of these lesions remained hypointense on follow up scans. Additional analyses with single VOI MRS examining NAA/Cre were also performed. Conclusions: In contrast to small REL, Large REL appear to have a greater potential for remyelination and repair. This observation sheds light on potential histopathologic differences based on patterns and volume of GdE depicted with in-vivo imaging employing MTR and MRS. This may also provide further insight into substrates of disability and potential of repair in MS.

P607

MRI changes predict response to IFN-beta in relapsing-remitting multiple sclerosis patients

Background and objective: To evaluate whether MRI performed at IFN beta onset and after twelve months allow us to identify RRMS patients with a disability increase in the first two years of therapy.

Methods: This is a prospective and longitudinal study of patients with RRMS treated with IFN beta. All patients included underwent brain MRI before the onset of therapy with IFN and 12 months after. Number and volume of T2 and contrast-enhanced T1 lesions and brain parenchymal fraction (BPF) at baseline and follow-up scans, and number of new T2 lesions at follow-up scans were measured. EDSS was scored every 3 months. We defined increase of disability as the increase of at least 1 EDSS point confirmed and sustained during the first two years of therapy with IFN beta. Regression analysis was performed in order to identify MR variables of response. Results: We included 178 RRMS patients with baseline and 12 months MRI examinations. One hundred and fifty-two patients were followed-up for at least two years. After two years of therapy, 24 patients (16%) had an increase of disability. Patients with a disability increase were comparable in clinical and demographic variables (age, sex, disease duration, EDSS and relapse rate) at baseline. Nevertheless a different MRI pattern at the onset of IFN beta was found. Patients with a disability increase after two years of therapy had a higher T2 and contrast-enhanced T1 lesion volumes at baseline (p < 0.05). BPF was not different among patients. Number of new T2 lesions after 12 months of therapy was the variable with the best value for predicting an increase of disability after 24 months of treatment. Conclusions: In RRMS patients treated with IFN beta the MRI changes occurred during the first year may have a prognostic value for identifying patients with a confirmed increase of disability after two years of therapy.

P608

The evaluation of black hole volume evolution as it relates to lesion load, extent of enhancement, and treatment with intramuscular interferon-beta-1a in two relapsing-remitting multiple sclerosis studies
E.W. Radue, M. Salatradin, A. Pace, R. Hyde; University Hospital Basel (Basel, CH); Biogen Idec (Cambridge, MA, USA); Biogen Idec International GmbH (Zug, CH)

Background: Damage to the CNS and loss of function in relapsing MS patients are triggered by inflammatory attacks. Pathological progression is followed on MRI but the relation to disability is not one to one. The “elocuence” of lesions and plasticity of the CNS may explain this. Chronic T1 hypointense lesions (T1 black holes) are areas
of severe damage to myelin and axons. They have been proposed as a closer correlate of impending disability. **Objective:** The aim of this evaluation is to understand the precursor characteristics for the formation of T1 black holes in MS patients and to further examine the effects of IM interferon beta-1a (IFN-1a) on this specific measure. **Methods:** A retrospective analysis of MRI data from the MS Collaborative Research Group (MSCRG) trial and the dose comparison trial (DCI) were used to evaluate the influence of baseline MRI characteristics on the evolution of black-hole volume. Data were assessed for normality, and correlations were determined based on the Spearman rank statistic. A non-parametric, rank-based regression model was used to identify characteristics that were predictive of change in T1 black holes and to determine the treatment effect of IFN-1a. **Results:** Correlations between baseline T1 volume and both baseline T2 volume and enhancing lesion volume revealed a closer cross-sectional relationship with T2 volume (r = 0.80 for MSCRG and r = 0.84 for DCI) than Gd lesion volume (r = 0.25 for MSCRG and r = 0.21 for DCI); however, all were highly significant. In the MSCRG trial, the baseline number of enhancing lesions was shown to be associated with the change in T1 black holes in placebo-treated patients. There was a significant association whether parametric or non-parametric analyses were employed (p < 0.001). The treatment effect of IFN-1a was previously evaluated without considering enhancing lesions as a covariate. In patients in the placebo group of the MSCRG study, median T1 hypointense lesions increased by 124.5 mm³ over 2 years compared with a median 40 mm³ in the IFN-1a -treated patients. This 68% decrease was statistically significant after adjustment for baseline Gd+ activity (p = 0.045). **Conclusion:** The development of T1 black hole volume is strongly influenced by the number of enhancing lesions. Methodology used to evaluate black holes should always consider this covariate in the analysis.

**P609**

Effect of mitoxantrone therapy on T1 lesion load and atrophy in multiple sclerosis patients

A. Walczak, T. Berkowicz, K. Wartolowska, M. Siger, K. Zaleski, K. Selmaj; Medical University (Lodz, PL)

**Background:** Mitoxantrone (MTX) is an antineoplastic agent with potent immunosuppressive activity that has been approved to slow progression of neurologic disability and reduce the relapse rate in patients with agressive multiple sclerosis (MS). Despite of positive results of clinical trials the MRI data showed that MTX does not clearly reduce the number of Gd-enhanced lesions. In this study we have evaluated the effect of MTX on T1 lesions load and atrophy in MS patients treated with 10 mg/m² of MTX. **Goals of the study:** To investigate development and evolution of T1-weighted lesions, PD-weighted lesions and brain atrophy in MS patients treated with MTX for 18 months. **Methods:** Twenty three patients with worsening relapsing-remitting or primary or secondary progressive MS were assigned for mitoxantrone treatment in the dose of 10 mg/m² every 3 months, to the cumulative dose of 120–140 mg and fifteen control, non-treated with any immunomodulating or immunosuppressive drug. MS patients were analyzed. MRI was performed at the study entry and at the end of the treatment period. MRI parameters used for analysis included: total brain T1-weighted lesion volume (TIV), total PD-weighted lesion volume (PDV) and brain parenchymal fraction (BPF). **Results:** TIV increased by 66.6% in control group whereas in the MTX treated group increased by 43.5%. PDV decreased by 34% in controls and by 29.9% in the MTX group. BPF values were decreased by 0.581% in controls and 0.53% in the MTX group. MTX (10 mg/m²) was generally well tolerated, the short-term side effects observed in this study were mild. No patient experienced symptoms of congestive heart failure or other clinically significant cardiac dysfunction. **Conclusions:** MTX therapy in MS patients decreased the rate of new lesion development observed on T1-weighted images, and decreased development of brain atrophy. The MTX effect on PD-weighted images was moderate and showed positive trend to decrease accumulation of PD-weighted lesion volume.

**P610**

Whole-brain magnetisation transfer imaging changes in relapsing-remitting multiple sclerosis receiving interferon-beta and glatiramer acetate

M. Mackenzie, Z. Latif, C. Caon, O. Khan; Wayne State University School of Medicine (Detroit, USA)

**Objective:** To determine whole brain tissue (WBT) MTR changes in RRMS patients receiving either IFN beta or glatiramer acetate (GA). **Background:** Previous studies have shown no significant effect of IFN beta on WBT MTR in patients with SPMS and RRMS. However, one study reported an increase in mean WBT MTR after one year of therapy with glatiramer acetate and IFN beta-1b in RRMS patients which was significant only with GA. We performed a similar pilot study to examine the effect of IFN beta and GA in previously treatment naive RRMS after one year of therapy. **Results:** Previously treatment naive 10 RRMS started treatment with GA and 12 with IFN beta (250 mcg SC qod or 44 mcg SC TIW). Patients underwent MRI and MT imaging using a standard protocol on a 1.5 T scanner. Both groups were well matched at baseline with respect to clinical demographics and MRI lesion load. Imaging was performed at baseline and one year of therapy. After one year of therapy, compared to baseline treatment with GA significantly increased mean WBT MTR by 1.54% while IFN beta treated group showed a non-significant improvement in mean WBT MTR by 0.94%. **Conclusions:** This study provides evidence that disease modifying therapies may positively influence WBT MTR. Furthermore, it also indicates that the effect of disease modifying therapies on WBT MTR may be independent of effect at the blood brain barrier level. Finally, it also indicates that WBT MTR may be a useful outcome to monitor underlying tissue damage and response to therapy. Larger studies with longer follow up are warranted to validate this MT imaging-based surveillance strategy.
patients, GM average MTR was significantly associated with disability (r = –0.63, p = 0.03). No correlation was found between cord cross-sectional area and GM average MTR. Conclusions: This preliminary MT MRI study suggests that, similarly to what is observed for the brain, cervical cord GM is also not spared by MS pathology. The quantification of cord GM damage might yet be another factor contributing to the locomotor disability associated to this condition.

P612

Evaluation of cortical lesions in patients with multiple sclerosis by multimodal MRI sequences

S. Nollet, Y. Chevalier, F. Cattin, E. Berger, J.P. Arnschpay, J.F. Bonneville, L. Rambuch; University Hospital (Besançon, F); INSERM (Strasbourg, F)

Background: A neurodegenerative component and the presence of cortical lesions have been showed for over a century from post-mortem studies. More recently advanced MRI techniques demonstrated grey matter involvement in Multiple Sclerosis (MS). Detection of cortical lesions in vivo by using MRI is challenging. Their identification would be of great importance and might provide more insight in explaining some clinical signs. Objective: To identify cortical lesions by morphological multimodal MRI sequences in patients with Multiple Sclerosis (MS). Methods: A study has been carried out in 25 patients with relapsing remitting, secondary progressive and primary progressive at different stage of the disease. MS Brain MRI was performed at 3.0 T; protocol included: Fast SE T2-weighted images, FLAIR T2-weighted images, 3D FLAIR T2 sequence and a T1 weighted 3D spoiled gradient (FSFGR). MRI examinations were analyzed (topography and number of lesions) by 2 neuroradiologists and 2 neuropsychologists, to determine the best sequence. These results were matched to the disease duration in one hand and to the clinical impairment in the other hand. Results: Cortical lesions were best demonstrated on 3D FSFGR images. The lesions appeared as small hypointense areas or dark spots in the cortex. 3D FLAIR T2 was less effective: in some cases lesions demonstrated on 3D FSFGR images were not detected on FLAIR T2-weighted images. Lesions are present from the onset of the disease whatever is the topography of MRI lesions is in accordance with the classification of Bo et al. (2003). Conclusion: T1–SPGR is a short duration sequence that allows better in vivo identification of cortical lesions in clinical routine. These results are on going to better quantify the lesions and to evaluate modifications in longitudinal studies and correlation with atrophy and clinical impairment.

P613

Multiple sclerosis patients with high number of cortical lesions are at risk of seizures

M. Calabrese, M. Atzori, V. Bernardi, L. Rinaldi, A. Morra, L. Barachino, P. Perini, P. Gallo; Multiple Sclerosis Center (Padua, I); Euganea Medica, Neuroradiology Unit (Padua, I)

Introduction: Epilepsy is three to six times more frequent in Multiple Sclerosis (MS) than in the reference adult population. Cortical and juxtacortical inflammatory lesions probably constitute the pathological substrate of seizures. However, cortical pathology is not depicted by conventional MR techniques that allow the identification of only 5 to 9% of demyelinated CLs that are visible in post-mortem tissue. The application of a Double Inversion Recovery sequence (DIR) has significantly increased the detection of CLs in MS brain. We applied these methodology to investigate the occurrence of CLs in MS patients suffering from epilepsy. Methods: 20 Relapsing Remitting (RR)MS patients who had seizures during the course of the disease and 50 RRMS without history of seizures were included in the study. The two groups were matched for age, disease duration, EDSS and T2 Lesion Volume (T2LV). They underwent MR examination (1.5 Tesla) including DIR sequence, electroencephalogram (EEG), accurate neurological examination and cerebrospinal fluid (CSF) analysis. Pure intracortical lesions, brain parenchyma fraction (BPF), grey matter fraction (GMF), white matter fraction (WMF), T2LV, and global and regional cortical thickness (CTh) were analyzed. Results: Patients with seizures showed four times more CLs (6.9±8.6) than patients without history of seizures (1.5±1.9), and this was significant (p = 0.025). CLs were observed in 17/20 (85%) of patients with seizures and in only 30/50 (60%) patients without history of seizures (Fischer Test, p = 0.05). The BPF and GMF were significantly reduced in the group of MS with seizures (p = 0.04 and p = 0.05, respectively). Interestingly, the female/male ratio in the MS group with seizures was 0.7 (9/11). Discussion: Our findings suggest that MS patients with more severe cortical pathology, in particular high number of CLs, are at high risk for seizures. This in vivo study confirms the hypothesis that demyelinating lesions in cortex is the cause of seizures in MS. Although a MS relapse is defined as symptom(s) lasting more than 24 hours, seizures – as expression of a demyelinating event- should be regarded as a clinical relapse, and therefore included among the diagnostic criteria for MS.

P614

Benign multiple sclerosis and relapsing-remitting MS differ in cortical pathology

M. Calabrese, V. Bernardi, M. Atzori, L. Rinaldi, L. Barachino, A. Morra, P. Grossi, P. Perini, P. Gallo; Multiple Sclerosis Center (Padua, I); Euganea Medica, Neuroradiology Unit (Padua, I)

Introduction: Although histological and MRI studies have disclosed neocortical and neuronal pathology in Multiple Sclerosis (MS) and suggested a relationship with disability, only a few studies have considered cortical pathology in the benign form of MS (bMS). Our study evaluated cortical thickness (CTh) and the number of cortical lesions (CLs) in bMS. Methods: 30 patients with bMS (i.e., disease duration > 15 years, and Expanded Disability Status Scale (EDSS) score ≤ 3.0, normal neuropsychological evaluation) and 50 RRMS were included in the study. The two groups were matched with regard to gender, EDSS and T2 lesion volume (T2LV). They underwent a MRI examination (1.5 Tesla), including Double Inversion Recovery sequence, accurate neurological examination, and cerebrospinal fluid (CSF) analysis for demonstration of intracerebally synthesiz ed IgG. Pure intracortical lesions, brain parenchyma fraction (BPF), grey matter fraction (GMF), white matter fraction (WMF), T2LV, and global and regional cortical thickness (CTh) were analyzed. Results: Mean age and mean disease duration were significantly higher in bMS group (43.9±7.4 and 18.1±4.3 years (range 15–30), respectively) than in RRMS group (35.2±10.0 and 4.9±3.4) (p < 0.0001 for both parameters). The number of CLs was significantly lower in bMS group (1.3±1.1) than in RRMS group (4.7±6.5; p < 0.001). No significant differences in BPF, GMF, WMF, and mean CTh were observed. Discussion: We demonstrate that bMS are characterized by a significantly lower number of CLs than RRMS patients. Moreover, the lack of difference in brain atrophy (i.e., BPF, GMF and CTh) between bMS and RRMS, in front of a significant difference in disease duration, suggest that bMS is characterized by a less severe neuro-pathological process.

P615

Cortical atrophy is relevant in multiple sclerosis at clinical onset

M. Calabrese, A. Morra, C. Romualdi, V. Bernardi, M. Atzori, L. Rinaldi, M. McAuliffe, R. Reynolds, L. Barachino, P. Perini, B. Fischl, P. Gallo; Multiple Sclerosis Center (Padua, I); Euganea Medica (Padua, I); CRIBI (Padua, I); Biomedical Imaging Research Services Section NIH (Bethesda, USA); National Institute of Mental Health NIH (Bethesda, USA); Artificial Intelligence Laboratory MIT (Cambridge, USA)

Introduction: Although recent studies have shown a cortical grey matter pathology in patients with Multiple Sclerosis (MS), it
is not yet clear how early this phenomenon begins, its impact on clinical disability or which cortical areas are primarily affected.

Methods: 100 consecutive patients (10 Clinically Isolated Syndrome (CIS), 32 possible MS (p-MS), 42 Relapsing Remitting MS (RR-MS), 16 Secondary Progressive MS (SP-MS)), and 40 age/gender-matched healthy volunteers (HV) underwent a neurological examination (Expanded disability status scale, EDSS) and a 1.5T MRI scan. Global and regional Cortical Thickness (CTh) measurements, brain pachyma and T2 lesion load were analysed. Results: We found a significant global cortical thinning in p-MS (2.22 ± 0.09 mm), RR-MS (2.16 ± 0.10 mm) and SP-MS (2.11 ± 0.12 mm) compared to CIS (2.51 ± 0.11 mm) and HV (2.48 ± 0.08 mm). The correlations between mean CTh and white matter (WM) lesion load was only moderate in MS (r = −0.393, p = 0.03) and absent in p-MS (r = −0.147, p = 0.422). Analysis of regional CTh revealed that the majority of cortical areas were involved not only in MS, but also in p-MS. Cortical pathology of the primary motor and visual area strongly correlated with motor and visual disability as evaluated by means of the corresponding Neuro-status Functional Systems. Discussion: Cortical thinning is a diffuse and very early phenomenon in MS, correlates with clinical disability, and is largely independent from WM inflammatory pathology.

P616

Effects of mid sagittal plane selection on corpus callosal area
O. Ishaq, G. Hamarneh, R. Tam, A. Traboulsee, D. Li; Simon Fraser University (Vancouver, CAN); UBC MS/MRI Research Group (Vancouver, CAN)

Background: Atrophy of the corpus callosum (CC) can occur in multiple sclerosis (MS) patients at a faster rate (−4.5% versus −1%) than loss in whole brain volume. It could be useful as another measure of neuro-degeneration. However, reliable identification of the mid sagittal plane (MSP) is difficult due to interhemispheric asymmetry, lack of landmarks and imprecise repositioning. These sources of error may confound the interpretation of changes in CC area. Objectives: To determine the effects of shifting (perturbation) the MSP selection on CC cross sectional area for MS patients. Methods: MR brain volumes of 5 MS patients with 3 scans each were acquired using a 3-D inversion-prepared spoiled gradient echo (SPGR) MRI sequence. Each sagittal slice was a 256 x 256 matrix, re-sampled to an isotropic pixel resolution of 0.97 mm x 0.97 mm x 0.97 mm. 10 sagittal slices containing the CC were segmented semi-automatically and used to generate a 3D volume of the CC. The centre of each volume was selected as reference MSP. The position of a plane embedded in a volume is dependent on its elevation and azimuth angles and its translation from the centre of the volume. 6615 perturbed MSP were generated for each volume by varying the orientation and translation parameters. The range of elevation and azimuth angles was from −2.0 to 2.0 degrees and the translation range was 0.97 mm on both sides of the reference MSP. Interpolation was used to correct for image intensities at points in the perturbed planes which did not lie on the discrete position grid of the volume. The cross sectional CC areas in the altered planes were measured and compared against the CC area in the reference MSP. Results: The mean CC area was 569 mm² (SD 38 mm²). The mean CC area change due to shifting (any perturbation) the MSP was 2.67%, (Median 1.98%, SD 2.47%). A translation shift of one slice (0.97 mm) changed CC area by mean 2.55% (SD 2.22%). 2 degrees shift of elevation changed CC area by mean 3.59% (SD 3.06%). 2 degrees shift of azimuth changed CC area by mean 3.26% (SD 2.56%). The maximum change in CC area was 17.14%. Conclusions: Imprecise selection of the MSP on follow-up MRI scans can occur because of patient repositioning and cerebral asymmetry. Shifts in the MSP between serial MRI studies may falsely increase or decrease the cross sectional CC area. This potential source of error should be taken into consideration when using CC area to monitor disease progression or treatment effects in multiple sclerosis.

P617

Biplanar whole cord MRI using parallel imaging in multiple sclerosis: a 1-year follow-up study
K. Weier, Y. Naegelin, A. Thoeni, J. Hirsch, L. Kappos, D. Leppert, E.W. Radue, A. Gass; University Hospital Basel (Basel, CH)

Introduction: Despite its functional importance and its common involvement in MS few studies have evaluated the spinal cord (SC) in MS patients serially. In a combined brain and whole SC MRI protocol we were following MS patients. The SC protocol part included assessment in the sagittal and transverse plane of the whole SC by using multi-array-coils and parallel imaging. We were interested in the evolution of SC pathology as it may occur in MS, which has also been considered an additional measure of treatment trials. Methods: A cohort of 256 MS patients from the outpatients clinic (178 women, 78 men, 24–74 years old, EDSS 0–7.0) with different MS subtypes (CIS, RRMS, SPMS and PPMS) treated with best individual selected standard treatments were investigated. Examinations were performed on a SIEMENS I.5T Avanto MRI system which offers multi-array-coils and parallel imaging techniques. SC pathology was assessed on both sagittal and transverse images. Results: Abnormal SC MRI was noted in 75% MS patients, in more than half of them only focal lesions were identified. Focal lesions were primarily located in the cervical SC (39%) and about 2/3 showed more than 2 lesions. Diffuse cord abnormalities were found in 21% and in almost one third of these diffuse abnormalities in all levels. On f/u, new brain lesions were demonstrated in 16% of patients, while SC changes were noted in 8% of patients. In half of these patients this was easy to interpret new pathology, while more subtle changes were seen in the other half. New pathology consisted of focal lesions and/or new or increasing diffuse abnormalities and confluent lesions. Transverse MRI was useful to confirm or suspect changes compared to the previous examination, particularly as exact repositioning and identical slice positioning on sagittal images is extremely difficult. Conclusion/Discussion: Follow-up examinations of the SC to assess disease activity in MS are still challenging. Exact repositioning and identical slice positioning on sagittal images is very demanding. Unless high contrast new lesions are visible the assessment of disease activity on cord MRI suffers from the lack of strong contrast. Our cohort included a relatively low percentage of patients with inflammatory disease activity and the rate of SC abnormality may therefore be lower than that of untreated cohorts.

P618

High-resolution MRI of the hypothalamus in multiple sclerosis
R.J. Mills, C.A. Young, T. Smith; Walton Centre for Neurology and Neurosurgery (Liverpool, UK)

Background: Autonomic dysfunction in MS is common yet overt involvement of the hypothalamus in MS is not often seen on magnetic resonance imaging (MRI). Indeed, by routine, diagnostic MRI at 1.5T, only 4% of patients have plaques in the hypotalamnic region, however by light microscopy at post mortem, 94% of patients have evidence of hypothalamic disease. One reason for this apparently wide discrepancy is the suboptimal scan geometry of routine MRI which often uses slice thickness of 5 mm with or without interslice gap. The use of thin, contiguous slices and higher field strength, which improves signal to noise ratio (SNR), may yield greater detection of abnormalities, while more subtle changes were seen in the other half. New pathology consisted of focal lesions and/or new or increasing diffuse abnormalities and confluent lesions. Transverse MRI was useful to confirm or suspect changes compared to the previous examination, particularly as exact repositioning and identical slice positioning on sagittal images is extremely difficult. Conclusion/Discussion: Follow-up examinations of the SC to assess disease activity in MS are still challenging. Exact repositioning and identical slice positioning on sagittal images is very demanding. Unless high contrast new lesions are visible the assessment of disease activity on cord MRI suffers from the lack of strong contrast. Our cohort included a relatively low percentage of patients with inflammatory disease activity and the rate of SC abnormality may therefore be lower than that of untreated cohorts.
neuropathologist. **Results:** Initial analysis suggested that abnormal signal can be detected in approximately 20% of patients. The architecture of the diencephalon appeared to be abnormal in nearly all patients especially regarding the shape of the third ventricle. Segmental volume analysis was undertaken to determine whether this was due to differential atrophy in either hypothalamic or surrounding structures. **Conclusion:** High resolution MRI at 3T improves detection of hypothalamic abnormality in MS.

**P619**

**MR imaging of hippocampal lesions in multiple sclerosis**

S.D. Rosendal, J.J.G. Gruets, B. Moraal, H. Vrenken, F.J.W. Pouwels, J.A. Casteljus, F. Barkhof; VU University Medical Center (Amsterdam, NL)

**Introduction:** Neuropsychological impairment, especially memory dysfunction, is prevalent in Multiple Sclerosis (MS). Hippocampal pathology can therefore be reasonably expected. Little is currently known concerning hippocampal lesions in MS, since conventional Magnetic Resonance Imaging (MRI) notoriously underestimates grey matter (GM) lesion numbers. Cortical GM lesion conspicuity can be improved using 3D-Double Inversion Recovery (3D DIR). In this study, we evaluated whether 3D-DIR also enables hippocampal lesion detection in MS. **Patients and Methods:** Imaging was performed using a 1.5T scanner (Siemens Sonata, Erlangen, Germany). Sagittal 3D-DIR images (TR/TE/T11/T12: 6500/349/2350/350 ms; voxel dimensions: 1.2 x 1.2 x 1.3 mm3) and 3D-T2-weighted turbo spin-echo images (3D-T2: TR/TE: 4300/349 ms; voxel dimensions: 1.2 x 1.2 x 1.3 mm3) of 16 patients (9 females; mean age: 39.5 years, range: 24–56) and 9 control subjects (3 females; mean age: 32.0 years, range: 22–53) were acquired cross-sectionally. Lesions were anatomically classified on 3D DIR as being: white matter (WM, i.e. total of periventricular and deep WM), cortical (total of intracortical, juxta-cortical and mixed WM-GM lesions), or hippocampal lesions. For optimal anatomical viewing, hippocampal lesions were assessed on orthogonally reformatted coronal 3D-DIR images. Other lesion categories were assessed on reconstructed oblique axial images. Hippocampal and cortical lesions were scored in consensus and defined as hyperintense with respect to surrounding normal GM, though less hypointense than WM lesions. A retrospective scoring for hippocampal lesions on coronal 3D-T2 was also performed. Pearson’s correlation coefficient was used to evaluate associations between hippocampal and cortical and between hippocampal and WM lesion numbers. **Results:** The mean number (±SD) of 3D-DIR hippocampal lesions was 2.6 ± 1.8 in MS patients, with 14 out of 16 patients having at least 1 hippocampal lesion. No hippocampal lesions were detected in control subjects. Hippocampal lesion count correlated with total cortical lesion number (r: 0.58, p = 0.02), but not with total WM lesion count (r: 0.22, p = 0.4). Retrospectively, 55% of hippocampal lesions could also be detected using 3D-T2. **Conclusion:** Hippocampal lesions can be detected in MS using 3D-DIR and are related to the number of cortical lesions. Further research is indicated to study clinical and cognitive effects of MR-visible hippocampal pathology.

**P620**

A voxel-based morphometry study of grey and white matter density changes in MS patients with different clinical phenotypes

A. Cecarelli, M.A. Rocca, E. Pagani, F. Agosta, B. Benedetti, B. Colombo, G. Comi, M. Filippi; Scientific Institute San Raffaele (Milan, I)

**Background:** Diffuse brain atrophy, as well as grey matter (GM) and white matter (WM) volume reductions, have been demonstrated in multiple sclerosis (MS) patients with different disease phenotypes. **Objectives:** To assess regional atrophy changes in GM and WM in a large cohort of MS patients with different clinical phenotypes, using statistical parametric mapping (SPM) and voxel-based morphometry (VBM). **Design/Methods:** Dual-echo and magnetization-prepared rapid acquisition gradient echo (MP-RAGE) scans were obtained from 31 patients with clinically isolated syndromes (CIS) suggestive of MS, 37 relapsing-remitting (RR) MS patients, 23 primary progressive (PP) MS, 43 secondary progressive (SP) MS patients, and 29 control subjects. Using SPM2, customized GM and WM templates from MP-RAGE scans of both healthy controls and MS patients were produced. Regional differences in GM and WM densities were assessed using an optimized version of VBM analysis and SPM2. Between-group comparisons were assessed using an ANCOVA model, where age, disease duration and intracranial volume were included as covariates. The following a-priori contrasts were tested: controls vs. CIS, CIS vs. RRMS, RRMS vs. SPMS, SPMS vs. PPMS. Correlation of GM and WM changes with expanded disability status scale (EDSS) and lesion load (LL) was performed using basic models and linear regression analysis. SPM maps were thresholded at p < 0.001. **Results:** Compared with controls, CIS patients had WM density reduction around the left (L) lateral ventricle, the splenium of corpus callosum, L middle frontal gyrus (MFG), right (R) middle and inferior temporal gyrus, while no GM density changes were found. Compared with CIS patients, RRMS patients had GM density reduction of the R postcentral gyrus and L MFG. Compared with RRMS, SPMS patients had GM density reduction of the R MFG, caudate nuclei, bilaterally, L insula and L anterior cerebellar lobe. Compared with SPMS, PPMS had GM density reduction of the bilateral orbital frontal cortex and WM reduction of the dorsal mesencephalon, while compared with PPMS, SPMS had GM density reduction of the L parietal cortex and L insula. No correlations were found between GM and WM changes and EDSS and LL. **Conclusions:** Different MS phenotypes show different patterns of regional distribution of brain tissue volume decrease. While WM damage seems to be predominant in the early phases of the disease, GM atrophy seems to be more evident in the progressive forms of the disease.

**P621**

Dirty-appearing white matter in multiple sclerosis: relationship to T2 disease burden increase and brain volume decrease with 8-year long-term follow-up

V. Miroplys, T. Vertinsky, G.J. Zhao, Y.S. Zhao, A. Traboulsee, D.K.B. Li; University of British Columbia (Vancouver, CAN)

**Background:** Dirty-appearing white matter (DWM) has been described in patients with multiple sclerosis (MS) as diffuse areas of slightly increased signal intensity in white matter distinct from the typical focal high signal intensity MS lesion. **Objective:** To determine the relationship of DWM to changes in T2 lesion volume (burden of disease, BOD) and brain volume (whole brain ratio, WBR) in a group of MS patients followed for up to 8 years. **Methods:** 348 relapsing-remitting MS patients originally enrolled in a randomized 2-year placebo-controlled treatment trial of subcutaneous interferon beta-1a (PRISMS) were studied at baseline and at long-term follow-up (LTFU)8 years later. Five mm thick transverse proton density and T2-weighted conventional spin echo scans were obtained according to a protocol with strict repositioning criteria. Presence of DWM and the change over time (reduced/same/increased) was determined qualitatively by two radiologists who reviewed the scans independently and then together (by consensus, with disagreements settled by a third radiologist). T2 BOD and WBR were determined quantitatively using a semi-automated algorithm by trained technicians. **Results:** DWM was seen in 88 of 348 (25.3%) patients at baseline. At LTFU, only 3 additional patients (0.9%) developed DWM. For those with DWM at baseline, DWM was the same in 69.3%, reduced in 28.4% and increased in 2.2% at LTFU. Patients with DWM had lower T2 BOD at baseline (p = 0.0014). While BOD change at LTFU was not different as a group between those with and without DWM, larger T2 BOD increase was seen in the subgroups (p = 0.03) who had increased or new DWM and those with reduced DWM. Patients with DWM had greater reduction in WBR at baseline (p = 0.0011) but greater reduction in WBR at LTFU (p = 0.038, adjusted for baseline WBR), compared to those without DWM. Reduced DWM patients had the greatest reduction
in WBR (p = 0.008). **Conclusion:** The 25.3% prevalence of DWM increased minimally over 8 years. At LTFU, the extent of DWM was more likely to be the same or reduced than increased. DWM patients showed greater decrease in WBR at LTFU than those without DWM. The largest BOD increase occurred in those with DWM increase, followed by those with reduced DWM. The finding that patients with reduced DWM also had the greatest WBR decrease supports the observation that DWM decrease was due to ventricular enlargement and replacement of DWM by typical T2 hyperintense lesions.

P622

**Evidence for grey matter atrophy in patients with clinically isolated syndrome suggestive of multiple sclerosis: a voxel-based morphometry study**

J.L. Cox, V. Yella, R. Zivadinov; Buffalo Neuroimaging Analysis Center (Buffalo, USA)

**Background:** Gray matter (GM) atrophy has been shown to occur in patients early in the disease course of multiple sclerosis (MS), and in individuals with clinically isolated syndromes (CIS) suggestive of MS. Previous studies have shown reduced thalamic volume in patients with MS compared to control subjects. Voxel based morphometry (VBM) is a whole-brain, unbiased method which can be used to detect atrophy differences between individual groups. **Objective:** To investigate regional GM differences between CIS patients and healthy control subjects using VBM. **Methods:** High resolution T1-weighted 3D SPGR images from 41 patients with CIS (mean age 39.9; mean disease duration 2.7 years; 8 male, 33 female) and 22 healthy control subjects (mean age 36.5; 11 male, 11 female) were collected with a 1.5T MRI. Expanded Disability Status Scale score was 3.5 or lower for all CIS patients (mean 1.6, SD 0.87). VBM analysis was performed using Statistical Parametric Mapping (SPM2; Wellcome Department of Cognitive Neurology, London, UK). Optimized VBM methods were used following the methods of Good et al., (Neuroimage; 2001;14: 21–36) and Gaser (http://dbm.neuro.uni-jena.de/vbm.html). A study-specific template was created to provide prior information for segmentation. The MR images were smoothed with a 8 mm full-width at half maximum kernel. Whole brain GM and white matter (WM) volumes were also calculated. The SPN analysis of covariance tool was used to assess group differences after controlling for age and gender. **Results:** VBM analyses revealed that patients with CIS had reduced GM volume compared to control subjects in the right thalamus and left inferior frontal gyrus (p < 0.001, uncorrected). Whole brain GM or WM volumes were not significantly different in MS patients compared to controls (GM: t(62) = 0.714, p = 0.48; WM: t(62) = 0.33, p = 0.74). **Conclusions:** Few studies have used VBM analyses in MS research. This study provides further evidence that deep GM structures are affected in patients with early RR MS. Further studies are needed to determine whether the regional GM volume reductions found in this study are correlated with higher risk of development of clinically definite MS. Furthermore, it suggests that regional atrophy measures may be more sensitive in revealing early changes in CIS patients than those based on whole brain tissue compartments.

P623

**Evidence for cortical atrophy in patients with clinically isolated syndrome**

R. Zivadinov, V. Yella, M.G. Dwyer, S. Hussein, J.L. Cox; University of Buffalo (Buffalo, USA)

**Background:** There is increasing evidence that gray matter damage in patients with multiple sclerosis (MS) is present from the earliest stages of the disease. **Objective:** To investigate the severity of cortical volume loss in patients with clinically isolated syndrome (CIS) and in normal controls (NC). To investigate whether the number, volume and regional distribution of hyperintense T2 lesions determines the extent of cortical pathology at clinical onset. **Methods:** Thirty-six consecutive CIS patients (mean age 39.9 SD 9.6 years, mean disease duration 3.7 SD 6, mean EDSS 1.6 SD 0.9) and 28 age- and sex-matched NC underwent MRI scan on 1.5T unit. Cortical atrophy was obtained by measuring normalized cortical volume (NCV) on 3D-SPGR-T1-WI (2.5 mm slice thickness) using SFINAX software. Number, volume and regional distribution of T2 lesions was assessed according to the McDonald criteria. Patients were sub-classified according to the onset of symptoms in those with supratentorial (S), infratentorial (T), optic nerve (N) and spinal cord (C) onset. **Results:** Patients with CIS showed lower NCV than NC (593.2 ml vs. 606.4 ml, p = 0.053). CIS patients who fulfilled McDonald criteria for dissemination in space (10) demonstrated significantly lower NCV than NC (572.2 ml vs. 606.4 ml, p = 0.03). There was a trend for lower NCV in patients who presented <9 hyperintense T2 lesions when compared to those with ≥9 T2 lesions (580.6 ml vs. 596.3 ml, p = 0.074). Patients with infratentorial onset of symptoms showed significantly lower NCV than patients with optic nerve onset (p = 0.037) and a trend for lower NCV when compared to supratentorial (p = 0.085) and spinal onset (p = 0.094). Partial correlation and regression analyses co-adjusted for age differences revealed a significant relationship between higher number and volume of periventricular T2 lesions and lower NCV (r = −0.37, p = 0.03 and R2 = 0.18, p = 0.01, respectively). **Conclusions:** This study suggests that patients with CIS exhibit mild-to-moderate cortical atrophy when compared to age- and sex-matched NC. Those patients with high risk for developing clinically definite MS are more likely to show more advanced cortical pathology. Infratentorial onset of symptoms related significantly to development of cortical atrophy in CIS patients. Macroscopic inflammatory white matter pathology may explain only partially the extent of cortical pathology in patients with CIS.

P624

**Evidence for cortical atrophy in a large sample of patients with multiple sclerosis**

K. Zivadinov, N. Bergland, J.L. Cox, F.E. Munschauer, N. Abdelrahman, N. Garg, M. Meyer, S. Hussein, B. Weinstock-Guttman; University of Buffalo (Buffalo, USA)

**Background:** There is increasing evidence that cortical damage in patients with multiple sclerosis (MS) is more severe than previously thought. Histopathological studies using new immunohistochemical methods show that cortical subpial lesions can be detected more frequently. The severity of cortical atrophy according to disease course has not been investigated in patients with MS. **Objective:** To investigate the severity of cortical volume loss in a large sample of MS subtypes and age- and sex-matched normal controls (NC). **Methods:** We studied 532 consecutive MS patients (mean age 46.1 ± 9.6 years) and 28 normal controls (NC) using 1.5 T brain MRI. Clinical disease subtypes were as follows: 351 relapsing-remitting (RR), 142 secondary-progressive (SP), 21 primary-progressive (PP) and 18 with clinically isolated syndrome (CIS). Mean disease duration was 12.8 ± 8.7 yrs. Mean Expanded Disability Status Scale (EDSS) was 3.3 ± 2.1. Cortical atrophy was calculated by measuring normalized cortical volume (NCV) on 3D-SPGR-T1-WI (2.5 mm slice thickness) using SFINAX software. Gadolinium (Gd), T2- and T1-lesion volumes (LVs) and EDSS were also assessed for all patients. General linear model analysis adjusted for age and gender differences was performed. **Results:** We found MS patients differed significantly from NC in NCV (553.2 ml vs. 611.6 ml, p < 0.0001). All disease subtypes differed significantly in their NCV from NC (p < 0.0001), with the size of the largest effect being for SP MS (d = 2.3). SP patients showed significantly lower NCV when compared to all other disease subtypes (p < 0.001), with the size of the largest effect being for PP MS (d = 1.26). There was no significant difference between RR, PP and CIS patients regarding mean and median NCV. **Conclusions:** This study demonstrates that all MS disease subtypes have significantly lower NCV than NC. The severity of cortical atrophy is the most evident in patients with SP MS. This may suggest that Wallerian degeneration from white matter lesions may be an important contributor to
development of cortical atrophy in the long-term. There was no difference for NCV between RR, PP and CIS patients, although PP and CIS patients showed significantly lower Gd-, T2- and T1-LVs ($p < 0.0005$) when compared to RR patients. This may suggest that other mechanisms of neurodegeneration in the gray matter may contribute to cortical atrophy in CIS and PP MS, including development of cortical subpial lesions and independent axonal transection.

**P625**

Increasing cord atrophy in clinical isolated syndrome group: a 5-year study

S. Dospinescu, T. Swanton, D. Altmann, K.T.M. Fernando, C. Dalton, A.J. Thompson, G. Plant, D.H. Miller; NMR Research Unit (London, UK); London School of Hygiene and Tropical Medicine (London, UK); National Hospital of Neurology and Neurosurgery (London, UK); Department of Neuro-ophthalmology (London, UK)

**Objective:** To report a 5 years serial MRI study of upper cervical cord area (UCCA) measures in clinically isolated syndrome (CIS) patients and healthy volunteers.

**Background:** Involvement of the spinal cord in multiple sclerosis (MS) is extremely common and very important in the development of disability but little investigation of atrophy has been undertaken in patients in the earliest stage of the disease.

**Methods:** For this prospective MRI study, 142 patients and 37 controls were investigated at baseline. After 1 year, 124 patients and 25 controls were imaged. After 3 years, 97 patients and 19 controls were imaged and at 5 years, 46 patients and 13 controls were imaged. After 1 year, 124 patients and 25 controls were imaged. After 3 years, 97 patients and 19 controls were imaged and at 5 years, 46 patients and 13 controls were studied. The EDSS and MS Functional Composite Measure (MSFC) were assessed in each patient. All subjects had a volume acquired inversion prepared fast spoiled gradient echo (FSPGR) of the spinal cord. A single observer (SD), blinded to both to the subjects details and the scan acquisition order, calculated the mean area of the slices using a technique previously described by Losseff et al. Statistical models adjusted for covariates including gender.

**Results:** At baseline, the presenting symptom was optic neuritis (ON) in 117 patients (82.4%), a brainstem syndrome (BS) in 16 patients (11.3%) and a spinal cord syndrome (SC) in 8 patients (5.6%). In cross sectional study no significant difference was found in UCCA between patients and controls at any time but the SC subgroup had significant difference at 3 years compared to both controls ($-5.88 \text{ mm}^2; p = 0.08$) and ON subgroup ($-6.27 \text{ mm}^2; p = 0.03$) and at 5 years (respectively $-12.7 \text{ mm}^2; p = 0.04$ and $-11.1 \text{ mm}^2; p = 0.07$). Longitudinal analysis showed a greater decrease of UCCA in patients than controls at 3 years ($-1.08 \text{ mm}^2; p = 0.08$) and 5 years ($-2.26 \text{ mm}^2; p = 0.03$). In subgroup of patients with lesions in spinal cord at baseline, we observed a greater decrease over 1 year than in patients without such lesions ($-0.94 \text{ mm}^2; p = 0.04$), confirmed at 3 years ($-1.87 \text{ mm}^2; p = 0.001$). In subgroup of patients with a SC CIS, the changes in UCCA were greater than both controls and ON subgroups at 3 years (respectively $-4.5 \text{ mm}^2; p = 0.001$ and $-3.5 \text{ mm}^2; p = 0.001$) and 5 years (respectively $-7.25 \text{ mm}^2; p = 0.005$ and $-4.9 \text{ mm}^2; p = 0.05$). No significant correlation was found between UCCA and EDSS or MSFC.

**Conclusion:** In summary, serial UCCA measurement over 5 years detects the development of spinal cord atrophy in CIS group, especially for patients with SC syndrome or spinal cord lesions at baseline.

**P626**

Grey matter atrophy in the visual pathway of multiple sclerosis patients

J. Sepulcre, J. Gállego, J. Gottl, N. Vélez de Mendizábal, P. Villoslada; University of Navarra (Pamplona, E)

**Background:** Visual impairment is frequent in multiple sclerosis (MS). Indeed, grey matter (GM) atrophy could represent a critical stage of irreversible impairment. Thus, the study of the GM atrophy in the visual pathway could provide useful clinical information and also clarify the different mechanism involved in visual disability. **Aims:** The aim of our work was to study the GM atrophy in the visual pathway in order to investigate its relationship with MS condition, previous optic neuritis (ON) and/or specific white matter (WM) lesions topography.

**Methods:** We study a cohort of 61 MS patients (McDonald criteria(22M/39F); age: 36±3.9 years; EDSS: 2.5 (0–7.0); 50RR/SP/6PP) and 20 matched controls. A 3D T1 weighted scan ($2$-mm thick contiguous axial slices; $256 \times 256$ matrix, in-plane voxel size $0.98 \times 0.98 \text{ mm}^2$). We used the MRicro and SPMS software for the image analysis. The lesion burden and the whole GM and WM volume were obtained for each patient. The WM lesions topography was analyzed using 3D lesion probability maps, and the GM atrophy using optimized voxel-based morphometry method. A parcellation method using Marsbar toolbox was used to assess GM regions of interest of the visual pathway. To perform all the statistical analysis with used the framework of the general linear model in the SPM2 and SPSS software ($p < 0.05$ corrected by multiple comparisons).

**Results:** Twenty one patients had a previous ON (13 right; 5 left; 3 bilateral). We found a volume decrease in the main components of the visual pathway (chiasm, optic tract, lateral geniculate nucleus (LGN), calcarine cortex and superior, inferior and middle occipital cortex) in patients compared with controls ($p < 0.05$ in all cases). Patients with previous ON have a statistically significant volume decrease in LGN and whole WM ($p = 0.022$ and $p = 0.04$ respectively), independently of their visual impairment. Finally, lesions in a specific WM topography of optic radiations correlate with LGN volume decrease (no voxels: $8592$; $p$-FDRcorr: 0.03; f: 22.49; max. coord: 39, − 36, 15).

**Conclusion:** We described GM atrophy in the visual pathway in MS patients that was mainly associated with disease condition. LGN could play a central role in visual disability because is related with previous ON and with a specific WM lesions topography.

**P627**

The relationship between focal cortical thinning and white matter degeneration in patients with multiple sclerosis

C. Mainiero, F. Caramia, D. Salat, V. Calabri, T. Benner, L. Prosperini, B. Rosen, C. Pozzilli; A.A. Martinos Center, MGH (Charlestown, USA); University "La Sapienza" (Rome, I)

**Objective:** To what extent cortical pathology described in multiple sclerosis (MS) is secondary to white matter (WM) damage, or may represent an independent neurodegenerative process is still an open question. Imaging studies have found weak correlations between lesion burden on conventional magnetic resonance imaging (MRI) and cortical atrophy. Using diffusion tensor imaging (DTI) that provides more sensitive indices of the underlying WM tissue micro-structure than conventional MRI and quantitative morphometric MRI, we assessed the relation between WM degeneration and cortical thickness in MS. **Methods:** We acquired T1-weighted MPRAGE scans for cortical thickness analysis and high-resolution diffusion tensor images in 23 patients with MS (mean age = 39.3 years, mean Expanded Disability Status Scale, EDSS, score = 3.63) and 11 matched healthy controls (HC, mean age = 39.1 years) on a 1.5 Tesla Philips scanner. In patients, we also collected conventional T2- and post-gadolinium T1-weighted scans. Cortical thickness difference maps were obtained using FreeSurfer. Areas showing significant thinning were defined as regions of interest (ROIs). ROIs’ thickness values were used for further analyses. Maps of fractional anisotropy (FA) were calculated from each participant’s DTI scan. Analyses were performed by voxel-based statistical comparisons. In patients we quantified T2 and T1 lesion load using Alice (Hayden Solutions).

**Results:** We focused our preliminary investigation on two ROIs that showed significantly thinning in patients with MS versus HC ($p < 0.05$, t-test). These ROIs included the primary motor cortex (M1) of the right hemisphere (mean thickness: patients $2.04 \text{ mm}$, HC $2.33 \text{ mm}$), and the primary visual cortex (V1) of the left hemisphere (mean thickness: patients $1.27 \text{ mm}$, HC $1.44 \text{ mm}$). In patients right M1 thickness significantly correlated with decreased FA in the right corticospinal tract, bilateral thalamus, and corpus callosum; thinning of the left V1
correlated with decreased FA in WM regions of the visual pathway of the ipsilateral hemisphere including the thalamus/lateral geniculate nucleus, part of the optic radiation, but also with reduced FA in the bilateral putamen/thalamus (p < 0.001). We did not find any relation between either M1 or V1 thickness and T2 or T1 lesion load.

**Conclusions:** The combined use of high resolution DTI and morphometric MRI can disclose a specific relation between focal cortical thinning and biophysical changes in selective WM regions.

**P628**

**Inter-site agreement of brain atrophy measurement in MS using SIENAX and SIENA**


**Background:** Brain atrophy is an important feature of multiple sclerosis (MS), thought to reflect the neurodegenerative component of the disease. To further elucidate the role of brain atrophy in MS, large multi-centre cohorts need to be studied. SIENAX and SIENA [1] are accurate and robust methods to quantify brain volume and brain atrophy rate respectively. Although these are automated methods, errors may arise in the classification of brain and non-brain tissue, which is frequently corrected by manual adjustment. Assessment of inter-site agreement is needed to justify exchange of manually edited brain atrophy data between sites. **Goal:** To investigate the inter-site agreement of brain volume measurement using SIENAX and SIENA with and without manual editing. **Methods:** 20 MS patients from 2 different sites underwent MR examination at baseline and follow-up. Standard spin-echo T1-weighted image sets were dispatched to 5 sites participating in the MAGNIMS study group. All sites had the latest version of SIENAX and SIENA installed, and performed all analyses with identical parameter settings. Each site performed fully automated and manually edited analyses for both SIENAX and SIENA, yielding Normalised Brain Volume (NBV) and Percentage Brain volume Change (PBVC) for each subject. Variation between sites was analysed using variance component analysis. The extent of absolute agreement between sites was expressed as the Concordance Correlation Coefficient (CCC), which was calculated from the variance components [2]. **Results:** As expected, excellent agreement between sites was observed for both fully automated NBV and PBVC (both CCC = 1.0). Manual editing decreased agreement between sites (CCC = 0.94 for NBV, 0.95 for PBVC). Mean NBV values for each site decreased after manual editing, while PBVC values remained similar on average. Interestingly, the total variance for PBVC decreased after manual editing (total variance for fully automated SIENA = 2.8, and for manually edited SIENA = 1.5), suggesting that the manual removal of non-brain tissue may improve statistical power for SIENA. **Conclusions:** NBV and PBVC values from different sites show good agreement, even after manual editing. For SIENA, manual adjustment appears to increase statistical power.

**References**


**P629**

**Regional brain atrophy rate is related to disability progression in early relapse onset multiple sclerosis**

B. Jasperse, H. Vrenken, V. de Groot, E. Sanz-Arigita, C.H. Polman, F. Barkhof; VU Medical Centre (Amsterdam, NL)

**Background:** Brain atrophy is a well-known feature of MS, and is thought to reflect irreversible tissue damage. Previous studies have demonstrated a relation between global brain atrophy rate and disability progression. Little is known on how disability progression in MS relates to the rate of atrophy in specific brain regions. **Goal:** To evaluate the association between disability progression and the regional distribution of brain atrophy rate in early relapse onset MS.

**Methods:** 80 relapse onset patients underwent clinical examination (Kurtzke’s Extended Disability Status Scale (EDSS) score and Multiple Sclerosis Functional Composite (MSFC) score) and MRI examination at the time of diagnosis and two year follow-up. SIENA [1] was used on T1 weighted images to produce an image that reflects the displacement of the brain edge between two scans, referred to as the displacement map. Voxelwise non-parametric statistics were performed on the displacement maps [2] to identify regions in which atrophy rate was significantly related to changes in EDSS and MSFC. P-values were uncorrected for multiple comparisons and considered statistically significant at the 0.01 level. **Results:** Baseline EDSS ranged from 0.0 to 4.0, indicating that our patient group was mildly disabled. Our results suggest that clinical progression as measured by change in EDSS and MSFC scores was significantly correlated with atrophy rate in several specific regions. Both EDSS and MSFC progression were significantly related with atrophy rate of the brainstem. MSFC progression was significantly related to widespread atrophy around the ventricular system, whereas EDSS progression appeared to be related more specifically to the atrophy rate of the corpus callosum. Atrophy rates in several cortical regions were significantly related to EDSS and MSFC progression. **Conclusion:** In recently diagnosed, mildly disabled, relapse onset MS patients, atrophy of specific brain regions is significantly related to clinical progression. Progression on EDSS and MSFC are correlated with atrophy rate of brainstem and several cortical regions. MSFC progression correlated with widespread ventricular widening.

**References**

progressive cervical spinal cord cross sectional area in relapsing-remitting multiple sclerosis
R.S. Manu, C.S. Constantinescu, C.R. Tench; University of Nottingham (Nottingham, UK)

The upper cervical cord is often affected in multiple sclerosis (MS) patients, and its cross sectional area (CSA) has been shown to correlate with disability. Accurate and reproducible image analysis techniques, applied to magnetic resonance imaging (MRI) acquisitions, have been developed to measure CSA. While reduced CSA has been detected in progressive MS, it has not been observed in relapsing remitting (RR) MS despite significant reduction in CSA being reported longitudinally in this patient group; in our previous study we have shown a 5% reduction in CSA over 48 months in RR MS patients. We hypothesised that this paradoxical result may be due both to a lack of measurement sensitivity and to small subject numbers in previous cross sectional studies. We used a previously published method that corrects for major sources of error in the CSA measured using MRI: partial volume averaging and cord orientation. Using this technique we measured CSA in a relatively large, compared to other studies, number of subjects: 35 RR MS patients (21 female, 14 male), and 35 normal controls (21 female, 14 male). The patients and controls were matched for age and gender. We also measured total intracranial volume (TICV) and used it to normalize the CSA to reduce the normal population variance. Power studies were performed to verify the usefulness of the normalization. Median disease duration in patients was 9 (interquartile range 5–131) years. Mean TICV in patients (1897 ± 34 ml) was not different from that in controls (1886 ± 34 ml). The mean CSA, before normalisation, was 77.97 ± 0.95 mm² in the controls, and 80.13 ± 1.36 mm² in the patients. The mean normalised CSA was 77.97 ± 0.95 mm² in the controls, and 80.38 ± 1.32 mm² in the patients. Statistical power analysis indicated that an assumed 5% reduction in CSA in the patient group could be detected with estimated power 0.74 before normalization and 0.9 after. The mean CSA in the patient group was not reduced compared to controls after

Abstracts

Progressive cervical cord damage in patients with multiple sclerosis: a longitudinal study using diffusion tensor MRI
M. Absinta, F. Agosta, B. Benedetti, M.A. Rocca, A. Ghezzi, E. Montanari, A. Bertolotto, G. Comi, M. Filippi; Scientific Institute San Raffaele (Milan, I); Ospedale di Gallarate (Gallarate, I); Ospedale di Fidenza (Fidenza, I); Ospedale di Orbassano (Orbassano, I)

Background: Cord damage is an important contributor to irreversible disability in multiple sclerosis (MS). Recently, a diffusion tensor magnetic resonance imaging (DT-MRI) sequence tailored for cervical cord imaging has been developed and has proved to be able to grade the extent of cervical cord damage associated to demyelinating conditions. Goals: To define the nature and the temporal evolution of the DT-MRI detectable cervical cord injury in MS patients with different disease phenotypes. Methods: Forty-two MS patients (13 relapsing-remitting (RR), 14 secondary progressive (SP), and 15 primary progressive (PP)) and eight matched healthy controls were studied at baseline and after a median follow-up of 2.4 years. At the two time-points, the following cervical cord sequences were acquired: fast short-tau inversion recovery (fast-STIR); diffusion-weighted sensitivity-encoded echo planar; T1-weighted 3D magnetization-prepared rapid acquisition gradient echo. Brain dual-echo scans were also performed and brain T2-visible lesion volume (LV) calculated. Cord lesions were identified on the fast-STIR images. Cord cross-sectional area as well as mean diffusivity (MD) and fractional anisotropy (FA) histograms were calculated. An analysis of variance model, adjusted for patients’ age, was used to compare cord DT-MRI parameters between baseline and follow up. Univariate correlations were assessed using the Spearman rank correlation coefficient. Results: During the study period, all MRI metrics from healthy controls remained stable. At follow up, eight MS patients showed an increase of the number of cord lesions. No change was detected in cord cross-sectional area in the entire MS cohort. In MS patients, a significant increase of average cervical cord MD (p < 0.001), independent from patients’ age, was found. In addition, a significant decrease of average cervical cord FA (p = 0.0009), significantly associated to patients’ age (p = 0.02) and disease phenotype, was detected. FA decrease was more pronounced in PPMs patients (19.0%) than in patients with SPMs (9.8%) and RRMS (4.4%). No significant correlation was found between brain and cord MR derived metrics changes. Conclusions: DT-MRI reveals progressive microstructural changes in the cervical cord tissue of MS patients, at least partially independent from the concomitant accrual of brain damage. Such diffusivity changes show different rates of temporal evolution in according to disease phenotypes.
clinical parameters are associated with disease progression. We were
able to test this.

P634
Non-conventional MRI measures of brain tissue integrity fail to explain the preservation of function in some MS patients with an extensive lesion load but a benign course of the disease
M. Wallner, C. Enzinger, S. Ropele, S. Strasser-Fuchs, F. Fazekas; Medical University Graz (Graz, A)

Background: T2-weighted MRI has greatly advanced our ability to detect MS lesions in the brain, but the correlation between lesion load and clinical disability is modest at most. Non-conventional quantita-
tive MRI metrics like the loss of brain volume, the magnetisation transfer ratio (MTR) and levels of N-acetylaspartate (NAA) have been suggested to partly explain what has been termed “a clinicoradiolog-
ical paradox”. Goals: We hypothesised that less pronounced abnormality on non-conventional MRI might explain preserved clinical functioning of some patients despite a large T2 lesion load.

To test this assumption, we obtained quantitative MRI metrics in MS patients with a benign relapsing-remitting MS (B-MS) despite a large lesion load and a secondary-progressive disease course (SP-MS) with similar disease duration. Results: We studied 13 patients with B-MS (median EDSS 2.0; range 0–3.0; mean disease duration 18.2 ± 7.9 yrs) and 15 patients with SP-MS [median EDSS 6.0 (range 4.0–8.5); mean disease duration 18.1 ± 8.2 yrs], by means of conventional MRI, magnetization transfer imaging and chemical shift imaging at 1.5 T. Global MTR metrics were calculated for brain parenchyma. The brain parenchymal fraction (BPF) was determined using SIENAX. Thirteen subjects received immunomodulatory/suppressant therapy (B-MS: 4/13 vs. SP-MS 9/15).

Results: The mean volume of hypointense lesions was non-significantly higher in B-MS compared to SP-MS patients (41.2 ± 27.1 cm³ vs. 27.9 ± 24.8 cm³; p = 0.19). The mean BPF in both groups was almost identical (B-MS and SP-MS: 0.71 ± 0.03). Also, there were no significant differences between both groups regarding the mean MTR of NABT (40.8 ± 1.1 vs. 40.7 ± 3.1, p = 0.85). NAA levels relative to creatine (1.52 ± 0.20 vs. 1.55 ± 0.15, p = 0.62), and ratios of choline and myoinositol were also comparable.

Conclusion: In this selected group of patients with a clinically benign course of MS despite a large T2 lesion load, preservation of function could not be explained by less brain atrophy, a higher integrity of NABT as measured by the MTR or the absence of metabolic changes in comparison to patients that had developed SP-MS with a similar lesion load over the same time period. This finding supports the eminent role of brain plasticity in the compensation of MS related brain damage while morphologic changes appear tightly linked independent of the clinical phenotype.

P635
Measurement of cerebral atrophy has added value in predicting development of disability in early multiple sclerosis
A. Minnebo, B. Jaspers, F. Barkhof, R.M.I. Uitdehaag, D.L. Knol, V. de Groot, C.H. Polman, J.A. Castellpu; VU University Medical Center (Amsterdam, NL)

Context: MRI parameters and clinical parameters are associated with disease progression, the added value of MRI is unknown. Objectives: examine which brain- and spinal cord-based MRI parameters and clinical parameters are associated with disease progression. We were particularly interested whether adding MRI parameters to a only clinical model could improve these associations. Methods: 89 patients (55 women) with recently diagnosed MS had clinical and MRI evaluation at baseline (time of diagnosis) and at follow-up (FU) after 2.2 years (Inter Quartile Range (IQR): 2.0–2.4). Detailed clinical data were available including disease type (relapse-onset or progressive-onset) and disability (EDSS). MRI parameters: Normalized Brain Volume (NBV) at baseline, percentage brain volume change (PBVC), baseline and FU T2 lesion loads (T2LL, T2LLfu), T1 lesion loads (BHL, BHLfu), baseline volume of gadolinium enhancing lesions (GADLL), ratio of BHL and T2LL (Black Holes Ratio (BHR, BHRfu)). Further, number and size of focal/diffuse spinal cord abnormalities were scored. Patients were dichotomized according to progression of disability: progression was defined as change in EDSS ≥1. To find parameters with the strongest associations with progression, several models were composed using stepwise logistic regression. Firstly a model containing only MRI (1) or clinical (2) parameters was composed. Secondly, for a model containing only clinical parameters, the added value of MRI parameters was tested. Results: At FU but not at baseline, T2LL, BHL and BHR were significantly higher in the group with progression. Of the changes in MRI parameters during FU, only rate of atrophy (PBVC/year) was significantly higher in the group with progression (−1.3 (IQR −1.7 −0.5) compared to −0.8 (−1.3 −0.3), p = 0.0011). Rate of atrophy was the only independent explanatory MRI parameter in model 1 (Odds ratio (OR) 0.41, 95% CI 0.21−0.78, p = 0.007). Type of disease (OR 9.8, 95% CI 2.17–44.27, p = 0.003), age (OR 1.06, 95% CI 1.00–1.12, p = 0.066) and EDSS at baseline (0.41, 95% CI 0.21–0.80, p = 0.009) were included in model 2. Adding PBVC/year to model 2 strengthened the model, indicating that MRI parameters added independent information (p<0.001, area under receiver operating characteristics curve increasing from 0.72 to 0.82).

Conclusions: rate of atrophy (PBVC/year) is the MRI parameter best associated with progression of disability. Combining clinical and MRI parameters results in stronger models.

P636
Relationship between inflammatory lesions and cerebral atrophy in multiple sclerosis
I. Fedotova, I. Feklina; Kharkov Medical University (Kharkov, UK)

Objective: To investigate the temporal relationship between inflammatory and cerebral atrophy in a longitudinal study of 32 patients with relapsing-remitting multiple sclerosis (RRMS) using serial monthly contrast enhanced Magnetic Resonant Imaging (MRI) ex-
aminations and monthly measurements of brain fractional volume (BFV) for an average of 5 (range 2.5 to 10) years. Methods: In this retrospective study, all patients had an active MRI scan at entry with a minimum of two new contrast enhancing lesions (CEL) on baseline MRI examinations. Patients were followed for a minimum of 6 months during a baseline (pretreatment) phase and subsequently followed during treatment with recombinant interferon beta (IFN) and various other immunomodulatory agents. Pre- and post contrast axial images were obtained using 3-mm slice thickness and a gadolinium contrast dose of 0.1 mmol/kg. Monthly CEL were sequentially numbered on hardcopy films and monthly BFV was determined on precontrast T1W images using a semiautomated program. For BFV measurements, all T1W scans were registered to the entry examination, which served as a mask image. Cerebral atrophy was measured as percent brain fractional volume change (PBVC) compared to the entry baseline scan. Results: The results demonstrate that cerebral atrophy paralleled that of contrast enhancing lesion accumulation. The correlation between cumulative CEL and PBVC ranged from R² = 0.56 to 0.71. Immunomodulatory agents that effectively reduced CEL accumulation also slowed the rate of atrophy. Conclusions: The correlation between contrast enhancing lesions (CEL) and atrophy suggests that patients who are not responding to therapy with a decrease in CEL may also be at risk for developing increased atrophy.
Abstracts

P637

Application of single-slab 3D-FLAIR, 3D-DIR and 3D-T2
B. Moraal, P.J.W. Pouwels, H. Vrenken, C.R.G. Gottmann, D.S. Meier, R.A. van Schijndel, F. Barkhof; VUMC Amsterdam (Amsterdam, NL); Harvard Medical School (Boston, USA)

Introduction: The clinico-radiological correlation between MRI indices and clinical disability is moderate at best [1]. It might improve with high-resolution, multi-contrast, MR imaging that better depicts abnormalities in both white matter (WM) and gray matter (GM) structures. A single-slab 3D method for multiple contrasts has been developed [2] including a DIR contrast [3] since a previous study showed increased detection of intracortical lesions using this technique(4). The goal of the current study was to perform a cross-sectional analysis, comparing the 3D sequences to the routinely used 2D-T2SE.

Patients and methods: Sixteen patients (nine females) with clinically definite MS were examined using a 1.5T whole body scanner (Siemens Sonata, Erlangen, Germany), using a standard circularly polarized head coil. 40 2D-T2SE and 30 3D-FLAIR and 3D-DIR images were acquired randomly in order by a single observer. Lesions were scored and characterized according to their anatomical localizations (mixed WM-GM, infratentorial). A cross-sectional analysis, comparing the 3D sequences to the oblique axial 2D images and all images were analysed in random order by a single observer. Lesions were scored and characterized according to their anatomical localizations (mixed WM-GM, infratentorial). Results: The total number of lesions identified on the axially resliced single-slab 3D images were 945 (DIR), 1031 (FLAIR) and 825 (T2), compared to 959 for the 2D sequences. Mean lesion counts per topographic region (data not shown) show an increased detection of intracortical and mixed WM-GM lesions using 3D-DIR images. The highest number of lesions was identified on the 3D-FLAIR images, especially in the deep WM. By comparison, the 3D-T2 performed relatively poorly, especially in detecting cortical and mixed WM-GM lesions. Discussion: The single-slab, multi-contrast 3D dataset allows for a better differentiation of MS lesions according to location, which may improve the clinico-radiological correlation. Furthermore, scanning times and artefacts are reduced compared to multi-slab 3D techniques [4], facilitating future clinical applications. Finally the high, near isotropic, resolution is advantageous for post-processing methods such as registration, subtraction and segmentation.

References

P638

Registration and subtraction of 2D-T2SE images in a routine multi-centre multiple sclerosis trial setting
B. Moraal, D.S. Meier, H. Vrenken, P.A. Poppe, R.A van Schijndel, P.J.W. Pouwels, C.R.G. Gottmann, F. Barkhof; VUMC Amsterdam (Amsterdam, NL); Harvard Medical School (Boston, USA)

Introduction: Detection of disease activity using serial conven- tional MRI is a labour intensive process, complicated by reposi- tioning errors and a background of unaltered non-active lesions [1]. This leads to a high level of inter-observer variance that is not significantly improved by consensus rules [2]. Subtraction images were deemed reliable, time efficient and informative in an earlier study [3] but have not yet been tested in a multi-centre trial. This study explored the feasibility and sensitivity of subtraction images in detecting active lesions in the setting of a multi-centre trial.

Patients and Methods: Images were selected from a clinical trial. 46 scan pairs were randomly selected from 41 patients, originating from 8 different sites. This sample was stratified for lesion number and enhancement status at baseline. MRI protocol included 3 mm interleaved dual-echo T2-weighted scans obtained at a 3-monthly interval. Images were registered to a halfway position and intra-scan [4] and inter-scan [5] intensity normalization was applied. Original and subtraction images were analyzed by a single observer for active T2 lesions, these were characterized as new or enlarging (positive activity) or resolved or shrunken (negative activity). A change of 50% or more defined a change in size (for enlarging and shrinking lesions). Artefacts on subtraction images were identified by simultaneously viewing registered images. Results: On the subtracted images 77% more active lesions (positive change) were detected in about two-thirds of the time (ca. 2 hours). The biggest sensitivity gain for change detection was observed for juxtacortical lesions, especially near the vertex. Sensitivity of negative change was decreased by 56%.

Discussion: Subtraction of registered images is a faster and more sensitive technique for detecting positive disease activity (new or enlarging lesions) in MS. The detection of negative disease activity might improve by adopting a two-way colour scheme that raises the contrast between lesions and background. These results strengthen our belief that subtraction images provide a method for MS disease activity assessment that is fast, reliable and feasible in a multi-centre trial.

References

P639

Improved visualisation of intracortical lesions in multiple sclerosis by phase-sensitive inversion recovery in combination with fast double inversion recovery MR imaging
F. Nelson, A. Poonawalla, P. Hou, P. Narayana, J. Wolinsky; University of Texas (Houston, USA)

Background: Detection of purely intracortical lesions with conven- tional MR imaging remains a challenge. While double inversion recovery (DIR) techniques have been shown to improve the detection sensitivity of intracortical lesions, this sequence is prone to image artifacts and poor lesion border delineation. We demon- strate that intracortical lesions can be identified with greater confi- dence by combining DIR with phase sensitive inversion recovery (PSIR) images. Methods: 16 subjects with MS by McDonald criteria (1 PPMS, 3 SPMS and 12 RRMS) were included in this study. FLAIR, DIR and PSIR, images acquired were inspected visually by three experts, with identification and classification of cortical lesions by consensus. Percentage difference in number of lesions detected per category was compared between techniques. The PSIR and DIR images were jointly used to conservatively classify lesions into intracortical, mixed grey/white matter, and juxtacortical categories. Lesions not seen in both PSIR and DIR were excluded from the study. Results: Combined PSIR+DIR showed a 337% improvement in total number of lesions detected (306) compared with FLAIR (70). Intracortical lesion detection (124) was improved by 417% compared with FLAIR (24). Detection of mixed grey/white matter and juxtacortical lesions (144 and 39) were improved by 396% and 130% respectively compared to detection on FLAIR (29 and 17). PSIR consistently confirmed the intracortical location of the lesions visualized on fast DIR, allowing a much clearer delineation of lesion borders and more confident classification between purely intracortical, mixed grey/white matter and juxtacortical lesions. Conclusion: Reliability in visualization of intracortical lesions in multiple sclerosis can be greatly improved by combined use of PSIR and DIR techniques. Accurate detection and classification of these lesions is important in understanding their behavior, role in disease progression and impact in the clinical manifestations of the disease.
Detection of subpial demyelination in vivo: methodology and evaluation in normal subjects and patients with multiple sclerosis

I.T. Chen, D.L. Collins, M.S. Freedman, H.L. Atkins, D.L. Arnold; Montreal Neurological Institute (Montreal, CAN); The Ottawa Hospital-General Campus (Montreal, CAN)

Background: Patients with MS often have a substantial burden of subpial demyelinating lesions in their cortical grey-matter on post-mortem histopathology. The vast majority of these lesions are undetected on conventional MRI. Goals: To develop and validate an image-processing method to detect subpial demyelination using co-registered T1-weighted (T1w) and magnetization transfer ratio (MTR) MRIs. Methods: We acquired proton-density, T2-weighted, T1w, and MTR images on a 1.5 T MR scanner with 1 mm in-plane resolution and 3 mm slice thickness. For each neocortical surface voxel, the boundary between the extra-cerebral cerebrospinal fluid and the cortical grey-matter was defined as the maximum of the derivative of the T1w intensity profile along the unit surface normal. Along the same surface normal, a boundary reflecting the gradient in cortical myelination was defined as the maximum of the derivative of the corresponding T1w and MTR intensity profiles followed by quadratic interpolation at the peak, and thresholding at >1 mm to account for the normally hypo-myelinated outer layer of the cortex, the percentage of the brain surface with increased thickness of sparsely myelinated subpial cortex could be quantified. To evaluate the method, we analyzed 2 normal subjects who had been scanned twice with a 2-month interval and 15 MS patients with variable follow-up. Results: In normal subjects, the measurement noise was found to affect 4.3 to 5.6% of the cortical surface and there was very little variation over time. In the MS patients, 5 to 26% (median 10%) of the brain surface analyzed had characteristics consistent with subpial cortical demyelination. Some patients showed a 2-fold increase over 2 to 3 years in the area of the cortical surface affected. Conclusions: We describe an image-processing method to detect cortical regions on T1w and MTR images with characteristics consistent with subpial demyelination. In MS patients, 5 to 26% of the cortical surface area was affected, and some patients showed significant increases over time.

Myelin content is similar in isointense and hypointense T1 lesions

I. Vavasour, C. Laule, A. MacKay, A. Traboulsee, D. Li; University of British Columbia (Vancouver, CAN); UBC MS/MRI Research Group (Vancouver, CAN)

Background: MS lesions are readily seen by conventional MRI and are pathologically heterogeneous. Lesions can undergo many changes including inflammation, edema, demyelination, axonal loss, and gliosis. Hypointense lesions, or “black holes” (BH) on T1-weighted (T1) scans histopathologically represent areas of increased extracellular fluid with matrix destruction and axonal loss. They correlate better with clinical disability than the typical hyperintense lesions seen on proton density (PD) T2-weighted (T2) scans. The present study compared the ability of different MRI scanners to demonstrate T1 BH. Material and Method: Non contrast enhanced 3-mm thick transverse PD and T2 and pre- and post-gadolinium enhanced T1 scans were obtained according to a protocol with strict repositioning criteria for a double-blind placebo-controlled multiple sclerosis treatment trial involving 34 different centres. Using a qualitative scale (1–5, very poor to excellent), the ability to visualize T1 BH was assessed by a single radiologist blinded to site and MRI vendor. T1 BH were defined as MS lesions (evident on the corresponding PD T2 scans) with signal intensity similar to or decreased compared to that of gray matter on T1 scans. Results: Of the 34 MRI scanners, 11 were from Vendor A, 7 from Vendor B and 16 from Vendor C. All scans obtained employed a conventional spin echo sequence. Basic scan parameters were similar: for Vendor A, repetition time (TR) 600 – 660 and echo time (TE) 9 msec were used; for Vendor B, TR 550 – 616 and TE 9 – 11 msec were used; and for Vendor C, TR 550 – 680 and TE 12 – 17 msec were used. The visualization of T1 BH was judged to be very poor/poor (scales 1 and 2) for 8 of 34 scanners (6 for Vendor A, 2 for Vendor C), good (scale 3) for 12 of 34 scanners (3 for Vendor A, 4 for Vendor B, 5 for Vendor C) and very good/excellent (scales 4 and 5) for 14 of 34 scanners (2 for Vendor A, 3 for Vendor B, 9 for Vendor C). Preliminary assessment of differences between the quality of scans from the same vendor suggests one possible factor that may reduce the visualization of T1 BH was the inadvertent magnetization transfer like effect produced by the use of additional radio frequency pulses such as a flow saturation pulse to reduce flow artifacts. Conclusions: This study has found that the visibility of T1 hypointense lesions differs not only between scanners from different vendors but also with scanners from the same vendor. The possible magnetization transfer effect of flow saturation pulses should be controlled in the design of MRI studies monitoring T1 BH.
with white matter (WM) lesions. However, there is no direct evidence (voxel-by-voxel) about specificity of WM lesions topography and memory performance in MS patients. **Aim:** The goal of our study was to determine how the topography of WM lesions was related to memory impairment in MS using a voxel-by-voxel analysis and an unbiased approach about their target locations. In addition, we study the relations between memory-specific WM lesions and grey matter (GM) atrophy. **Methods:** Forty-six patients with MS (McDonald criteria) (38RR/4SP/4PP; EDSS 2.5 (0 to 7.0); disease duration 3.7 years (0.75 to 36)) were assessed with the Buschke’s Selective Reminding test and the 10/36 Spatial Recall test. A 3D T1-weighted magnetic resonance image (axial 2-mm slices; 236 x 256 matrix, TE 4.6, voxel size 0.98 x 0.98 mm²) was also obtained from each of them. The WM lesions topography was analyzed using 3D lesion probability maps, and the GM atrophy using a modified protocol of the optimized voxel-based morphometry method to avoid the influence of MS lesions in the normalization and segmentation procedures. We used voxel-by-voxel multiple linear regressions for the statistical analysis (p < 0.05 corrected by multiple comparison). **Results:** We identified five significant WM lesion locations that were negatively correlated with verbal memory storage. These were located in each temporal lobe, in the left thalamus, in the anterior limb of the left internal capsule and in the left parietal centrum semiovale. We also identified another five negatively correlated with verbal memory retrieval in the left temporal lobe, the left fronto-parietal and right parietal centrum semiovale, the left thalamus and the right anterior limb of the internal capsule. Finally, the verbal memory-specific WM lesion topography was associated with the atrophy in certain memory-related GM regions, particularly in the thalamus (anterior, midline and dorsomedial nuclei) and prefrontal cortex (inferior frontal gyrus, pars triangularis). **Conclusions:** We identified the WM lesions topography related to memory impairment in MS. Our study highlights the importance of the selective damage of short WM tracts for memory storage, and of short and long WM tracts for memory retrieval. Finally, we described the contribution of the memory-specific WM topography in the atrophy of GM regions.

**P644**

**Longitudinal study of diffusion weighted MRI in relapsing-remitting multiple sclerosis**

S. Latourte, E. Salort-Campana, M. Deloire, M. Bonnet, K.G. Petry, V. Douset, B. Brochet; University V. Segalen (Bordeaux, F)

**Background:** Diffusion weighted MRI (DWI) provides quantitative measurements of different aspects of tissue microstructure obtained by using the properties of water diffusion in the brain. It may reflect measurements of different aspects of tissue microstructure obtained in normal appearing white matter (NAWM) and in normal appearing grey matter (NAGM). Mean ADC was calculated. **Results:** EDSS remained stable in RRMS but worsened significantly in progressive MS during the three years. In the whole group, mean NABT ADC decreased significantly (p < 0.001) during the first year and remained stable the second year. In progressive MS but not RRMS, we observed correlation between mean NAWM ADC at baseline and EDSS change during 3 years (r = 0.33, p = 0.02).

**Conclusion:** NAWM ADC measure close to MS diagnosis is correlated with progression of disability. This work is supported by grants of ARSEP and Schering France SA.

**P645**

**Fibre tracking detects corticospinal tract changes due to inflammatory lesions**

K. Boehnans, J. Kaufmann, N. Bodammer, H. Heinzé, W. Brack, M. Sailer; Otto-von-Guericke University (Magdeburg, D); Georg-August University (Gottingen, D)

The present study was designed to analyze the time course of fibre tracking results in corticospinal tracts (CST) affected by prominent inflammatory lesions. Based on conventional magnetic resonance imaging (MRI) two patients with large paraventricular lesions of comparable size including CST fibres were selected. T1-weighted imaging after administration of Gd-DTPA showed hyperintense signal in both lesions. Both patients reported a sudden onset of clinical symptoms including hemiparesis and Babinski sign. Over a period of 16 months, we sequently performed tractography of CST fibres based on diffusion tensor MR imaging (DTI), which was carried out on a 1.5 T GE tomograph. A self-written Monte-Carlo-based fibre tracking algorithm allows for counting tracks that are outgoing from start regions at the level of the medulla oblongata. In case of 16-year old patient A, a partial regeneration of CST fibres was detected compared to the initial examination shortly after the onset of symptoms. This finding might indicate a tissue repair process and corresponds to an improvement of the patient’s clinical outcome. In contrast, the 28-year old patient B showed a conspicuous continued reduction of the number of CST tracks during the whole examining period at the level of the cerebral peduncle and the posterior limb of the internal capsule. Additionally, for patient B the quantitative diffusion parameters within the CST caudal of the large lesion showed an increase of the apparent diffusion coefficient (ADC) and a decrease of the fractional anisotropy (FA). These changes were observable clearly before any evidence for Wallarian degeneration was given by T1-weighted images. Brain biopsy revealed an inflammatory process consistent with a multiple sclerosis in both patients. Immunohistopathological examination showed an immunosubtype I for patient A and an immunosubtype III for patient B, corresponding to the classification scheme by Lucchini et al. (Ann Neurol, 2000). This finding, suggesting a higher chance for neuronal regeneration in patient A, is in accord with the fibre tracking results. To summarize, fibre tracking based on DTI is a potential technique to detect changes in CST fibres outside of the lesion and might be useful to differentiate heterogeneous pathologic substrates of individual lesions. According to the patients’ clinical outcome it may serve as a predictor before CST fibre changes are detectable in conventional MR imaging.

**P646**

**Development of optic nerve diffusion weighted magnetic resonance imaging for translational research**

T. Kilpatrick, H. Butzkueven, P. Mitchell, Q. Wang, S Kolbe, C. Kokkinos, G. Edgar; University of Melbourne (Melbourne, AUS); Howard Florey Institute (Melbourne, AUS); Royal Melbourne Hospital (Melbourne, AUS)

Axonal degeneration probably drives disability in multiple sclerosis, leading to interest in regenerative therapies but methods to test efficacy are lacking. Phenotypic analysis in animal models is an imperfect measure of response but histological analysis is time consuming, indicating need for non-invasive surrogates, in particular of axonal pathology. Ideally, the same surrogates could be used in early phase clinical trials. Optic neuritis is a good focus for translational research. The optic nerve is affected in experimental

Downloaded from msj.sagepub.com by Shula Edelkind on October 1, 2010
allergic encephalomyelitis (EAE) and in humans, optic neuritis can be rigorously phenotyped but whereas clinical recovery is often complete, residual axonal degeneration is common. Surrogates, such as magnetic resonance imaging (MRI), and in particular diffusion imaging which can identify axonal structure, could increase sensitivity to identify a therapeutic response. Methods: Healthy and EAE animals were studied via a Bruker 4.7 Tesla MR system with apparent diffusion coefficient (ADC) mapping via spin echo diffusion tensor imaging (DTI). Histology was assessed by toluidine blue staining. Healthy humans had a three orthogonal direction diffusion weighted MRI scan on a GE 1.5 Tesla MR system. Diffusion perpendicular to the nerve was directly measured. Due to variable angulation in the y axis, parallel diffusion was calculated as ADC/cos². Results: Mean ADC for healthy animals (n=10) parallel to the nerve was 1.64 × 10⁻³ mm² s⁻¹ (SD = 0.18) and in EAE (n=12), 1.24 × 10⁻³ mm² s⁻¹ (SD = 0.14; p = 1.4 × 10⁻⁷). In EAE, there was a correlation between disease grade and parallel DTI reduction (R² = 0.63). No change was identified in ADC perpendicular (healthy 0.83 × 10⁻³ mm² s⁻¹ (SD = 0.14); EAE 0.72 × 10⁻³ mm² s⁻¹ (SD = 0.11; p = 0.32). Histology revealed axonal degeneration, without demyelination. In the humans, mean ADC values parallel and perpendicular to the nerve (n=6) were 1.38 × 10⁻³ mm² s⁻¹ (SD = 0.31) and 0.85 × 10⁻³ mm² s⁻¹ (SD = 0.17; p = 0.004). Conclusion: We have identified similar patterns of diffusion in healthy murine and human optic nerves and changes in ADC parallel in EAE, consistent with axonal degeneration. Values of diffusion using a standard clinical scanner available for multiscientific use were similar to those previously generated using diffusion tensor sequences on research scanners. We will now assess putative regenerative therapies by MRI in EAE and assess correlations between diffusion imaging and clinical indices in human optic neuritis.

P647
Diffusion tensor imaging in multiple sclerosis is more sensitive in the detection of active lesion pathology than in normal appearing white matter
D. Jeffery, Y Feng; Wake Forest University School of Medicine (Winston-Salem, USA)

Objective: To determine whether diffusion tensor imaging (DTI) metrics are able to distinguish between homogeneously and ring enhancing lesions and to determine whether there is a difference in fractional anisotropy (FA) and apparent diffusion coefficient (ADC) in normal appearing white matter (NAWM) and lesions in relapsing-remitting and secondarily progressive (SP) MS. Background: Conventional MRI has been informative in MS but lacks pathological specificity. Diffusion tensor imaging measures the diffusion of water molecules along fiber tracts and may provide a more sensitive measure of tissue integrity. It is unclear whether DTI is a more sensitive measure of degenerative NAWM pathology or inflammatory change.

Design: This cross sectional study enrolled 29 patients and 6 healthy controls at the time of this abstract submission. Patients were scanned on a 3T GE magnet. Sequences included a T1 FLAIR, T2 FLAIR, post contrast SPGR, and DTI. FA and ADC were measured in enhancing lesions, nonenhancing T2 lesions, and in NAWM. Results: Twenty-nine patients were studied (24 RR and 5 SP) and compared to six controls. Disease duration was significantly longer in SP MS than in RR MS (14.5 ± 4.9 vs. 7.2 ± 5.3). NAWM FA was significantly decreased and ADC increased in RR and SP MS compared to controls but neither ADC nor FA in NAWM differed between RR and SP MS patients. In contrast, ADC values were significantly increased in ring enhancing lesions versus homogeneously enhancing lesions (1.08 ± 0.18 vs. 0.88 ± 0.1 p = 0.05, respectively). No change in T2 lesion FA and ADC did not differ between RR and SP MS. Conclusions: Diffusion tensor imaging is more sensitive in the detection of inflammatory changes in lesions than to degenerative changes in NAWM. Ring enhancing lesions showed a greater increase in ADC than homogeneously enhancing lesions but neither ADC nor FA was able to differentiate between NAWM in RR versus SP MS.

P648
A longitudinal study of T1 relaxation time in normal appearing white matter and grey matter in early primary progressive multiple sclerosis

Introduction: Increased T1 relaxation time (T1-RT) has been related to inflammation and axonal loss. Longitudinal changes in T1-RT have not been studied in patients with primary progressive MS (PPMS). Aims: To detect T1-RT changes in normal appearing white (NAWM) and gray matter (NAGM) in early PPMS over 2 years; to analyze whether T1-RT changes correlate with clinical changes; to investigate whether T1-RT at baseline predicts clinical outcomes. Methods: Twenty-five patients (11 male, 14 female, mean age 47.8, range 26–60) with early PPMS (within 5 years of symptom onset) and 12 controls (6 male, 6 female, mean age 34.0, range 27–52) were studied at baseline and 2 years. EDSS and MSFC (including time walk test) scores were assessed at baseline and two years. All subjects underwent 2D dual echo FSE, 2D T1 and 2D PD-weighted gradient echo scans, from which T1-RT maps were generated, on a 1.5 T scanner. T1-RT histograms of NAWM and NAGM, normalized for brain volume, were obtained at each time point. Mean T1-RT, peak heights (PH) and peak locations (PL) of the histograms were measured. Paired t-tests were used to compare baseline and 2 year histogram values in patients and controls. Spearman’s correlation coefficient was used to assess the relationship between T1-RT and clinical changes. To investigate whether T1-RT predicts clinical changes multiple linear regression analyses were applied. Age and sex were kept as covariate in the statistical model. Results: There were no changes in T1 parameters in controls. In the MS cohort there were increases in NAWM and NAGM mean T1-RT (p = 0.002 and p = 0.009 respectively) and PL (p = 0.009 and p = 0.047 respectively). Moreover, NAWM and NAGM PH decreased significantly (p = 0.015 and p = 0.026 respectively). NAWM and NAGM mean T1-RT changes over two years correlated with changes in the EDSS (p = 0.001; p = 0.003) and time walk test (p = 0.016, p = 0.027). Lower MSFC scores at two years were predicted by higher baseline mean NAWM T1 values and lower baseline NAWM histogram peak heights. Conclusion: T1 relaxometry detects substantial changes in NAWM and NAGM over two years in early PPMS and these changes correlate with increased disability. T1-RT parameters at baseline predict clinical outcome at 2 years. These results suggest that T1 relaxometry is a good marker of disease progression and has prognostic potential in PPMS.

P649
Optic nerve head topography in optic neuritis
S.A. Trip, P. Schlothmann, S. Jones, D.F. Garway-Heath, A. Thompson, G. Plant, D.H. Miller; Institute of Neurology (London, UK); Moorfields Eye Hospital (London, UK); National Hospital for Neurology and Neurosurgery (London, UK)

Background: Optic neuritis is a useful model for studying the relapse in MS as non-invasive imaging can be combined with electrophysiological and clinical measures of anterior visual pathway function to provide insights into the pathophysiological mechanisms associated with relapse and recovery. Scanning laser tomography performed with the Heidelberg Retina Tomograph II (HRT-II) can be used clinically to produce three dimensional images of the optic nerve head including measures of axonal loss from the optic nerve neuroretinal rim and retinal nerve fibre layer (RNFL). Although the main current clinical application for the HRT-II is to monitor progression of glaucomatous changes over time, it could potentially be used to study optic nerve head topography in other optic neuropathies. Objective: To investigate optic nerve head topography in patients with optic neuritis compared to controls using the HRT-II and determine if detected changes are related to visual function and electrophysiology. Subjects/Methods: 25 patients with a previous
single episode of unilateral optic neuritis and 15 controls were studied with HRT-II, visual evoked potentials and pattern electroretinogram. Patients also had testing of visual acuity, visual field and colour vision.

**Results:** In affected eyes compared to fellow eyes, there was reduction of both the mean RNFL thickness at the disc edge (p=0.009) and the neuroretinal rim volume (p=0.04). In affected eyes compared to control eyes, the three dimensional optic cup shape was increased (p=0.01), indicative of an abnormal cup shape. There were no other significant differences in HRT-II measures. Within-patient interocular difference correlation was used to investigate the functional relevance of these changes and demonstrated associations between RNFL thickness change and changes in visual acuity, visual field and colour vision. Colour vision change was also associated with change in neuroretinal rim volume. **Conclusions:** HRT detects functionally relevant changes in RNFL thickness and neuroretinal rim volume between eyes affected by optic neuritis and unaffected fellow eyes.

**P660**

*In vivo* detection and monitoring of inflammatory lesions in experimental autoimmune encephalomyelitis using SPIO-enhanced MRI

A. Oweida, E. Daun, P. Foster; Roberts Research Institute (London, CAN)

Multiple sclerosis (MS) is characterized by breakdown of the blood brain barrier (BBB) and the formation of focal areas of inflammation and demyelination in the brain and the spinal cord. Currently, gadolinium-enhanced (Gd-enh) MRI is the most common tool for diagnosis and monitoring of MS lesions and assessing brain inflammation. However, the simple movement of Gd across a transiently broken BBB does not provide insight on the cellular components of inflammation. Disease severity in experimental autoimmune encephalomyelitis (EAE) has been shown to correlate with the absolute number of macrophages invading the brain parenchyma. Various studies have shown that superparamagnetic iron oxide (SPIO) can be used to label macrophages in vivo during active inflammation and to detect the label in the CNS using MRI. In the present study our goal was to analyze lesion number and size longitudinally in EAE animals by giving multiple SPIO injections. SPIO was systemically injected into the circulation of EAE-induced mice on day 11 post-immunization and at multiple time-points thereafter (days 13,15,17,19,21). Imaging was performed 24 hours after each SPIO injection. On the final scanning session (day 22), animals were sacrificed and brains were excised for histopathology. Staining was comprised of Perl’s Prussian blue stain for iron and hematoxylin and eosin to detect cellular infiltrates. Our results show that regions of signal loss can be observed even prior to initiation of clinical symptoms. Multiple SPIO injections revealed increasing number of lesions over the course of EAE. The average number of lesions detected at disease onset (11 lesions, day 14) was much less than that detected during later stages of EAE (28 lesions, day 18). Lesion number and size was variable at different time-points during the disease course and even within the same clinical score. In some cases, the maximum size of lesions occurred 1-2 days after the animals reached their peak clinical score. Histological analysis showed excellent correspondence between regions of signal loss on MR images and Perl’s-positive cellular infiltrates on histology. Our results demonstrate that SPIO is a reliable marker for assessing the degree of inflammation in the CNS. Future studies will involve cross-sectional histopathological analysis for validation with MR results.

**P651**

USPIO-enhanced cellular MR imaging of lesion activity in multiple sclerosis

M.M. Vellinga, J.J.G. Geurts, S.M.A. van der Pol, C. Pering, C.H. Polman, H.E. de Vries, F. Barkhof; VU University Medical Center (Amsterdam, NL); Schering AG (Berlin, D)

**Background:** New therapies in multiple sclerosis (MS) aim at preventing cellular infiltration. In evaluating their therapeutic efficacy, ultrasmall superparamagnetic particles of iron oxide (USPIO), taken up by macrophages and transported into inflammatory MS lesions, may be a more specific MRI marker than Gadolinium-DTPA (Gd), which only visualizes breakdown of the blood-brain-barrier. **Objective:** Visualizing cellular infiltration in MS inflammatory lesions using USPIO and comparing it to Gd enhancement in a phase II setting. **Patients and Methods:** Relapsing-remitting MS patients are currently screened monthly using TI-weighted spin-echo, dual-echo T2-weighted spin-echo, and T2 gradient-echo. In case of a Gd-enhancing lesion (GEL), USPIO (SH U 555 C, Schering AG, Berlin (Germany), diameter: 25 nm, T1/2 6-8 h) are administered (single intravenous bolus injection of 40 micromol Fe/kg BW) within 24-48 hours. Twenty-four hours after injection, MRI is performed (same protocol) and blood is withdrawn to evaluate monocyte activation level and USPIO-uptake. Follow-up of lesion progression consists of 3 monthly scans. EDSS and relapses are registered. **Results:** So far, 12 patients have been included, 4 of which have received SH U 555 C. More patients will be included. In our preliminary dataset, USPIO-enhancement (hyperintense on T1) occurred in 4 different patterns: 1) ring-like, surrounding a GEL; 2) as small clusters around original GEL; 3) as an isointense signal in originally hypointense lesions (pre contrast T1); 4) as enhancement not visible as GEL on prior images, some Gd enhancement at 1 month follow-up. Not all GEL showed USPIO enhancement. No signal changes were observed on the gradient-echo T2. Results on evaluation of monocyte activation level and USPIO-uptake are pending. **Conclusion:** USPIO-enhancement is based on infiltration of labelled macrophages into inflammatory lesions, and shows patterns that are distinct from Gd enhancement in MS. SH U 555 C may be used to increase specificity when evaluating efficacy of future MS therapies such as cellular infiltration prevention.

**P652**

A diffusion-tensor MRI study of brain and cervical cord in benign multiple sclerosis patients

E. Judica, M. Rovaris, E. Perego, B. Benedetti, P. Valdsasina, V. Martellini, R. Capa, A. Ghezzi, A. Pulzzi, G. Comi, M. Filippi; Scientific Institute San Raffaele (Milan, I); Spedali Civili di Brescia (Brescia, I); Ospedale di Gallarate (Gallarate, I)

**Background:** Diffusion tensor (DT) MRI is able to quantify the severity of brain and spinal cord damage in multiple sclerosis (MS), thus providing us *in vivo* with information about the extent of tissue damage that can be undetected by conventional MRI. **Goals:** To investigate the DT MRI patterns of brain and cervical cord structural damage in benign (BMS) versus secondary progressive MS (SPMS) patients, to achieve a better understanding of the nature of disability in MS. **Methods:** Conventional and DT MRI scans of the brain and the cervical cord were acquired from 55 patients with BMS, 36 with SPMS and 15 healthy controls. After the creation of mean diffusivity (MD) and fractional anisotropy (FA) maps, histograms of MD and FA values were produced for the brain normal-appearing white matter (NAWM) and gray matter (GM), as well as for the cervical cord tissue as a whole. T2-hyperintense brain lesion volume (LV) and normalized brain volume (NBV) were also computed. **Results:** Median expanded disability status scale (EDSS) score was 2.0 (range: 0.0-3.0) for BMS and 6.0 (range: 4.0-8.0) for SPMS patients. Compared to healthy controls, BMS patients had lower average NAWM FA (p<0.001), higher average GM MD (p<0.001) and lower NAWM and GM MD peak height (p<0.001). When compared to BMS patients, SPMS patients had higher T2 LV (p=0.004), lower NBV (p=0.002), higher average GM MD and lower GM MD peak height (p=0.0019 and 0.007). In comparison with healthy subjects, BMS patients also had higher average cord MD (p<0.001) and lower cord MD peak height (p=0.005). SPMS patients had lower cord average FA (p=0.009) when compared to those with BMS. **Conclusions:** The severity of brain
Immunomodulation-Part II

P653

Validating diffusion tensor imaging as a measure of physiologic disruption
R.J. Fox, R. McColl, J.C. Lee, T. Frohman, E. Frohman; Cleveland Clinic Foundation (Cleveland, USA); University of Texas Southwestern (Dallas, USA)

Background: Diffusion tensor imaging characterizes MS tissue injury, although it has remained unknown whether DTI changes in disease have functional consequences. The median longitudinal fasciculus (MLF) is a key brainstem pathway for ocular adduction. Injury to the MLF causes internuclear ophthalmoplegia, which is common in MS. We evaluated the relationship between MLF pathology and oculomotor dysfunction using DTI and oculography.

Methods: The MLF was identified in 6 MS patients with ophthalmoplegia and 10 healthy controls (HC) through a brainstem atlas overlay. Average mean diffusivity (MD) and lattice index (LI, a measure of anisotropy) was measured within the pontine MLF. Ocular dysmotility was characterized by the versional dysconjugacy index (VDI), which is the ratio of abduction/adduction eye movement parameters, measured using quantitative infrared oculography. Group differences between MS and HC were evaluated using repeated measures mixed model, while Pearson correlation coefficients evaluated the relationship between VDI and DT measures in the combined MS and HC groups.

Results: The MD within the pontine MLF was increased compared to HCs (p < 0.003), while the pontine LI was decreased (p < 0.03). As expected from patient selection, VDI was significantly increased in MS patients with INO compared to HC (p < 0.0001). Correlations were observed between the VDI and MD (left: r = 0.65, p < 0.01; right: r = 0.46, p = 0.07). Similar correlations were found between VDI and LI (left: r = 0.43, p = 0.09; right: r = 0.65, p = 0.001). Discussion: We identified DTI evidence of pathological disruption of a small brainstem fiber pathway which is crucial for accurate lateral eye movements. Measures of both overall diffusion (MD) and anisotropy (LI) were altered in the pontine MLF, where 12 prolongation was also observed. More importantly, we observed correlations between DTI changes and oculomotor dysfunction. To our knowledge, this study is the first to correlate pathology measured by DTI with electrophysiologic evidence of impaired CNS function. Our observations support the hypothesis that the structural measures of DTI represent pathologic disruption with significant physiologic consequences. These cross-sectional studies provide validation that DTI may be a useful outcome measure in therapeutic studies.

P654

Long-term favourable response to immunomodulatory treatment in Hungarian patients suffering from multiple sclerosis
T. Csépány, Z. Mezei, D. Bereczki, S. Sipka, L. Csiba; University of Debrecen (Debrecen, HUN)

Background: Interferon beta and glatiramer acetate (Copaxone) since 1996, intra-muscularly administered interferon beta 1a (Avonex) since 1999 and subcutaneously administered interferon beta 1a (Rebif) since 2001 are available for treating RRMS patients in Hungary. Goal: The primary endpoint of our study was to evaluate the response of the long-term immunomodulatory treatment on relapse number and different immunological laboratory data of peripheral blood (cells counts, levels of different immunoglobulins, antimuclear antibodies, T, NK cells and cytokine levels). We evaluated the safety of long term therapy also. Patients and methods: 79 relapsing-remitting multiple sclerosis (mean age 35.4 ± 10.6 years, duration of the disease 3.8 ± 5.7 years) patients were treated with immunomodulatory drugs between 1996 – 2005 for 53.9 ± 35.4 months at the Dept of Neurology, Debrecen University (group A: n = 20, Betaferon subcutaneously (s.c.) injected 8 MIU every other day, group B: n = 25, Copaxone s.c. injected 20 mg every day, group C: n = 27, Avonex intramuscularly (i.m.) injected 30 ug once a week, group D: n = 7, Rebif subcutaneously (s.c.) injected 44 ug three times a week). The duration of the treatment 7 ± 4.2, 5.2 ± 2.1, 3.1 ± 0.7 and 3.4 ± 0.4 years were in the groups, respectively. The clinical status (expanded disability status score (EDSS), relapse, side effects were controlled every 3 months and laboratory control was performed every 6 months. Results: The relapse number was reduced in every group. The relapse rate was changed from 2.8 to 1.4 in group A, from 1.8 to 0.2 in group B, from 2.4 to 0.9 in group C and 2.1 to 0.3 in group D comparing the pretreatment and treatment 2 years period of disorder. The EDSS increased from 3.4 ± 1.8 to 4.8 ± 1.1 after 81.9 ± 6.4 months in group A and from 3.0 ± 0.7 to 3.8 ± 0.4 after 52.7 ± 7.1 months and 7 – 7 patients changed the course of disorder to secondary progressive. The EDSS was not changed in the other 2 groups, but the duration of those patients treatment was shorter than in group A and B. Decreased white cells counts was more frequent in the group A than the other groups. Discussion: Our results support the long-term favorable effect of immunomodulatory treatment in MS patients. The possible relation of laboratory changes (the percentage of CD4+, CD8+ cells, TNF alfa, interferon gamma, interleukin 10 and TGF beta levels in the peripheral bloods) to clinical status will be discussed in the presentation.

P655

Raised urinary neopterin excretion is associated with a better clinical outcome in PP-MS patients treated with interferon beta-1a
K. Réjak, S.M. Leary, A. Petzold, Z. Stebniak, A.J. Thompson, D.H. Miller, G. Giovannoni; Medical University of Lublin (Lublin, PL); Institute of Neurology, UCL (London, UK)

Background: Primary progressive multiple sclerosis (PP-MS) has clinical characteristics distinct from other MS subtypes and the immunomodulatory treatment does not affect the course of the disease. However, the biomarkers of a therapeutic effect are needed to assess therapeutic efficacy even without apparent clinical effects. The aim of this study was to assess the utility of monitoring the urinary excretion of neopterin and nitric oxide (NO) metabolites in subjects with PP-MS treated with interferon beta-1a (IFNBeta-1a).

Methods: Fifty subjects with PP-MS enrolled in a phase II trial of IFNBeta-1a (Placebo n = 20; Avonex® 1 × 30 µg/week (INF-30), n = 15; Avonex® 1 × 60 µg/week (INF-60) n = 15), were studied. Study participants were assessed using the EDSS and with MRI. Urine samples were collected every 3 months for two years. Nitrite/nitrate (NOx) and neopterin/Cr (NOxCR) quotients were measured by vanadium based colorimetric assay, and neopterin and creatinine (Cr) using an HPLC technique (NOx/µmol Cr (µmol Cr) (NOxCR) and neopterin/Cr (µmol Cr) (UNCR) quotients were calculated. Results: There was no significant difference between pre-treatment baseline levels of UNCR or NOxCR between the three study groups. For the longitudinal analysis, only subjects who completed the study on their assigned dose of IFNBeta-1a were analysed (placebo, n = 20; INF-30, n = 12; INF-60, n = 6). There was a significant overall difference in UNCR levels between the placebo-treated group (278.2 ± 32.7; mean ± SD) and the INF-30 (431.0 ± 63.5)
Abstracts

P656

10 year follow-up of the European Study of interferon-beta-1b (EUSPMS) in secondary progressive multiple sclerosis – Interim report


Objective: The European study of interferon beta-1b (IFNb-1b) in secondary progressive MS (EUSPMS) provided evidence that IFNb-1b delays neurologic deterioration in patients in this phase of the disease. Data on long-term effects and adherence to IFNb-1b treatment in SPMS are scarce. We present an interim report of the ongoing 10 year follow-up (LTFU) of the EUSPMS Study. Methods: After the double-blind phase of this trial (DB) which lasted up to a maximum of 36 months, patients were offered participation in an open-label treatment study (OL) that lasted another 18 months. Since then patients are followed in 6 or 12 monthly intervals in the participating centres. From the initial total of 718 patients in 35 centres, until May 1st 2006 we have collected 10 year data including EDSS, relapses and treatment history of 239 patients (73.5% of 325 originally randomised in these centres) from 15 centres. Results: 120/239 received placebo and 119 IFNb-1b during the DB phase. At month 120 in A was 2.21/1.53 and in B 1.87/1.9 (p = 0.03) and INF-60 treated groups. The mean UNCR of the INF-30 and INF-60 treated groups did not differ. Conclusion: Urinary neopterin may be a candidate marker to monitor one of the in vivo effects of IFNb-1a and has a potential of identifying subgroups of MS patients responding to the therapy.

P657

Axonal damage and inflammation in early multiple sclerosis: effects of subcutaneously interferon-beta-1a treatment

A. Pascual-Lozano, M.C. Martínez-Bisbal, I. Bosca, C. Valero, F. Coret, B. Martínez-Granados, L. Martí-Bonmatí, B. Celda, B. Casanova; Hospital la Fe (Valencia, E); Universidad de Valencia (Valencia, E); Hospital Clínico (Valencia, E); Hospital Quiron (Valencia, E)

Background and Objective: Several multicenter, randomized, double-blind and placebo-controlled trials have demonstrated the effect of interferon beta in reducing inflammatory activity in multiple sclerosis (MS), but a possible effect on accumulated neuroaxonal damage is still unknown. This is a prospective two-year follow-up study designed to examine the effect of interferon beta-1a (IFNb1a) over relapse rates, total brain T2 lesion volume and brainstem metabolic changes, comparing treated and untreated MS patients. Methods: Sixteen early relapsing-remitting MS patients treated with IFNb1a, twenty RRMS patients untreated and ten age-matched healthy subjects were studied for two years. Between treated and untreated patients there were no basal differences in sex, age, EDSS and evolution time. Disability was rated in each scheduled visit using the EDSS. Relapse rate (RR) was calculated annually. T2-weighted MR and 1H-MRS imaging were acquired at recruitment and at year two. The brain T2-hyperintense lesion volume (T2LV) was calculated with a semiautomatic program; NAA, Cr and Cho resonances areas were integrated with MRUI program and the ratios calculated for the sum of the volume elements that represented the brainstem. Results: The basal NAA/Cr ratio at brainstem was significantly decreased in MS patients compared with controls. After two years, there was a decrease in the NAA/Cr (p = 0.002) and the NAA/Cr ratio (n.s); p = 0.000), meanwhile controls subjects had no significant metabolic changes. Pre-treatment annualized RR was 1.9 in the treated group, and it decreased to 0.98 after treatment. EDSS did not change significantly within two years (untreated group: 1.2 vs. 1.0; treated group 1.7 vs. 1.5). In untreated patients, T2LV increased significantly over two years (mean 2.4 ml vs. 3.4 ml, p = 0.025), whereas in treated patients T2LV remained stable (5.1 ml vs. 5.3 ml, ms). The metabolic study showed a decrease in the NAA/Cr ratio in both groups of patients (untreated group: 4.9 vs. 4.0, p = 0.006; treated group: 4.4 vs. 3.8, p = 0.007). Conclusions: IFNb1a was successful in reducing the annual RR and the accumulation of disease burden on T2-weighted images but, treated and untreated MS patients suffered similar metabolic decrements. Therefore IFNb1a treatment did not be effective in delaying neuroaxonal damage in this short-term study. Analyses with longer periods of observations are still needed to confirm these preliminary findings.

P658

Evaluation of topical interventions in the reduction of injection-site reactions after interferon treatment for multiple sclerosis

D. Mikol for the ASSIST Study Group

Background: Injection-site reactions (ISRs) are common in treatment with injected multiple sclerosis (MS) therapies. Although ISRs are typically mild, the risks associated with decreased adherence are important, as it is essential to maintain continuous active treatment to reduce inflammatory activity and ultimately slow disease progression. Based on anecdotal evidence, over-the-counter (OTC) topical anti-inflammatory agents are often recommended to treat ISRs, but their efficacy has not been studied in well-controlled clinical trials. Objective: To evaluate three OTC topical medications’ abilities to reduce ISRs in patients with relapsing-remitting MS treated with Rebif (registered trademark [R] (interferon-beta-1a sc, tin). Design: This study planned to enroll 100 subjects with an interim analysis to assess patients that completed the study. Two independent, 9-week, multicentre, open-label, crossover studies were designed to compare Cortizone-10(r) (hydrocortisone) and Tucks(r) Pads (witch hazel) to Lubriderm(r) Lotion, which served as a control. Patients with ISRs (defined as redness at least 20 mm in diameter 48 – 72 hours [h] post-injection) were randomly assigned to apply a test agent or Lubiderm(r) immediately post-injection for 2 weeks. Participants then switched from control to test agent or vice versa for the final 2 weeks. ISRs were measured 48 – 72 h and 7 days (d) post-injection. The primary outcome measure was the difference in the mean diameter of redness 48 – 72 h post-injection. Results: At interim analysis (n=60), 29 of 100 expected subjects had completed the study. Subjects were typically female (88.9%) and Caucasian (94.4%), with a mean age of 41.4 years. In the hydrocortisone/Lubiderm(r) cross-over
P659

Product enhancements decrease the incidence of injection site reactions and pain resulting in improved adherence to therapy in patients with multiple sclerosis

J. Scanzillo, R. Bennett, P. Biancucci, V. Divan, S. Sherman, A. Al-Sabbagh/Serono, Inc. (Rockland, USA); Pfizer Inc. (New York, USA)

Background: Injection site reactions (ISRs) are a common cause of treatment discontinuation in patients with multiple sclerosis (MS). Support organizations such as the MS LifeLines program (www.mslifelines.com) may help patients to manage ISRs more effectively by providing support, education, and training on injection technique; however, simplification of the injection process through use of autoinjectors and finer gauge needles may also help to decrease ISRs. Objective: Evaluate the impact of product enhancements such as a new autoinjector (Rebiject II) and finer needles upon adherence to therapy for patients receiving subcutaneous (sc) Interferon-beta-1a (IFNβ-1a) 44 mcg tiw. Methods: Data were gathered by the MS LifeLines program between August 2003 and November 2004 (before product enhancements) and again between December 2004 and March 2006 (after product enhancements). Patients were contacted by nurse educators at regular intervals and asked a series of questions to determine whether they were adherent to therapy or had discontinued, and, if they had discontinued, why they had done so. Reasons for discontinuation were recorded and divided into 3 categories: ISRs, pain and/or burning at the injection site, or other reasons. Interim data from this ongoing analysis are presented. Results: Between August 2003 and November 2004, 11,783 total patients received subcutaneous (sc) IFNβ-1a 44 mcg tiw. Of the 2079 patients who discontinued therapy (17.6%), 1714 (82.4%) cited reasons other than ISRs or injection pain and burning. Of the remaining discontinuations, 190 (9.1%) were due to ISRs and 175 (8.4%) were due to pain and/or burning at the injection site. Between December 2004 and March 2006, when Rebiject II and finer gauge needles were incorporated, 12,968 total patients received sc IFNβ-1a 44 mcg tiw. Of the 1780 (13.7%) who discontinued therapy, 1648 (92.6%) cited reasons other than ISRs or pain or burning. Of the remaining discontinuations, 99 (5.6%) were due to ISRs and 33 (1.9%) were due to pain and/or burning at the injection site. Conclusions: These results demonstrate that product enhancements such as Rebiject II autoinjector and finer gauge needles have dramatically decreased discontinuation of therapy due to overall ISRs and injection pain or burning in patients administering sc IFNβ-1a 44 mcg tiw. The decreased discontinuation to therapy may potentially improve long-term outcomes.

P660

Effectiveness of interferon-beta in delaying the age at secondary progression and at assignment of irreversible disability milestones in multiple sclerosis

M. Trojano, M.P. Anzato, F. Pellegrini, D. Paolicelli, A. Fuiani, V. Zippoli, E. Di Monte, E. Portaccio, G.B. Zimatore, F. Livrea; University of Bari (Bari, I); University of Florence (Florence, I); Consorzio Mario Negri Sud (Santa Maria Imbaro, Chieti, I)

Background: Recent studies suggest that the reaching of disability landmarks in Multiple Sclerosis (MS) is age-related, at least in part. Survival techniques which take account of the assessment of ages at assignment of Secondary Progression (SP) and irreversible disability milestones may provide accurate outcome for therapeutic trials. Objective: To evaluate the effectiveness of IFN-beta in a cohort of Relapsing Remitting (RR) MS patients on two milestones: age at SP and age at EDSS 4. Methods: A sample of 1,452 RR MS patients (1046 IFN-beta treated and 406 untreated) was prospectively followed, at the MS Centers of Bari and Florence, for at least 1 year from first visit up to 7 years (median follow-up = 4.7 years). Ages at milestones were calculated as time from birth to each milestone and considered as survival time. To account for baseline imbalances between treated and untreated groups, a Cox regression model adjusted for propensity score (PS) quintiles was used. Estimated ages at milestones were obtained using PS adjusted survival curves. We also performed a sensitivity analysis to account for potential residual confounding deriving from the effect of an unmeasured binary covariate. Results: PS adjusted Cox models for age at milestone SP showed that IFN-beta group vs. untreated group had a highly significant 70% reduction of incidence of SP during 7 years (HR = 0.30, 95% CI 0.17 – 0.52, p < 0.0001). Milestone EDSS 4 also reported a significant 36% difference in favour of IFN-beta group (HR = 0.64, 95% CI 0.43 – 0.97, p < 0.05). PS adjusted survival curves translated HRs into the estimated delay of age at the two milestones. Milestones SP and EDSS 4 were reached by 30% of patients with 11 years (48.6 for untreated vs. 59.8 for treated) and 4.2 years (45.7 for untreated vs. 49.9 for treated) of difference respectively between the two groups. Sensitivity analysis for milestone SP showed that significant effect of IFN-beta might be altered by an unmeasured confounder with a HR of at least 4 with a prevalence imbalance between treated and untreated groups of at least 40%. As to milestone EDSS 4, an unmeasured confounder with HR ≥2 and 10% of imbalance would be sufficient to alter the significant effect of treatment. Conclusions: The results of this observational study in a large MS population demonstrate that IFN-beta treatment delays the age at assignment of SP and irreversible EDSS 4. However appropriate statistical analyses need to properly estimate the real treatment effectiveness.

P661

Intramuscular interferon beta-1a delays conversion to clinically definite multiple sclerosis in patients with a clinically isolated syndrome: subgroup analyses based on new diagnostic criteria

R.P. Kinkel, P.W. O’Connor, M. Kronenchutzky; Harvard Medical School (Boston, USA); St. Michael’s Hospital (Toronto, CAN); University Hospital London Health Sciences Centre (London, CAN)

In CHAMPS, treatment with intramuscular (IM) interferon (IFN) beta-1a (30 mcg once weekly) significantly delayed conversion from clinically isolated syndrome (CIS) to clinically definite multiple sclerosis (CDMS) (adjusted hazard ratio 0.49, 95% CI 0.33, 0.73; p < 0.0001). Patients who participated in CHAMPS were characterized as monosymptomatic on the basis of the predominant symptom of their presenting syndrome; asymptomatic signs identified upon neurologic examination reflecting multifocal involvement were not taken into account. Recent changes to diagnostic criteria and interpretations of presenting symptoms have been incorporated into the description of patients participating in some early treatment trials. The purpose of this study was to use a new standardised classification scheme for a reanalysis of the CHAMPS study population. Patients’ presenting syndromes were assessed using an algorithm derived from Uditehaag and colleagues (2005), which employed a stepwise neurologic evaluation. Patients were reclassified as monofocal or multifocal. The ability of IM IFN beta-1a to delay progression to CDMS in subgroups based on disease onset (monofocal, multifocal) and baseline magnetic resonance imaging (MRI) characteristics, including number of T2 lesions (< 9, ≥ 9) and presence of gadolinium-enhancing (Gd+) lesions (0, ≥ 1) was assessed. Although all patients were...
Abstracts

considered monosymptomatic at CHAMPS randomisation, 30% of patients were classified as multifocal on reanalysis. Over 3 years of IM IFN beta-1a treatment, monofocal patients experienced a greater reduction in risk of developing CDMS than multifocal patients (52% vs. 24%). Patients with <9 T2 lesions at baseline experienced a greater reduction in risk of developing CDMS than those with ≥9 baseline T2 lesions (58% vs. 38%). Risk reductions were lower for patients lacking Gd+ lesions at baseline than for patients with at least 1 baseline Gd+ lesion (37% vs. 66%). The treatment effect was consistent for all different subgroups (p > 0.05 for the interactions). Two-year results will also be presented. IM IFN beta-1a was effective when initiated at the time of a first demyelinating attack whether the disease was clinically monofocal or more disseminated, either by clinical or MRI criteria. IM IFN beta-1a effectively prolongs time to conversion to CDMS in patients with early disease as defined by an updated evaluation of presenting symptoms. This finding is consistent with that of the original CHAMPS analysis.

P662

Comparison of the efficacy and tolerability of interferon-beta in the treatment of relapsing-remitting multiple sclerosis: Spanish results from the international QUASIMS study

O. Sánchez-Solino, M. Guerrero-Esteo on behalf of the QUASIMS Spanish Group

Objective: To compare the efficacy and tolerability of 4 different preparations of interferon beta (IFNb) in the treatment of relapsing-remitting multiple sclerosis (RR-MS). Patients and methods: Observational, multicenter, retrospective, longitudinal, open, four-arm comparative study. Inclusion criteria: patients with RR-MS treated for at least 2 consecutive years with IFN b-1a 30 mg IM/week (A), IFNb-1b 250 mg SC/48 h (B), IFNb-1a 22 mg (R22) or 44 mg SC (R44) three times a week. Efficacy and tolerability variables studied were percentage of progression-free and relapse-free patients in 2 years, relapse rate, and reasons for switching therapy. Results: In total, 313 patients were included in 6 centers. Baseline characteristics: 1) Distribution of patients by treatment arm: A = 86, B = 70, R22 = 113 and R44 = 44; 2) sex: 65.5% women; 3) mean of age at onset of MS: 33.8 ± 9 years; 4) Mean ± SD baseline EDSS: A = 1.7 ± 1.1; B = 2.2 ± 1.0; R22 = 2.1 ± 1.4; R44 = 3.0 ± 1.6. Relapse rate at 2 years: A = 0.43; B = 0.5; R22 = 0.53; R44 = 0.79. Relapse-free patients at 2 years: 49.8% (A = 57.8%; B = 50.5%; R22 = 50.5%; R44 = 32.6%). Progression-free patients at 2 years: 85.8%, with no significant differences between the 4 groups (A = 90.6%; B = 88.2%; R22 = 85.7% and R44 = 73.3%). In all groups, the most common reason for changing treatment was perceived lack of efficacy and the second most common reason was the patient’s decision. Conclusions: IFNb therapy was well tolerated. The efficacy of IFNb treatments as measured by the percentage of relapse- and progression-free patients at 2 years was similar for all 4 products.

P663

Multiple sclerosis treatment: interferon-beta-1b

J. Kraja, M. Rakacoll, V. Prifti, S. Mijo, I. Zekja, E. Kika; UHC Mother Theresa (Tirana, AL)

Objective: To evaluate the effect of Interferon Beta 1b in Multiple Sclerosis Albanian patients. Methods: We studied the course of disease in 148 MS patients, treated with Interferon beta 1b - Betaferon (9.6 MIU S/C every other day) for a period 6 - 24 months (mean 18.8 months (SD 5.9). All of them had the diagnosis according McDonald criteria. We evaluated the EDSS at the baseline, and every 6 months (EDSS1, 2, 3, 4, 5). Results: There were 54 males (35.8%) and 94 females (64.2%). The mean age at the beginning of the treatment was 36.6 years old (18 - 57 SD 8.6). The age of disease varies from 1 - 25 years. There were 102 patients with RR form and 46 with SP form. In total, we had 24 relapses during Interferon treatment in 20 patients. The mean EDSS1 was 3.68 (2.0 - 5.5 SD 0.85); EDSS2 − 3.4 (1.5 − 5.5 SD 0.9); EDSS3 − 3.29 (1.5 − 5.5 SD 0.95); EDSS4 − 3.3 (1.5 − 5.5 SD 1.02); EDSS5 − 3.08 (1.0 − 5.0 SD 0.89). We used the paired sample tests and Kendall coefficient to evaluate our data. Conclusion: The use of Interferon Beta 1b results helpful in our patients.

P664

Interferon beta-1b 16-year long-term follow-up study: patient-reported outcomes


Background: Patient-reported outcomes (PRO) and assessment of cognitive function are increasingly recognised as significant contributions to the understanding of the impact that multiple sclerosis (MS) has on patients’ lives, yet very limited data exist. Insights from PRO can improve quality of life (QoL), patient management and treatment response. The 16-Year Long-Term Follow-up (16-Year LTF) of the original cohort from the interferon beta-1b (IFNB-1b; Betaseron™) pivotal trial, aims to evaluate the long-term effects of IFN-1b treatment on patient-reported outcomes and cognition, and relate these to clinical and imaging parameters. Design/Methods: The 16-Year LTF is a multicentre, open-label, observational study that uses cross-sectional data collection from patients having participated in the original pivotal trial. A comprehensive battery of tests was selected to assess the health-related (HR) QoL and cognitive status of the patients. These included MS-specific PRO questionnaires, such as the Functional Assessment in MS (FAMS) and EuroQol (EQ-SD). The results have been analysed according to the length of exposure to treatment over the 16 years, i.e. “always” = IFNB-1b >80% of the time; “ever” = IFNB-1b >10–80% of the time; “never” = IFNB-1b ≤10% of the time. Results: 328 of the original 372 patients (88.2%) have been identified and they are almost equally distributed across the original treatment groups. Analysis of the FAMS total score and trial outcome index (TOI) indicated that patients from the “always” group had an improved score compared with those from the “ever” and “never” groups. This trend was also seen in the EQ-SD (rating scale and visual analogue scale) total scores. Assessment of EQ-SD subscales demonstrated that more patients from the “always” group reported “No problem in walking about”, “No problem with self-care” and “No problem performing usual activities”. Further PRO and cognition data (currently being analysed) will be presented. Conclusions: 85.2% of the original patients have been located even after 16 years. Preliminary results indicate that self-reported QoL is better for the “always” patient group compared to the “ever” and “never” groups, and this finding is consistent across different assessments. Furthermore, the FAMS score shows clinically meaningful differences between the groups. These results suggest that early and long-term treatment sustains HRQoL and self-reported functional dependence in patients with MS.

P665

Interferon beta-1b 16-year long-term follow-up study: MRI outcomes

D. Li, G. Ebers, A. Traboulsee, R. Tam, D.S. Goodin, A. Konieczny for the Betaseron/Betaferon LTF Study Group and the UBC MS/MRI Research Group

Background: The interferon beta-1b (IFNB-1b; Betaferon®) pivotal study demonstrated efficacy, safety and tolerability of IFNB-1b in patients with relapsing-remitting (RR) multiple sclerosis (MS), including a persistent beneficial effect on MRI lesion burden over 5 years. The 16-year Long-Term Follow-up (16-Year LFT) study is hypothesis generating, with the aim to assess the long-term treatment effects of IFNB-1b on clinical outcomes and relate these to changes in MRI outcomes. Design/Methods: The 16-Year LTF is a multicentref, open-label, observational study utilising cross-sectional data collected from patients from all 11 centres who
participated in the original pivotal trial. MRI scans were analysed at a single centre and MRI outcome measures assessed included T2 burden of disease (BOD), normalised brain volume (NBV), gadolinium-enhancing lesions, black holes and cervical cord area at C2 (CCA). The results have been analysed by stratification according to the original treatment assignment of the pivotal trial (placebo, 50 mcg IFNB-1b subcutaneously [sc] every other day [eod], 250 mcg IFNB-1b sc eod) and also according to the length of exposure to treatment over the 16 years, i.e. “always” = IFNB-1b >80% of the time; “ever” = IFNB-1b >10–80% of the time; “never” = IFNB-1b ≤ 10% of the time. Results: 328 of the original 372 patients have been identified (i.e. 88.2%). 192 patients underwent MRI studies. Technically adequate C2 CCA data was available in 81 patients. A trend was observed between T2 BOD and increasing disability within the stratified groups. Furthermore, patients with a higher EDSS score tended to have a smaller NBV and reduced CCA. When SPMS status was used as a covariate with T2 BOD in the “always” group than the “never” group. Further analyses are currently being performed, and results will be presented at the meeting. Conclusions: Although significant differences between the groups were not observed, preliminary results indicate that the changes in the different MRI measures over time in the whole cohort are in line with expected clinical progression. Moreover, covariates such as SPMS appear to be important when analysing these findings. Measurements of the cervical cord area at C2 provided results consistent with the brain measurements.

P666

Final results from the interferon beta-1b 16-year long-term follow-up study

G. Ebers, A. Traboulsee, D. Li, D. Langdon, D.S. Goodin, A. Reder, A. Konieczny for the Betaseron/Betaferon LTF Study Group

Background: The pivotal interferon beta-1b (IFNB-1b; Betaseron®) study demonstrated efficacy, safety and tolerability of IFNB-1b in patients with relapsing forms of multiple sclerosis (MS) and led to the first approved immunomodulatory therapy for MS. The 16-Year Long-Term Follow-up (LTF) study is primarily hypothesis generating, and aims to evaluate the long-term treatment effects of IFNB-1b on clinical and imaging parameters, cognitive function and patient-reported outcomes. Design/Methods: The LTF is a multicentre, open-label, observational study that evaluates outcomes in patients having participated in the original IFN-1b pivotal trial which began in 1988. Survival, disease status, relapse rate, Expanded Disability Status Scale (EDSS) score, adverse events, magnetic resonance imaging parameters and other data have been collected. Results: The sample size is 1064 patients, and 816 have final results. Results: Of the original 372 patients, 328 have been identified (i.e. 88.2%); 293 of these patients are alive and 35 are deceased. Median time from diagnosis was 19 years. Data from 260 patients indicate that 78/260 (30%) patients are currently taking IFNB-1b, and the median length of exposure to IFNB-1b has been almost 10 years (3345 days). Wheelchair use is required by 44.2% of patients (NS). Two periods were divided into “never” = IFNB-1b ≤ 10% of the time; “ever” = IFNB-1b >10–80% of the time; “always” = IFNB-1b >80% of the time. Results: Of the original 372 patients, 328 have been identified (i.e. 88.2%); 293 of these patients are alive and 35 are deceased. Median time from diagnosis was 19 years. Data from 260 patients indicate that 78/260 (30%) patients are currently taking IFNB-1b, and the median length of exposure to IFNB-1b has been almost 10 years (3345 days). Wheelchair use is required by 44.2% of “never” patients and 29.4% of “always” patients. Median time to EDSS 6 was 6 years later for “always” patients than for “never” patients. Annualised relapse rates were lower in the “always” group, compared with the “never” group. Adverse events were uncommon. More detailed analyses are ongoing. Conclusions: This is the longest and most complete follow-up of any disease modifying drug for MS. After 16 years, 88.2% of patients from the pivotal IFNB-1b trial have been located. Long-term safety of IFNB-1b is excellent. Results are consistent with the hypothesis that continuous long-term treatment positively impacts on the disease course but selection bias cannot be excluded. Comparisons are being made to natural history data from the Ontario cohort. The lack of randomisation and blinding in observational studies may be offset by the clarity of their outcomes.

P667

Withdrawal of interferon-beta treatment in 140 consecutive relapsing-remitting multiple sclerosis patients: rate and reasons

G. Malanda, G. Castelnuovo, P. Labauge; CHU de Nimes (Nimes, F)

Objectives: To estimate the frequency and reasons of Interferon Beta treatment withdrawal in relapsing remitting (RR) MS patients.

Methods: Observational, retrospective and prospective study of patients in a MS clinic. Results: 140 RR MS patients were included in this study (female: 104, male 36). 69 (49.28%) patients were treated by Avonex®, 37 (26.42%) by Betaseron® and 34 (24.30%) by Rebif®. The frequency of withdrawal was 47.83% in Avonex® patients, 59.49% in Betaseron cases, and 41.18% in Rebif® patients (NS). Two periods were mainly concerned by the withdrawal: between the 6th and 2nd year (36.23%), and between the 2nd and 4th year (34.79%). Reason of withdrawal during the two first years was general intolerance (70.3%), most often myalgias (50% des cases) and hepatitis enzymes increase (11.5%). Local intolerance was observed in 26.94% of the cases. Lack of withdrawal (after the 2nd year) was mainly related to lost of efficiency (81.3% of the cases). Discussion and conclusions: Interferon Beta withdrawal in RR MS patients is frequent (50% of the patients). Reason of withdrawal is correlated with the length of treatment: general or local intolerance is observed during the two years, in contrast late withdrawal is related with lost of efficiency.

P668

Immunomodulatory treatment of multiple sclerosis with onset in childhood or adolescence

A. Ghezzi on behalf of the ITEMS Study Group

Background: Immunomodulatory drugs (IDs) are effective in MS, but they have not been tested in early onset MS (EOMS). Some studies have demonstrated that they are effective and well tolerated in EOMS (Pohl et al., Banwell et al., Ghezzi et al.). A co-operative study group was created in Italy to evaluate the safety, tolerability and effectiveness of ID drugs in MS subjects treated before 16 years of age. We present here the long term results of this study.

Methods: Patients were included if fulfilling the following criteria: 1) diagnosis of definite MS, 2) relapsing-remitting course, 3) disease onset and beginning of IDs before 16 years of age. All patients were submitted to clinical and laboratory evaluation every 3 months. A standardised form was used to record clinical and laboratory data. Results: 110 patients were included in our data-base but results were analyzed in subjects with a pre-treatment and a treatment duration > 6 months, obtaining a cohort of 84 patients: the mean age of onset was 12.1 years, the mean disease duration was 22.9 months. Fifty-two patients were treated with Avonex: after a mean follow up of 43 months the relapse rate was 0.32 (1.98), the EDSS 1.3 (1.4), with clinical side effects in 35/52 of cases and laboratory abnormalities in 11/52. Twenty-two patients were treated with Rebif/Betaferon: after a mean follow up of 47 months the relapse rate was 0.68 (2.25) the EDSS 1.7 (1.8), with clinical side effects in 12/22 of cases and laboratory abnormalities in 4/22. Ten patients were treated with Copaxone: after a mean follow up the relapse rate was 0.16 (2.5) the EDSS 1.2 (1.2), with clinical side effects in 3/10 of cases and laboratory abnormalities in 1/10. Side effects were transient in most cases. In the whole cohort, 23 subjects were shifted to other treatments (17, others 6), 8 subjects stopped the therapy.

Conclusions: Immunomodulatory treatment is effective, safe and well tolerated in EOMS.
Biomarkers of beta-interferon activity and prevalence of HHV-6 in multiple sclerosis patients
M. García-Montojo, R. Alvarez-Lafuente, V. De las Heras, M. Bartolomé, R. Arroyo Hospital Clínico San Carlos (Madrid, E)

Background: In a subset of multiple sclerosis (MS) patients, human herpesvirus (HHV-6) could be involved in the development or maintenance of the disease. The mRNA levels of the myxovirus resistance protein A (MxA), an interferon acute response protein, and matrix metalloproteinase 9 (MMP-9) and its endogenous tissue inhibitor (TIMP-1) are directly related with the bioactivity and bioavailability of beta-interferon, a cytokine with immunomodulatory and antiviral activities. Objectives: 1) To study the antiviral activity of beta-interferon treatment in MS patients, by measuring mRNA levels of MxA and its correlation with the HHV-6 DNA prevalence in blood and serum samples along a six-months follow-up. 2) To analyze the correlation between the mRNA levels of MxA and the expression levels of MMP-9 and TIMP-1. Methods: Four blood and serum samples of 54 MS patients were collected for 6 months: prior the onset of the beta-interferon treatment, (0) and 1, 3 and 6 months after the starting of beta-interferon treatment. Total RNA of PBMCs was extracted and then analyzed by quantitative real-time RT-PCR in order to determine mRNA levels of MxA, MMP-9 and TIMP-1. As internal control, levels of 18S rRNA were analyzed and the results were expressed in a relative way. DNA from blood and serum samples was extracted, and quantitative PCR with a standard curve was performed to detect HHV-6 genomes. Results: MxA levels increased during the study in the 89% of MS patients (p < 0.001). MMP-9 did not show any relevant result but the expression of its inhibitor showed an increment from 0 to 1 month in 70% of patients (p < 0.001) and from 1 to 3 months in 67.5% (p < 0.001); the 87.5% of patients presented a total increase in TIMP-1 from 0 to 6 months (p < 0.001). We found a correlation between MxA levels and expression of MMP-9 and TIMP-1 too (p < 0.001). Moreover, another correlation seems to exist between MxA expression and HHV-6 prevalence that decreased from 53.7% to 42.6% in blood samples and from 18.5% to 14.8% in serum samples. All patients presenting HHV-6 genomes in PBMCs or serum at the first visit but not six months later had a significant increase in MxA expression. Conclusions: MxA seems to be a good marker for bioactivity of beta-interferon. Increased antiviral activity of beta-interferon could be involved in the decrease of HHV-6 prevalence both in blood and serum samples. Further studies are needed to find a correlation with the clinical development of the disease.

Association of APOE with treatment response on betaferon
E. Dincic, M. Zivkovic, S. Popovic, D. Obradovic, A. Stankovic, D. Alavantic, R. Raicevic; Military Medical Academy (Belgrade, CS); Institute of Nuclear Sciences (Belgrade, CS)

Background: Betaferon (Interferon-beta 1b) is used in therapy of multiple sclerosis (MS), its mechanism of action as an immunomodulator is not fully understood. MS patients are considered poor or non responders to this therapy. Reason for that may be due complex genetic traits of MS. Some gene polymorphisms influence susceptibility of MS, other determine clinical course or progression of the disease, some of them maybe can influence different treatment response on the same therapy. Allele 4 of ApolipoproteinE (APOE) gene polymorphism is associated with more progressive disease in more different population of MS patients. Objective: To establish genotype and allele frequencies of polymorphism in APOE gene in MS patients of Serbia and Montenegro. Methods: The study included 57 patients with clinically defined relapsing-remitting MS who were receiving Betaferon. Responders and non-responders were determined according to number of relapse during one year Betaferon therapy and EDSS, which evaluation were performed before and after Betaferon treatment. Patients with one or more relapses and increased value of EDSS for one year were considered as non-responders. CTLA-4 genotypes were determined by PCR and SSCP analysis. Results: There were 22.65% of non-responders to Betaferon treatment in investigated sample of MS patients. The duration of the disease was not significantly different between two subgroups, responders and non-responders, of patients (5, 5 and 4, 9 years, respectively). There were no significant differences in genotype and allele frequencies between responders and non-responders to therapy. Still, the most frequent genotype in responders was AA (46.67%), and in non responders AG (38, 33%) genotype. None of the non-responder patients had CTLA-4 +499G genotype. We did not found any significant difference for CTLA-4 gene polymorphism genotypes or alleles between responders and non-responders to one year Betaferon therapy in our sample. Conclusion: Our results suggest that this polymorphism does not influence the response to Betaferon treatment.

Association of polymorphism APOE with treatment response on betaferon
E. Dincic, M. Zivkovic, S. Popovic, D. Obradovic, A. Stankovic, D. Alavantic, R. Raicevic; Military Medical Academy (Belgrade, CS); Institute of Nuclear Sciences (Belgrade, CS)

Background: Betaferon (Interferon-beta 1b) is used in therapy of multiple sclerosis (MS), although its mechanism of action as an immunomodulator is not fully understood. MS patients could be responders or non-responders to this therapy. Reason for that heterogenic therapeutic response may be due to complex genetic traits of MS. Certain gene polymorphisms influence susceptibility to MS; others determine clinical course or progression of the disease. Some of them could influence different response to the treatment of the same therapy. Several gene polymorphisms in CTLA-4 (Citotoxic T lymphocyte antigen-4) have been associated with T cell mediated autoimmune disease. Expression of CTLA-4 on T cells negatively regulates CD4 and CD8 T cells response. Defect in CTLA-4 signaling could contribute to the immune disregulation in MS. Objective: To establish genotype and allele frequencies of exon 1 +99AG polymorphism in CTLA-4 gene in MS patients from Serbia and Montenegro on Betaferon therapy. To evaluate the potential influence of this polymorphism on response to one year Betaferon treatment. Methods: The study included 57 patients with clinically defined relapsing-remitting MS who were receiving Betaferon. Responders and non-responders were determined according to number of relapse during one year Betaferon therapy and EDSS, which evaluation were performed before and after Betaferon treatment. Patients with one or more relapses and increased value of EDSS for one year were considered as non-responders. CTLA-4 genotypes were determined by PCR and SSCP analysis. Results: There were 22.65% of non-responders to Betaferon treatment in investigated sample of MS patients. The duration of the disease was not significantly different between two subgroups, responders and non-responders, of patients (5, 5 and 4, 9 years, respectively). There were no significant differences in genotype and allele frequencies between responders and non-responders to therapy. Still, the most frequent genotype in responders was AA (46.67%), and in non responders AG (38, 33%) genotype. None of the non-responder patients had CTLA-4 +499G genotype. We did not found any significant difference for CTLA-4 gene polymorphism genotypes or alleles between responders and non-responders to one year Betaferon therapy in our sample. Conclusion: Our results suggest that this polymorphism does not influence the response to Betaferon treatment.
Conclusion: Our results suggest that this polymorphism does not impact to respond on Betaferon treatment,of MS patients in Serbia and Montenegro.

P672

Relative immunogenicity of a new formulation of interferon-beta-1a in an in vivo murine model

F. Belloni, A. Madu, G. Palmieri, L. Borraccetti, M. Mattei, A. Jaber, G. Antonelli; University La Sapienza (Rome, I); University "Tor Vergata" (Rome, I); Serono International S.A (Geneva, CH)

Background: Although there is general agreement that treatment decisions in multiple sclerosis (MS) should be based on clinical evidence, long-term studies indicate that development of neutralizing antibodies (NAbs) to interferon (IFN) beta may attenuate clinical efficacy in some patients. To reduce immunogenicity and improve tolerability, a new formulation of IFN beta-1a (Rebif® New Formulation; RNF) has been developed. Aims: To compare the immunogenicity of the IFN formulations RNF, Rebif® (R) and Avonex® (A) using identical dosing regimens and a single NAb assay in an in vivo murine model. Methods: Female BALB/c mice received RNF, R or A at 0.1 mcg/mL administered subcutaneously three-times-weekly (tiw), or no medication (control). Serum samples were collected 72 hours after the third weekly administration of IFN-beta (on days 7, 21, and 35), and were assayed for NAbs with the cytotoxic effect assay; titres were quantified using Kawade’s method and were expressed as t 1/10, namely the dilution of serum that reduces 10 laboratory units (LU/mL) of IFN to 1 LU/mL. The NAb titres are expressed in Log10 and represent the geometric mean (± standard deviation) of two independent determinations. The mean values were compared using a t test. Results: Eighteen mice received IFN beta (RNF, n = 6; R, n = 6; A, n = 6) and 6 mice were included in the control group. NAbs were not detected in the control group at any time point in the study. NAbs were not detected in any mouse at day 7. By day 21, 1 mouse had NAbs in the RNF group compared with 3 mice in the R group and 5 mice in the A group. At day 35 all mice had NAbs. Throughout the study NAb titres were lowest in the RNF group and highest in the A group. At day 35, NAb titres were significantly lower in the RNF group (1.91 t1/10 ± 0.55) than the R group (2.55 t1/10 ± 0.35; p = 0.037), which had a significantly lower NAb titres than the A group (3.10 t1/10 ± 0.38; p = 0.026). Conclusions: Time to development of NAbs and NAb titres indicate that RNF has a low immunogenic potential compared with equivalent dosing of R and A. Further pre-clinical experiments are ongoing. In addition, a full clinical study is investigating the profile of RNF in patients with MS.

P673

Reduced immunogenicity with two new formulations of interferon-beta-1a using ex vivo T cell assays

A. Jaber, M. Baker; Serono International (Geneva, CH); Balbawra Institute (Cambridge, UK)

Background: The significant benefits of subcutaneous (sc) interferon (IFN) beta-1a (Rebif®), 22 and 44 mcg three times weekly (tiw), are well established. Neutralizing antibodies (NAbs) to IFN beta-1a have been shown to impact on efficacy in a minority of patients, whereas other patients continue to experience significant treatment benefits. In order to minimize the impact of NAbs even further, IFN beta-1a sc tiw has been reformulated to reduce its immunogenic potential. Aims: To determine ex vivo the immunogenicity of current Rebif® (R), and two test formulations (Rebif New Formulation [RNF1 and RNF2]), versus IFN beta-1a control. Methods: Monocytes were isolated from peripheral blood mononuclear cells (PBMC) from healthy donors and incubated to induce mature dendritic cells (DC). On day 4, test antigen, IFN beta-1a control (recombinant protein in acetate buffer) or non-antigen control (growth medium) were added and incubated for ≥6 hours. DCs were washed to remove exogenous antigen and gamma irradiated. Autologous CD4+ T-cells, isolated from PBMC, were co-incubated with the antigen loaded DCs for 7 days. Secretion of interleukin (IL)-2 and IFN gamma were determined by Elispot assay. Proliferation was assessed with an 18-hour 3H [Thymidine] pulse. Each test antigen response was normalized to the mean Antigen control response to give the stimulation index (SI). Results: Expressed as mean SI of test antigen relative to the mean SI of IFN beta-1a control. Results: DCs from 26 healthy donors were isolated and incubated. The IL-2 secretory response was 78.7%, 66.2% and 108.2% of the IFN beta-1a control with RNF1, RNF2 and R, respectively. The SI of RNF2 was significantly lower than control or R (0.80 vs. 1.21 or 1.27 respectively, p < 0.05; ANOVA). IFN gamma secretion was highly variable between different donor cultures and there were no statistical differences between the test substances. The proliferation responses were 59.7%, 47.7% and 90.8% of the IFN beta-1a control with RNF1, RNF2 and R, respectively. The RNF2 response was significantly lower than both R and control (0.7 vs. 1.4 and 1.6, respectively, p < 0.05; ANOVA), while RNF1 response was significantly lower than control (0.9 vs. 1.6, p < 0.05). Conclusions: DCs treated with RNF2 have a reduced immunogenic potential to stimulate T-cells to secrete IL-2, and a lower proliferative response than current R. The reduced immunogenicity of RNF2 is now being assessed in a Phase IIb clinical trial (25632).

P674

Assessment of the safety and tolerability of a new formulation of subcutaneously administered interferon-beta-1a

C. Bradley, A. Jaber, A. Priestley, M. Seiberling; Serono International (Geneva, CH); LCG Bioscience (Cambridge, UK); Swiss Pharma Contract Ltd (Alschwill, CH)

Background: Application-site disorders are common adverse events (AEs) associated with subcutaneous (sc) injection. With high-dose high-frequency interferon (IFN) beta-1a (Rebif® R) these AEs are generally mild but may lead to some patients discontinuing therapy. New formulations of R, which are free from human and animal-derived components, are thus being investigated with the aim of reducing AEs and thereby improving compliance. Aims: To compare the safety and tolerability of two R new formulations (RNF1 and 2), the current formulation of RNF2 did not differ appreciably from those of R. Conclusions: The results from this study show that RNF2 has improved tolerability and comparable PK/PD characteristics relative to the current R formulation. RNF2 is now being assessed in a Phase IIb clinical trial (25632).
P675

Reduced immunogenicity with a new formulation of interferon-beta-1a (Rebiš®): 24-week results of a phase IIIb study

G. Giovannoni, O.L. Barbarash, A. Jaber, J. King, L.M. Metz, L. Mitchell, G. Pardo, J. Simmsarian, P.S. Ørensen, B. Stubainski on behalf of the RNF Study Group

Background: Although the significant benefits of interferon (IFN) beta-1a for MS patients are well known, the role of neutralising antibodies (NAbs) continues to be debated. Reducing NAbs may deliver benefits in some patients. A new formulation of IFN beta-1a (Rebiš®: New Formulation; RNF) has therefore been developed with the objective of reducing NAbs and improving tolerability. Aims: To compare the antigenicity and tolerability of RNF with historical data on the current formulation. Methods: This is the 24-week analysis of a 96-week phase IIIb multicentre single-arm open-label study (253632) in patients with relapsing forms of MS (18–60 years; EDSS <6.0). Patients self-injected RNF (44 mcg/0.5 mL, sc, tiw) after a standard 1–4 week dose titration period. Blood samples were collected at baseline and weeks 12 and 24 for analysis of NAbs with same time point historical data (E). PRISMS (P) and SPECTRIMS (S) studies.

Results: 260 patients enrolled and received at least one treatment dose; of these, 259 had at least one post-baseline NAb assessment. By week 24, 242 patients were still continuing on treatment in the study. Fewer patients on RNF were NAb+ at 24 weeks (2.7%; 7/259; 95% CI: 1.1–5.5%) compared with the same time point historical data (E). PRISMS (P) and SPECTRIMS (S) studies. Results: 260 patients enrolled and received at least one treatment dose; of these, 259 had at least one post-baseline NAb assessment. By week 24, 242 patients were still continuing on treatment in the study. Fewer patients on RNF were NAb+ at 24 weeks (2.7%; 7/259; 95% CI: 1.1–5.5%) compared with the same time point historical data (E). PRISMS (P) and SPECTRIMS (S) studies.

Conclusions: These interim data suggest that RNF has a low immunogenic potential and is overall better tolerated than the current formulation of Rebiš®. By limiting the occurrence and reducing the titre of NAbs and improving tolerability, RNF may provide even greater benefits for patients.

P676

TNF-alpha and IGF-1 variation in relapsing-remitting multiple sclerosis patients before and after one year treatment with high dose of IFN beta 1a

G. Las, G. Di Biase, M. Fratta, G. Maniscalco, R. Cotrufo; Second University of Naples (Naples, I)

Increased expression of both a proinflammatory cytokine, tumor necrosis factor alpha (TNF-alpha), and a survival peptide IGF-1 occurs in multiple sclerosis (MS). Conventional roles for these two proteins are neuroprotection by IGF-1 and neurotoxicity by TNF-alpha. To evaluate whether frequent TNF-alpha and IGF-1 assessments may predict clinical and MRI changes over 1 year treatment period with IFNbeta 1a 44 mcg tiw, we enrolled twenty-five previously untreated patients (17 females and 8 males) with relapsing-remitting (RR) MS. Study design includes monthly MRI scan (T2-LI, Gd-LI and T1 black holes LL, changes in brain volume from baseline to year 1) and blood sampling (TNF-alpha and IGF-1 determinations) for 6 consecutive months with a further combined assessment at month 12. Moreover, for each patients, number of relapses in the preceding 2 years, EDSS baseline, changes EDSS during the 1-year treatment period, relapses during 1 year treatment were also collected. We will show clinical, laboratory and MRI data of these patients during one year of treatment.

P677

Long-term clinical benefits from subcutaneous interferon-beta-1a: a phased analysis of the PRISMS long-term follow-up study

A. Traboulsee, D. Li, L. Kappos on behalf of the PRISMS LTFU Study Group and the UBC MS/MRI Research Group

Background: The PRISMS study of subcutaneous (sc) three times weekly (tiw) interferon (IFN) beta-1a (Rebiš®) has already shown differences between early and late treatment in on EDSS, relapse rate and burden of disease (BOD). Aims: To investigate the effect of early and late therapy with 44 or 22 mcg IFN beta-1a sc tiw on annualised relapse rate (RR), BOD and brain parenchymal volume (BPV) in different study phases. Methods: Patients were initially randomised to IFN beta-1a (44 or 22 mcg sc tiw) or placebo. After year 2, placebo patients were re-randomised to 44 or 22 mcg IFN beta-1a (‘late treatment’ group). Patients could continue on study medication to year 6. Between the patients’ last visit in PRISMS and LTFU any or no therapy could be taken. Study data are presented for the group of patients attending LTFU at year 7–8. Results: 382 (68.2%) of the 560 patients originally randomised returned for LTFU. In years 1–2, RR was lower for the 44 (0.86) and 22 mcg groups (0.90) compared with the late treatment group (1.27). Over years 3–4, RR was reduced in patients who converted from placebo to active therapy (0.63) and was similar to the 44 and 22 mcg groups (0.54 and 0.61). RR was sustained for all groups between year 4 and LTFU (0.40, 0.35 and 0.38 for 44, 22 mcg and late treatment). Median percent change in BOD was also lower in years 1–2 with 44 or 22 mcg compared with late treatment (−2.8, −0.73 and +6.5%). The late treatment group improved on starting active treatment in years 3–4 (+1.16, +2.25 and +1.70%), which was sustained to LTFU (+1.66, +2.32 and +1.65% for 44, 22 mcg and late treatment). Over years 1–2, median BPV decreased more in the 44 mcg (−0.88%) than the 22 mcg (−0.56%) or late treatment (−0.59%) groups. Over years 3–4, patients converting from placebo to active therapy had a greater reduction in BPV (median −0.85%) similar to the 44 mcg group in years 1–2. These results indicate the potent anti-inflammatory effects of the treatment. In years 3–4 (when pseudo-atrophy components of therapy have stabilized), BPV decrease for 44 and 22 mcg (−0.42 and −0.49%) was less than the natural history BPV of placebo over years 1–2. Up to LTFU, differences in BPV stabilised between the 44, 22 mcg and late treatment groups (−0.39, −0.65 and −0.51). Conclusions: These results indicate that the effects of early and late IFN beta-1a sc tiw on relapse rate and inflammatory MRI outcomes are marked on initiation of therapy and are sustained over the long term.

P678

Greater reduction of MRI T2 burden of disease with interferon-beta-1a 44 mcg administered subcutaneously three-times-weekly compared with 30 mcg administered intramuscularly once-weekly: analysis of 48-week data from the EVIDENCE study

A. Traboulsee, A. Al-Sabbagh, R. Bennett, P. Chang, R. Glanzman, H. Russell, D. Li on behalf of the Evidence Study Group and UBC MS/MRI Research Group

Background: The EVIDENCE (Evidence of Interferon Dose-response: European North American Comparative Efficacy) study established the superior efficacy of subcutaneous (sc) interferon (IFN) beta-1a 44 mcg three-times-weekly (tiw) over intramuscular (im) IFN beta-1a 30 mcg once-weekly (qw) in patients with relapsing–remitting multiple sclerosis (RRMS) by increasing the proportion of relapse-free patients and reducing the number of active MRI lesions at weeks 24 and 48. (Neurology 2002; 59: 1496–1506). Aims: To compare IFN beta-1a 44 mcg sc tiw with IFN beta-1a 30 mcg im qw over 48 weeks on reduction of T2 burden of disease (BOD) – a measure of the total brain MS lesion load. Methods: Patients with evaluable T2 MRI scans before start of dosing and at Week 48 were included. Percent (primary
analysis) and absolute change in BOD (mm²) from baseline to Week 48 were summarised by treatment group. Changes were compared using ANCOVA with baseline BOD as covariate. Analysis was also done by neutralizing antibody (NAb) status (NAb+ defined as >20 NU/mL) in the 44 mcg sc tiw group vs. the whole 30 mcg im qw group. **Results:** There were no significant baseline differences between treatment groups (44 mcg sc tiw, n=279; 30 mcg im qw, n=274) for age, gender, race or BOD. Median (range) percent change in BOD from baseline to Week 48 was −6.7% (−65, 431) for the 44 mcg sc tiw group compared with −0.6% (−61, 197) for the 30 mcg im qw group. Adjusted treatment mean difference [SE] was −4.6% [2.6], which significantly favoured the 44 mcg sc tiw group (p=0.002). The corresponding median (range) absolute BOD change was −189.5 mm² (−2345, 56869) and −19.0 mm² (−13337, 10161). When analysed by NAb status, there was a statistically significant adjusted mean change difference [SE] in percent BOD change in favour of NAb− 44 mcg sc tiw over 30 mcg im qw (−6.6% [2.8]; p<0.0001); treatment effects for NAb+ 44 mcg sc tiw and 30 mcg im qw were similar (adjusted mean change difference [SE] = 0.5% [3.9]; p = 0.583). **Conclusions:** Consistent with the other clinical and MRI findings from the EVIDENCE study, there was a significantly greater reduction in T2 BOD in the 44 mcg sc tiw group than in the 30 mcg im qw group over 48 weeks. Patients receiving 44 mcg sc tiw who were NAb+ demonstrated similar benefit on T2 BOD compared to the overall 30 mcg im qw group.

P679

**Double dose interferon-beta-1b (500 mcg) re-establishes efficacy and lowers neutralising antibodies in patients with breakthrough of multiple sclerosis disease on standard high-dose interferon-beta therapy**

N. Soucy, K. Cardinal, G. Theoret, H. MacLean, M.S. Freedman; The Ottawa Hospital General Campus (Ottawa, CAN)

Breakthrough of MS disease, as determined by recurring relapses, MRI activity or continued progression on standard interferon-beta (IFNβ) therapies is sometimes associated with the appearance of neutralizing antibodies (NAB). It is possible that by increasing the dose of IFNβ disease control can be renewed and NAB reduced. We describe 5 cases of relapsing-remitting (RR) MS patients experiencing disease breakthrough after a relatively stable period of disease control on standard high dose IFNβ therapy, in whom high titre NAB were detected. Patients were then treated with double dose IFNβ-1b (500 mcg) (obtained by compassion from Berlex Canada) and their clinical status as well as NAB titres were followed. NAB were assessed whenever possible using both the CPE assay (S. Grossberg, Milwaukee WI) and the Mx protein assay (LabCorp, San Leandro CA). All patients had RRMS and were started on either standard dose IFNβ-1b (Betaseron 250 mcg eod sc) (n=2) or IFNβ-1a (Rebif 22 or 44 mcg tiw) (n=3). Disease control (no perceived relapses, progression or MRI activity) was observed for periods of up to 58 months before breakthrough was noted. All patients with relapses also showed MRI worsening compared to previous studies as well as an increase in their Expanded Disability Status Scale (EDSS). At the time of disease breakthrough serum was drawn and sent for NAB testing. We were interested to see whether the two different NAB assays (CPE vs. MxA protein) conveyed similar information. Initially, both laboratories confirmed high NAB titres (range 290–5,764 NU/mL (N=20 NU/mL) in all patients. Patients were then started on double dose IFNβ-1b (500 mcg eod sc) and their clinical status as well as NAB titres followed regularly. The double dose IFNβ-1b regimen was well tolerated by all patients, with no significant new side effects or changes in laboratory values. All patients experienced substantial reductions in relapses and improvement in EDSS for up to 2 years. Individual patient results will be shown. In virtually all cases, titres of NAB fell precipitously with the start of IFNβ. The dose of IFNβ-1b regimen was well tolerated by all patients, with the CPE assay compared with MxA protein assay in that NAB titres were observed to decrease only with the CPE assay. Increasing the dose of IFNb in patients experiencing disease breakthrough associated with NAB may be a strategy for re-establishing disease control.

P680

**Increased endogenous catecholamine production, beta2-adrenoceptor and dopamine D5 receptor expression in circulating lymphocytes from patients with multiple sclerosis undergoing interferon-beta treatment**

M. Zaffaroni, M. Cosentino, F. Marino, M. Ferrari, E. Carcano, M. Persun, E. Rasinì, R. Bombelli, A. Bizzo, A. Ghezzi, G. Comi, S. Lechini; S. Antonio Hospital (Gallarate, I); University of Insubria (Varese, I); S. Raffaele Scientific Institute (Milan, I)

**Background:** One of the putative mechanisms of action of IFN-beta in multiple sclerosis (MS) is the modulation of activation-induced apoptosis of lymphocytes by endogenous catecholamines (CA) produced in peripheral blood mononuclear cells (PBMCs), which are involved in functional regulation of immune responses. We previously reported a dysregulated production of CA by PBMCs correlating with disease activity in MS patients and showed that in vitro IFN-beta enhances endogenous CA production in mitogen-stimulated human PBMCs and reverses the inhibitory effect of IFN-gamma. To assess the effects of in vivo IFN-beta on CA system in PBMCs, we included 48 relapsing-remitting MS patients in a one-year longitudinal study as they started IFN-beta treatment. **Methods:** PBMCs were cultured with 10 μg/ml PHA in standard conditions. CA levels were assayed by HPLC. mRNA for tyrosine hydroxylase (TH—the rate-limiting enzyme in CA synthesis), for beta2-adrenoceptors and for dopamine D5 receptors (D1-like) were determined by standard RT-PCR and western blot techniques. **Results:** The preliminary data from 44, 38, 36, 24 patients with 1, 3, 6, 12 months of follow-up respectively are here reported. PBMC CA increased up to 139.1–250.1% of pre-treatment levels at months 6–12 (p<0.01 vs. baseline). In the same cells, mRNA levels for TH showed a trend to decrease (~22% and ~17% at months 6 and 12 respectively), whilst mRNA for D2-adrenoceptors and D5 receptors increased to 177.0–166.7% and 150.0–169.2% of pre-treatment levels at months 6 and 12 respectively (p<0.001 vs. baseline). With treatment progression, IFN-beta added in vitro progressively failed to inhibit CA production by PBMCs and, speculatively, IFN-beta increased the antagonistic effect on IFN-gamma in vitro. **Conclusions:** Our data show that endogenous CA production by PBMCs is enhanced by IFN-beta treatment in MS patients and that the sensitivity of these cells to CA is possibly increased by therapy, as suggested by up-regulation of both beta2-adrenoceptor and dopamine D5 receptor. These effects are evident several months after starting the immunomodulatory treatment. We confirm the role of CA system in MS, and possibly provide a rationale for using adrenergic/dopaminergic agents as additional therapy. Supported by Italian Multiple Sclerosis Foundation (FISM), grant n. 2002/R/18, and USA National Multiple Sclerosis Society, grant n. PP0791.

P681

**Interferon-beta 1-b in secondary progressive multiple sclerosis**

N.S. Oztekin, M.F. Oztekin, R. Polat; SB Diskapi Hospital (Ankara, TR)

**Objective:** The aim of the study is to evaluate the efficacy and safety of Interferon beta 1-b in secondary progressive multiple sclerosis. **Method:** It is an open controlled study including 45 secondary progressive patients EDSS scores ranging from 4.5 to 6.5. Patients were randomly assigned either IFNBI-1b (250 mngms every other day/week) or exeeds 1.0 EDSS point (0.5 point if EDSS score was 6.0 to 6.5 at entry) confirmed at 6 months. Secondary outcomes are mean change in EDSS score from baseline, relapse rate, MRI activity and standard neurophysiological function tests. Follow up period is 24 months.
Results: There was no significant difference in time to confirmed progression of EDSS scores between IFNB1-b treated and untreated patients during the 24 months. However IFNB1-b treatment resulted improvement on secondary outcome measures involving clinical relapses, new active MRI lesions and accumulated burden of disease on T2 weighed MRI. Conclusion: The results of this open controlled study revealed no treatment benefit on the time to confirmed progression of disability between the treated and untreated groups. But relapse and MRI related outcomes showed statistically significant benefit (p < 0.005) in the IFNB1-b treated group compared to the untreated group and this result is consistent with the outcomes of earlier clinical trials. Further studies with more patients will enable us to make a more accurate deduction.

P682

Early treatment with interferon-beta-1a (Avonex®) in patients with relapsing-remitting multiple sclerosis with mild disability: results of a 6-year open-label follow-up

N.S. Oztekin, M.F. Oztekin; SB Dışkapi Hospital (Ankara, TR)

Background: Several studies revealed that initiation of disease modifying therapies early in the course of the disease delays disease accumulation, reduces relapse rates and MRI burden of disease in relapsing remitting multiple sclerosis (R-R MS). Objective: The aim of the study is to determine the clinical efficacy and effect on disease activity of IFNB1-a once weekly administered intramuscularly (Avonex) in patients with newly diagnosed R-R MS with mild disability. Disease activity is measured by reduction in number and volume of Gd-enhanced lesions on MRI three months apart. The efficacy on relapse frequency is also evaluated. The primary endpoint was time to sustained disability progression of at least 1.0 on EDSS. Method: After a baseline scan 71 patients with definite R-R MS EDSS between 0.5-3.5 were enrolled to the study and administered IFNB1-a (Avonex) 30 mgms once weekly. Fortyfour age and EDSS matched patients who did not receive any treatment were used as controls. The patients were evaluated monthly in the first 24 months of treatment and 3 months apart during 6 year follow up period. MRI was performed 3 months after 6 years of treatment, while the reduction was 20% in the control group. There was no statistically significant difference in terms of annual exacerbation rate (p > 0.01). Exacerbation frequency was mean 0.472 in the treatment groups whereas it was 0.91 in the control group (p < 0.01). There was a reduction in MRI disease activity up to 45% reduction in the number of active lesions per patient per MRI scan after 6 years of treatment, while the EDSS improved in 31.6%, remained stable in 21.7% and worsened in 28.3% of patients in the control group. Due to ethical reasons 40 patients in the control group did not receive any treatment. The proportion of patients discontinuing therapy was low (1.2%). There was a significant difference between the treatment and control groups in terms of number and volume of Gd-enhanced lesions on MRI (p = 0.002 and p = 0.05).

Conclusion: The results of this 6 year open labeled study comparing the immunomodulating effects of the four therapies in R-R MS patients have shown no statistically significant difference in terms of annual exacerbation rate, exacerbation frequency and MRI disease activity.

P684

The effect of interferon-beta-1b (Betaferon) therapy on relapses, progression of disability and brain MRI findings in patients with relapsing-remitting multiple sclerosis

M.R Motamed, S.M Fereshtehnejad, N. Najimi, M. Javdani; Iran University of Medical Sciences (Tehran, IR)

Introduction: Multiple Sclerosis (MS) is a demyelinating disease of the central nervous system. It may present in various clinical courses which remitting-relapsing MS (RRMS) consists about 60% of these patients. It seems that disease-modifying treatment in RRMS may reduce the frequency of relapses and the progression of disability.

On the other hand, as brain magnetic resonance imaging (MRI) has been found to yield findings with some prognostic value, this study was performed to evaluate the effect of disease-modifying treatment with interferon beta-1b (Betaferon) on relapses, progression of disability and brain MRI findings in patients with RRMS. Methods and Subjects: This randomized clinical trial study was conducted on 41 patients who had RRMS determined using McDonald criteria. They were evaluated during a 24-month study period from January 2004 to January 2006. Patients were assigned in two groups: 21 patients under interferon beta-1b (Betaferon) 250 lg subcutaneously every other day (groupB) and 20 patients without disease-modifying treatment (groupA). Neurological and clinical assessments were done at baseline, 12th, 18th and 24th month of follow-up. Number of new attacks, changes of brain MRI findings and Kurtzke Expanded Disability Status Scale (EDSS) were reported. Independent T-test, Repeated Measurement, Friedmann and McNemar tests were used in analysis. Result: At the time of inclusion in the study, mean age (± SD) was 26.41(± 6.53) years, mean EDSS (± SD) at baseline was 2.96(± 0.58). Patients in group A represent lesser number of attack (0.76, SD = 0.70 vs. 2.05, SD = 0.76; P = 0.000),more reduction in disability: results of 6-year open-label follow-up

N.S. Oztekin, M.F. Oztekin; SB Dışkapi Hospital (Ankara, TR)
Randomized controlled trials have proven the effectiveness of interferon beta-1a, interferon beta-1b and glatiramer acetate. Our result demonstrated that disease-modifying treatment for RRMS patients may lead to beneficial results. And also interferon beta-1b significantly delayed progression to disability, reduced incidence of new relapses and improved brain lesions.

P685

Impact of beta-interferon on disability progression in clinical practice: a Bayesian analysis
K.E.T. O’Rourke, C. Walsh, M. Hutchinson; St. Vincent’s University Hospital (Dublin, IRL); Trinity College (Dublin, IRL)

Objective: Observational studies of beta-interferon (IFNβ) are essential, but bias renders standard frequentist statistics unreliable. Bayesian analysis expresses data as a likelihood function which is integrated with a probability distribution representing a prior belief. It thus enables 1) evaluation of evidence from observational studies in the context of randomised trials, 2) multiple comparisons without correction, and 3) explicit incorporation and quantification of uncertainty and bias. This study used Bayesian analysis to evaluate an observational study of beta-interferon (IFNβ) therapy for relapsing-remitting MS (RRMS) in clinical practice. Methods: 175 RRMS patients treated with IFNβ were followed at 12 monthly intervals for a median of 5 years. An EDSS measurement was performed at the start of IFNβ therapy and yearly thereafter. Progression was defined as a one-point increase in the pre-treatment EDSS sustained for 6 months. The odds ratio (OR) of progression after 2 and 4 years of IFNβ was calculated by comparison with the progression rate in a matched group of 185 historical control subjects from the Sylvan Lawrey Centre for MS Research, and expressed as a likelihood function; the likelihood function was adjusted for a 30% point estimate and precision bias based on published estimates of bias. Evidence-based prior probability distributions (priors) for the OR of progression after 2 years of IFNβ were derived from a published meta-analysis of randomised trials which took into account possible outcomes in patients lost to follow-up, and defined as enthusiastic and sceptical priors; the PRISMS-4 trial was used to construct enthusiastic and sceptical posterior probability distributions (posteriors) for the OR of progression. Conclusion: Bayesian analysis indicates that disability progression is attenuated after 2 years of IFNβ therapy for RRMS in clinical practice, even given a sceptical evidence-based prior and allowing for observational bias; no IFNβ effect is evident after 4 years.

P686

Interferon-beta in multiple sclerosis: ten years’ experience in a multiple sclerosis specialist centre
M. Khalil, V. Zehrer, R. Egg, A. Lutterotti, R. Ebhing, C. Gneiss, I. Mayringer, B. Kuenz, M. Reindl, F. Deisenhammer, T. Berger; Innsbruck Medical University (Innsbruck, A)

Background: Randomized controlled trials have proven the effectiveness of interferon beta in patients with relapsing-remitting (RRMS) multiple sclerosis (MS), with a clinical isolated syndrome, and partially with secondary-progressive MS. Long-term observational studies may provide additional information about the long-term effectiveness, safety and tolerability of beta interferons in the post-marketing-period. Objectives: To investigate the long-term effectiveness, safety and tolerability of beta interferons (interferon beta 1a i.m. Avonex®, interferon beta 1a s.c. Rebif®, interferon beta 1b s.c. Betaferon®) in patients with RRMS. Methods: Ten years (1994–2004) long-term follow-up observational study in MS patients treated with interferon beta at the MS Clinic, Clinical Dept. of Neurology, Innsbruck Medical University. Clinical visits were performed every three months. At each clinical visit EDSS was assessed, and a routine laboratory testing was performed. Documentation at clinical visits included side effects, compliance, concomitant medication, concomitant diseases, reason for changing the dose or drug or stopping the therapy. Only patients with complete clinical documentation and/or follow-ups were included in this study. Results: 152 (112 female, 40 male) RRMS patients were eligible for this study. 55 (36.2%) patients received Betaferon®, 58 (38.2%) Avonex® and 39 (25.7%) Rebif®. 35 patients (23.0%) were treated more than 5 years. The proportion of relapse free patients after 12 months (64.7% Betaferon®, 71.1% Rebif® and 71.9% Avonex®) and 24 months (58.1% Betaferon®, 63.3% Rebif® and 67.6% Avonex®) was not statistically different between the treatment groups. The proportion of patients with sustained progression in EDSS after 24 months (15.8% Betaferon®, 0.0% Rebif® and 5.6% Avonex®) did also not significantly differ between the treatment groups. No unexpected or severe side effects occurred during the follow-up period. 34 (22.4%) patients withdrew from therapy and 35 (23.0%) patients switched treatment over the follow-up period. Most common reasons for stopping or changing the therapy were disease progression and occurrence of side effects. Conclusion: The data from the long-term follow-up study from the Innsbruck MS cohort confirm the findings from another large observational study. Interferon beta treatment is safe and well tolerated. All three interferon beta preparations provide a comparable efficacy in a large non-selected cohort of MS patients.

P687

Peripheral blood mononuclear cells from patients with multiple sclerosis show decreased activation of STAT4 by interferon-beta and reduced expression of interferon type 1 receptor
A.I. Falhey, A. Robins, C.S. Constantinescu; University of Nottingham (Nottingham, UK)

Background: The type I interferon, IFN-beta is used in the treatment of multiple sclerosis (MS). IFN-beta utilises IFNAR receptor and signals via signal transducer and activator of transcription 4 (STAT4). The type I IFN receptor is comprised of two chains, IFNAR1 and IFNAR2. Although type I IFN-beta can modify the course of MS, the effect is partial and the clinical response variable. Our aim is to determine the effects of interferon (IFN)-beta on the phosphorylation (activation) of signaling molecule STAT4 in MS patients compared to controls; to measure the interferon type 1 receptor (IFNAR) expression in MS patients and controls. Methods: Peripheral blood mononuclear cells (PBMC) were isolated from 22 clinically stable untreated patients with relapsing remitting MS and 12 healthy matched controls. PBMC were untreated or treated with 10 ng/ml IFN-beta. STAT4 was measured by intracellular staining with anti-phosphorylated STAT4 (pSTAT4) and 12 healthy matched controls. PBMC were untreated or treated with 10 ng/ml IFN-beta. STAT4 was measured by intracellular staining with anti-phosphorylated STAT4 (pSTAT4) and total STAT4 antibodies and flow cytometry. IFNAR was assessed by surface staining with IFNAR antibody and flow cytometry. Whole blood RNA was also isolated from both the MS patients and controls, the individual receptor subunits (IFNAR 1 and IFNAR 2) mRNA expression was measured using real time PCR. Results: IFN-beta stimulation increased pSTAT4 when compared to unstimulated cells for both MS and controls. No significant difference in total STAT4 was observed. pSTAT4 induction was higher (p<0.05) for controls than for MS patients. The level of IFNAR expression was lower (p<0.05) in MS patients compared to controls. MS patients and controls had
similar IFNAR2 mRNA expression, however IFNAR1 mRNA expression was significantly lower in MS patients when compared to control (p<0.05). A weak but still significant positive correlation (p<0.05) was observed between IFNAR protein expression and IFN-beta induced pSTAT4 by MS patients. **Conclusions:** The reduced pSTAT4 induction by IFN-beta for MS patients compared to controls indicates a decreased ability to activate IFN-beta signalling pathway via STAT4. This, combined with the reduction of IFNAR expression, suggests that untreated MS patients have decreased IFN-beta responsiveness. Whether disease modifying treatment restores IFN-beta responsiveness will be determined in future studies. Study supported by the MS Society of the UK and Northern Ireland

**P688**
**Differences in natural history and treatment effect of interferon-beta-1b in CIS patients with mono- vs. multifo cal presentations: subgroup analyses of the BENEFIT study**


The BENEFIT study examined patients with a clinically isolated syndrome (CIS) and at least two clinically silent brain MRI lesions. The risk of placebo patients developing clinically definite MS (CDMS) within a 2-year observation period was 45% and was significantly reduced in patients treated with 250 mcg interferon beta-1b (IFNB-1b). We studied the impact of key demographic, clinical and MRI parameters on the risk of CDMS and on IFNB-1b treatment effect in subgroups of the study population. Subgroups of the 468 patients (IFNB-1b: n = 292; placebo: n = 176) were created for demographic characteristics, clinical, laboratory and MRI findings at disease onset. The “natural” risk of CDMS was studied in placebo patients by Kaplan-Meier statistics and the IFNB-1b treatment effect analysed by Cox proportional hazards regression. The risk of CDMS was higher in younger placebo patients (<30 years: 60% vs. 33%), those with positive CSF findings (49% vs. 36%) and in patients who had steroid treatment of the first event (48% vs. 38%). MRI parameters implied a higher risk in patients with monofocal disease (>9 T2 lesions: 55% vs. 31%; presence of gadolinium (Gd) lesions: 63% vs. 36%) but not in those with multifocal disease onset. Treatment effects were consistent across subgroups with risk reductions of ~50%. Across the entire population, more pronounced treatment effects were observed in patients with less dissemination/disease activity at onset (monofocal: 55%, <9 T2 lesions: 60%, no Gd lesions: 57%). Within monofocal patients the treatment effect was more pronounced if there were >9 T2 lesions (61%) or Gd lesions (58%). The treatment effect was robust across subgroups including those with less dissemination/activity at disease onset. Importantly, the data indicate that a carefully performed neurological assessment of symptoms and signs is essential for better defining the impact of baseline MRI measures on risk of conversion to CDMS and treatment effect.

**P689**
**Extensive fat necrosis following subcutaneous interferon-beta injection to a patient with multiple sclerosis**

P. Vermersch, F. Lamarche, D. Testard, H. Zephir; University of Lille II (Lille, F); Rue Sainte Catherine (Abbeville, F); Centre Hospitalier de Boulogne (Boulogne, F)

**Objective:** We present a case of severe fat necrosis secondary to interferon beta-1b treatment. **Background:** most of the cutaneous reactions associated with interferon beta treatment have been reported to occur at injection sites, with lesions varying from sclerotic dermal plaques to erythematous plaques to cutaneous ulcers. We report a case of a 40-year-old woman treated with interferon beta-1b who developed a fat necrosis presenting as a large mass. **Methods:** The patient’s medical record was reviewed. **Results:** The patient was followed for a secondary progressive multiple sclerosis with superimposed relapses. Her expanded disability status score was 6.0. She was treated with subcutaneous injections of interferon beta-1b every other day. The patient said that the injections were slightly painful and she preferred to inject the drug mainly at the abdominal level. Several months after treatment onset, the patient first noticed a small, asymptomatic and subcutaneous mass in the right paraaortic region. The lesion grew slowly over several weeks in size and extended to her right iliac fossa. She progressively complainated of abdominal pain which became severe. The treatment was discontinued and the patient was referred to an emergency unit. Clinical examination, ultrasonography and CT-scan revealed a large mass measuring approximately 10 x 5 cm localized in the subcutaneous tissues extended to the deep fascia. She underwent a surgical debridement. Microscopic examination of the excised abdominal tissue showed degenerated and necrotic fatty tissue, non-specific inflammatory changes and calcifications. The patient recovered rapidly after surgery. **Conclusion:** Therapy with recombinant interferon beta-1b is associated with a large spectrum of cutaneous and subcutaneous reactions.

To our knowledge, this is the first report of fat necrosis associated with subcutaneous interferon beta 1b injection. This reaction may be related to the repeated injections at the same site.

**P690**
**Reduction of injection site reactions in multiple sclerosis patients starting interferon beta-1b therapy: Comparison of two different autoinjector devices: The EPICURE study, final results**

B. Brochet, G. Lemaire, A. Bediirafi and the EPICURE Study Group

**Objectives:** To compare occurrence of injection site reactions (ISRs) using 3 delivery methods in patients with relapsing remitting multiple sclerosis (RRMS) starting interferon beta-1b (IFNB-1b; Betaseron®/Betaseron®). **Study design:** A randomised, multicentre, phase IV, open label, cross-over study was performed at 82 sites in France. A total of 294 patients with RRMS beginning treatment with IFNB-1b were included. For the first month all patients used a standard injection technique. Patients then used an autoinjector, Betajeekt® or Betajeekt Light®, for 1 month each, according to the cross-over design. The primary outcome was the percentage of injection sites with ISRs as evaluated by the investigator. Secondary endpoints included grading of ISRs by both investigators and patients using a five-point scale, injection-related pain assessed by patients, percentage of patients without ISRs, and a global evaluation of the autoinjection devices by patients. **Results:** The occurrence of ISRs was significantly reduced when using either Betajeekt® or Betajeekt Light® (ISR 24.1% with both autoinjectors) compared to the standard technique (35.9%; p<0.0001 for both). No significant difference was observed between the two autoinjectors. Mean ISR intensity (assessed by physicians and patients) was significantly lower with the autoinjectors compared to the standard injection technique (both p<0.0001). In addition, the percentage of ISR-free patients was significantly higher with the use of either autoinjector (68.1% and 66.7% respectively; both p<0.0001) than with the standard injection technique (52.4%). A higher percentage of patients subjectively preferred Betajeekt® (53.7%) to Betajeekt Light® (46.3%), but this difference was not statistically significant. **Conclusion:** The results of this study show that autoinjector use reduces the occurrence of ISR during IFNB-1b therapy in RRMS.

**P691**
**Hepatic reactions during treatment with interferon-beta in patients with multiple sclerosis in Belgrade, Serbia**

S. Mesaro, D. Stojilovic, N. Stojavs/evic, T. Pekmezovic, J. Dradovic; Institute of Neurology (Belgrade, CS)

**Background:** Hepatic dysfunction, manifested as liver enzymes elevations, occurs frequently in patients with multiple sclerosis (MS)
 abstracts
early treatment on disease progression in a clinical setting, however, warrant further investigation in a prospective study. **Study design:** BEST is a prospective, 5-year, observational, international study of patients with early relapsing-remitting (RR) MS treated with IFNβ-1b 250 mcg subcutaneously every other day. Data collected every 6 months include Expanded Disability Status Scale (EDSS) scores and relapse assessments, together with health-related quality of life (HRQoL) and resource use information. **Results:** More than 3500 patients from 32 countries worldwide were included by the end of December 2005. We report on all patients recruited before Jan 2004 and completely documented by March 2006 (2-year cohort: N = 1324) and a subgroup recruited before Jan 2003 (3-year cohort: N = 554). Of these patients, 27.4% (363/1324) had dropped out in the 2-year cohort and 46.4% (257/554) in the 3-year cohort. After 2 years in the group of patients who remained in the study the mean age was 34.6 years (± 9.5 SD). 62.5% of the patients with relapsing-remitting multiple sclerosis (RRMS) total score was 0 for patients with stable EDSS. No new or unexpected adverse events were observed. **Conclusion:** The data confirm the safety and, to some extent, efficacy results of the pivotal study in an every day clinical practice setting. Drop out rates at 2 and 3 years are higher than those reported from clinical studies or centres specialised in MS care. This should motivate improved patient selection and better individualised care. HRQoL was correlated with clinical status as assessed by the treating neurologists and remained stable in progression-free patients.

**BEST PGx: Baseline data of a pharmacogenomic and pharmacogenetic study to identify predictors of treatment response to interferon-beta-1b**

L. Kapps, L. Achtichtich, E. Radue, S. Wu for the BEST PGx Study Group

**Background:** Markers allowing for the assessment of disease activity, prognosis and response to treatment are still urgently needed in multiple sclerosis (MS). The goal of the BEST PGx (Betaferon® in Early Relapsing-remitting Multiple Sclerosis Surveillance Trial-Pharmacogenomics and Pharmacogenetics) study is to search for and identify differences in the genetic and gene expression profile of patients with different treatment responses to interferon beta-1b (IFNβ-1b). **Study design:** Patients diagnosed with definite relapsing-remitting (RR) MS according to the Poser or McDonald criteria in the early phase of the disease course were included. After the decision to start IFNβ-1b therapy, patients were invited to participate in this study. Patients are followed for 2 years with evaluations every 6 months, including both clinical and MRI measures. Gene expression profiling using oligonucleotide microarray analysis will be performed on RNA from whole blood samples obtained at baseline, after 6–8 weeks and 10–14 months of treatment. Blood samples are only collected from patients while they are receiving treatment as prescribed. The microarray data will be analysed to identify gene expression profiles indicative of a benefit treatment response. Expression data of a limited number of genes of interest, identified from the microarray analysis, will be confirmed by real-time polymerase chain reaction (RT-PCR). **Results:** 147 patients had been enrolled in the study by the end of the recruitment phase on March 31, 2006 from 21 European centres. At baseline the mean age was 34.6 years (± 9.5 SD). 62.5% of the patients were female and 6.8% had a positive family history of MS. Mean time since the first neurological event was 3.4 years (± 4.3 SD, 1.0 median). Mean baseline EDSS step was 1.90 (± 0.8, 1.0). **Conclusion:** The BEST PGx study is expected to provide important genomic and genetic information in patients who respond differently to immunomodulatory therapy, as well as insights into the mechanism of action of IFNB-1b. **BEST PGx Investigators:** Germany: H. Peterleit, N. Sommer, H. Tumani, P. Vogel; Italy: R. Bergamaschi, R. Capra, L. Durelli, M. Leone; Netherlands: P.J.H. Jongen, B. van Oosten; Portugal: J. de Sa’; Spain: O. Fernandez; Switzerland: N. Goebels, L. Kappos, H. Mattle; Turkey: M. Eraksoy, E. Idiman, C. Irieke, R. Karabudak, A. Siva, O. Faruk Turan

**Expression of CD25, NK and CD4+ 45RO+ cells in multiple sclerosis patients before and after 9 months INF beta 1a and INF beta 1b treatment**

E. Bělník, A. Bojárská-Junák, K. Mitosék-Szewczyk, H. Bartosik-Pajzek, Z. Stelmiasiak, J. Rolinski; Medical University (Lublin, PL)

**Introduction:** Multiple sclerosis is a chronic, inflammatory, autoimmune, demyelinating disease of central nervous system. The pathological process underlying MS involve dysregulation of the immune system and it is predominantly T cell-mediated immune disorder. **Aim of the study:** The aim of our study was evaluation of some select T-cells subpopulations: CD4+ 45RO+ (memory cells), CD25 and CD316+ (NK cells) in patients with MS treated with INF beta1a (Avonex) and INF beta1b (Betaferon). **Material and Methods:** We investigated 21 patients (7 men and 14 women in mean age 32.3±8.4) with RRMS – 6 was treated with INF beta1a, 14 with INF beta1b. Peripheral blood samples were collected before therapy and after 9 months after therapy initiation. Comparisons were made with 20 control patients matched in age. **Results:** Compared to the controls the MS patients showed statistically significant (p< 0.001) lower expression of NK cells (22.8±4.0% vs. 45.4±2.0). This results doesn’t change significant during and after 9 months of INFbeta1b and INFbeta1a treatment. Compared to the controls the MS patients showed statistically significant (p< 0.01) higher expression of CD25 cells (5.3±2.7% vs. 12.0±11.7%) and higher expression (p<0.001) of CD4+ 45RO+ cells (1.53±2.6% vs. 26.7±7.4%). Expression of CD25 increased after 9 months of INFbeta 1a therapy and decreased in INF beta 1b treated group, but it was not statistically significant. Expression of CD4+ 45RO+ cells statistically significantly increased after 9 months of INFbeta 1a therapy, and does’n change in INFbeta1b treated group. **Conclusions:** Our findings suggest that after 24 months of INFbeta1b therapy there are no significant normalisation in disturbances in NK, CD4+ 45RO+ and CD25 cells expression in MS patients.

**Interferon-beta dose increase in daily practice for the treatment of patients with relapsing multiple sclerosis: improvement on relapse rate and good tolerance (the ADER study)**

W. Camu, T. Moreau, A. Al Khedher, B. Carlander, A. Danielli, A. Monjour; Centre Gui de Chauliac (Montpellier, F); Hôpital Général (Dijon, F); Hôpital Nord (Armentières, F); Centre Hospitalier de Montauban (Montauban, F); Hôpital Louis Pasteur (Colmar, F)

**Background:** Following previous positive results of interferon (IFN) beta-1a dose increase on relapse rate and disease activity in patients with relapsing–remitting multiple sclerosis (RRMS), this study evaluates the efficacy and tolerance of an IFN beta dose increase in a clinical practice setting over a 12-month period. **Methods:** 260 patients (mean age 39 years; 74% female) with RRMS (143 centres), treated with IFN and for whom the physician had decided upon an IFN dose increase (from low dose IFN beta-1a to 44 mcg subcutaneous [sc] three times weekly [twi]), were included in this study. Relapse rate, tolerance and quality of life (QoL) were assessed at baseline, 6 (M6) and 12 months (M12). Follow-up data from 260, 214 and 171 patients are available for study entry, M6 and M12, respectively. **Results:** At inclusion, the mean duration of MS was 7.8 years; 42% of patients had received IFN beta-1a > 30 mcg intramuscularly once weekly, 47%
22 mcg sc tiw and 11% 22 mcg sc once every other day. The mean duration of this treatment was 2.4 years. The percentage of relapse-free patients rose from 24% at baseline to 80% at M6 and 77% at M12. Mean relapse rates were 1.16 at baseline, and 0.23 and 0.25 at M6 and M12, respectively, with a 70% relative reduction at M12 post-conversion. Expanded disability status scale scores over the 6 months prior to baseline worsened for 43% of patients but remained unchanged between M6 and M12 for the majority. There was no impact of the dose increase on tolerance: at baseline 68% of patients treated with low-dose IFN had a good/excellent general tolerance profile; corresponding figures after conversion to 44 mcg were 77% at M6 and 78% at M12. The Short-Form 36 QoL showed poor values for both physical and mental scores at baseline, but scores improved for 59% and 62% of patients at M6 and for 56% and 60% at M12, respectively. At M12, treatment compliance (>90% of the injections) was high (95%), independent from the relapse rate. Only 5% of patients withdrew from treatment during the observation period.

**Conclusions:** These results from a ‘real life’ clinical setting confirm that the clinical activity of disease following an IFN beta dose increase is reduced in patients with RRMS. Relapse rate was reduced (70% at M12) and both local and general tolerance profiles were good. These data strongly support that high-dose, high-frequency IFN treatment provides a better outcome for patients with RRMS.

---

**P698**

**Early treatment with interferon-beta 1-a in children with multiple sclerosis**

M.R. Bardini, S. Lori, M. Morilla, M.P. Amato; A. Meyer Children Hospital (Florence, I); Careggi Dept. of Neurological & Psychiatric Sciences (Florence, I)

**Background:** multiple sclerosis (MS) is rare below 15 years of age (2.7–5% of total) and even rarer below 10 years (0.2–0.7% of total). Studies have demonstrated the efficacy and safety of Interferon Beta-1-a in adult relapsing-remitting MS, but a few data are available in children. **Objective:** To study the efficacy and tolerability of early treatment with Interferon Beta 1-a (Avonex) in subjects below 10 years with MS. **Methods:** We describe 3 white children (2 females and 1 male) with MS, diagnosed according to Poser’s criteria. At first observation the girls were 7 years (case1) and 6 years old (case2); the boy was 8 years old (case3). Case 1 follow up is of 19 years; Case2 of 3 years; Case3 of 4 years. We studied clinical, liquoral, MRI (T2 lesions and enhancing) and Evoked Potentials parameters at baseline in all. Brain MRI was repeated at any clinical relapse or every 3, 6, 12 months in the first year and every 12 months during follow up. Case1 had 14 clinical and 16 MRI relapses (6 in the first three years of illness). Case2 had 2 clinical and MRI attacks in the first 6 months. Case3 had 4 clinical and MRI attacks in the first year. All acute attacks were treated with Methylprednisolone (15 mg/kg for 5 days). Interferon Beta 1-a (1 vial/week i.m.) was started in case 1 when 16 years old (EDSS 5.5). Case2 began therapy with Interferon Beta 1-a (1/2 vial/week i.m.) 7 months after her first attack and Case3 (1 vial/week i.m.) 2 years after a relapse. Drug tolerability was studied monitoring blood levels of glucose, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, bilirubin, creatinine, protein electrophoresis, and blood erythrocyte, leukocyte and platelet count. **Results:** In case1 immunomodulating therapy induced a clinical improvement. In Case2 and in Case3 it stopped the illness progression. Tolerance was good; patients only suffered from light flu-like symptoms in the 6–8 hours after the injection, with no alteration of hematologic parameters. **Conclusions:** In these patients we obtained an arrest of illness progression after the introduction of Interferon Beta 1-a, with very good tolerance. Case1 had a worse outcome that seems in cases2 and 3. The latter, earlier treated, might face a better outcome. Follow-up is still short, however. Further multicentric studies and longer follow-up are necessary to verify the role of the Immunomodulating therapy in stopping or reducing the progression of the disability in early onset MS, and its tolerance in children.

---

**P699**

**SWABIMS: SWiss Atorvastatin and Interferon-Beta 1b Trial In Multiple Sclerosis:** efficacy, safety and tolerability of Atorvastatin® 40 mg in patients with relapsing-remitting multiple sclerosis treated with interferon-beta 1-b

C. Kamrn, J. Greve, A. Magglin, R. Bchler, C. Lientr, F. von Bredow, G. Schroth, H. Mattl on behalf of the SWABIMS study group

**Background:** Interferon-beta 1-b (IFNB-1b) subcutaneously (sc) every other day (ead) is an effective and well tolerated therapy for relapsing-remitting multiple sclerosis (RRMS). It reduces relapse rate, relapse severity and disease activity assessed by magnetic resonance imaging (MRI). Statins are lipid-lowering drugs that inhibit the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA-) reductase. In addition, statins have anti-inflammatory and immunomodulatory properties. Therefore, the purpose of SWABIMS is to investigate the efficacy, safety and tolerability of atorvastatin 40 mg in addition to IFNB-1b in patients with RRMS. **Methods:** Multi-center, randomized, rater-blinded, parallel-group-study in Switzerland (started 05/2005). 80 patients (18–55 years) with RRMS according to McDonalds criteria, EDSS between 0–3.5 and one or more relapses in the preceding two years will be enrolled. Three months after initiation of treatment with IFNB-1b, patients will be randomized into two equal-size parallel arms. Half of the patients will receive atorvastatin 40 mg/d in addition to IFNB-1b for 12 months. MRI, clinical and laboratory assessments will be rater-blinded. **Results:** Primary endpoint will be the proportion of patients with new T2 lesions. Secondary endpoints include Gd-enhancing lesion, change of total T2 hyperintense lesion volume, cortical atrophy, EDSS, MSFC, relapse rate and others. Sieric antibodies against myelin oligodendrocyte glycoprotein (anti-MOG) and myelin basic protein (anti-MBP), neutralizing antibodies (NAb), matrix-metalloproteinases (MMP-9) and tissue inhibitors of matrix-metalloproteinases (TIMP-1) will be determined. Peripheral blood mononuclear cells are prepared to investigate their migratory potential in dependence of treatment. **Conclusion:** SWABIMS will give information about the efficacy, safety and tolerability of the combination of IFNB-1b and atorvastatin in the treatment of relapsing-remitting multiple sclerosis and whether it is superior to IFNB-1b monotherapy. Furthermore important safety and tolerability data will be generated.

---

**P700**

**Effect of interferon beta-1a on changes of non-conventional MRI measures in patients with multiple sclerosis**

R. Zivadinov, D.L. Cookfair, F.E. Munschauer, M.G. Dwyer, J.L. Cox, S. Hussen, N. Abdelrahman, B. Weinstock-Guttman; University of Buffalo (Buffalo, USA)

**Background:** It is not clear whether interferon beta-1a (Avonex®) monotherapy (6.0 MU administered i.m. each week) may exert a treatment effect on lesion recovery and occult changes ongoing in normal appearing (NA) brain tissue (BT) in patients with relapsing-remitting (RR) and secondary-progressive (SP) multiple sclerosis (MS). **Objective:** To evaluate the effect of Avonex® on global and focal disease as measured by a variety of non-conventional and conventional MRI indices in a large sample of MS patients. **Methods:** This was an open label, single-blind, post-marketing, MRI observational study. Inclusion criteria were: age 18–65, RR or SP disease course, EDSS score ≤6.5, disease duration 1–20 years and presence of Avonex® monotherapy for a minimum of 6 months prior to study entry (mean exposure 5.4 years). 147 consecutive MS patients (RR = 131, SP = 16, with mean disease duration 11.1 yrs, mean age 42.8 yrs and mean EDSS 2.4) were included in the study and followed for a mean follow-up of 13.7 months. Patients received 1.5T MRI scan and clinical examination at baseline and at the end of follow-up. Changes were determined in a number of non-conventional and conventional MRI measures. **Results:** Out of 147 patients, 144 completed clinical and MRI follow-up. 13.6% progressed in their disability status and...
25.2% of the patients presented ≥1 relapse during the follow-up period. Treatment with Avonex® decreased accumulation of T1-lesion volume (LV) (-12.7%, p < 0.0001) and promoted recovery of magnetization transfer ratio (MTR) in T1-LV (+21.2%, p < 0.0001). No significant within-patient deterioration was observed in MTR measures of whole brain (-1.4%), NAIT (-1.3%), and NA white matter (WM) (-1.1%), except for the NA gray matter (GM) (-1.1%, p = 0.05). No significant subcortical atrophy was observed in NCV. A significant decrease of Gd-enhancement (p = 0.008) activity was observed. Subclinical disease activity, as evaluated by presence of Gd lesions at baseline, determined more advanced progression on normalized GM volume (-4% vs. -1.6%, p = 0.008) and cortical volume (-3.3% vs. -1.3%, p = 0.005) measures. The improvement-stabilization on a variety of MRI measures was related to the length of pre-study drug exposure to Avonex® (p = 0.014).

**Conclusions:** Avonex® slowed down accumulation and promoted lesion recovery of T1 hypointense lesions, and stabilized progression on a number of other MRI measures. This study was funded by a grant from Biogen Idec.

**P701**

**MRI results from a randomised, double-blind, placebo-controlled study of intramuscular interferon beta-1a, azathioprine, and corticosteroid combination therapy in patients with relapsing-remitting multiple sclerosis**

K. Zivadinov, D. Horakova, M.G. Dwyer, A. Dolezel, J. Krasensky, N. Berglund, J.L. Cox, I. Novakova, V. Ticha, S. Balachandran, A. Svobodnik, Z. Seidl, M. Vaneckova, E. Havrová; University of Buffalo (Buffalo, USA); Charles University (Prague, CZ); Masaryk University (Brno, CZ)

**Background:** To date, the efficacy of agents such as azathioprine (AZA) and corticosteroids in combination with IFN-beta has only been examined as treatment for breakthrough multiple sclerosis (MS) in small, uncontrolled trials. **Objective:** To determine the efficacy of IM IFNb-1a combined with AZA, or AZA plus low-dose corticosteroids compared with that of IM IFN beta-1a alone as initial therapy. **Methods:** The Avonex-Steroids-Azathioprine (ASA) combination study was a randomised, double-blind, placebo-controlled trial. A total of 181 patients with clinically definite MS, and EDSS score ≤3.5 at screening, and either 2 relapses in the previous 12 months or 3 relapses in the previous 24 months were equally randomised to 1 of 3 treatment groups. Group 1 received interferon beta (IFN beta-1a) 30 μg IM once weekly, Group 2 received IFN beta-1a 30 μg IM once weekly plus AZA 50 mg by mouth (PO) once daily (QD), and Group 3 received IFN beta-1a 30 μg IM once weekly plus AZA 50 mg PO QD plus prednisone 10 mg PO every other day; placebo was administered in place of AZA and prednisone in Group 1 and in place of prednisone in Group 2. MRIIs were obtained every 2 months for the first 2 years and once yearly thereafter for up to 5 years. Percent brain volume change (PBVC), normalised grey matter volume (NGMV), normalised white matter volume (NWMV), and normalised cortical volume (NCV) were measured bi-monthly for the first 2 years and once yearly thereafter for up to 5 years using SIENA and SIENAX software. In addition, T2-hypointense lesion volume (LV), lateral ventricle volume (LVV), and third ventricle width (3VW) were assessed every 6 months for the first 2 years and once yearly thereafter for up to 5 years. **Results:** Over 2 years, the addition of AZA alone, or in combination with prednisone, to IM IFN beta-1a did not improve any MRI outcome in patients with MS. The combination therapy favored the lower accumulation in T2-LV at 2 years in group 3, when compared with group 1 (p = 0.01). At 2 years the overall percent change in atrophy measures in all study participants was: PBVC -1.5, NGMV -3, NCV -2.7, NWMV -0.1, 3VW +24.1 and LVV +22.8. At 5 years the figures were: PBVC -4.7, NGMV -7.9, NCV -7.8, NWMV -1.8, 3VW +30.9 and +LVV 87.1. **Conclusions:** Combination treatment did not improve any of the MRI outcomes when compared with monotherapy. This study provides important information on longitudinal evolution of various atrophy measures in the long-term.

**P702**

**Comparison of Betaferon®, Avonex® and Rebiif® in treatment of relapsing-remitting multiple sclerosis**

V. Shaygannejad, M. Etemadifar, A. Fereshteh; Isfahan University (Isfahan, IR)

**Objectives:** To compare the relative efficacy of Betalferon, Avonex and Rebiif in the treatment of relapsing-remitting multiple sclerosis (RRMS). **Methods:** 90 patients with RRMS were randomly allocated to the three treatment groups. The first group received Betalferon, the second group received Avonex and the third group received Rebiif for 24 months. Response to treatment was assessed at 6, 12 and 24 months after start of therapy. **Results:** Of the 30 patients treated with Betalferon, the mean (SD) of relapse rate decreased from 2.2 (0.7) to 0.7 (0.7) episode. Correspondingly, in the 30 patients treated with Avonex, the mean (SD) of relapse rate decreased from 2.0 (1.2) to 1.2 (0.9) (p < 0.001). In the 30 patients treated with Rebiif, the mean (SD) of relapse rate decreased from 2.4 (1.0) to 0.6 (0.9) (p < 0.01). After 2 years, 43.3% of patients receiving Betalferon and 56.7% of patients receiving Rebiif remained relapse free compared with 20% of those given Avonex. **Conclusion:** Relapse rates decreased by 0.7 units in Betalferon-treated patients (p < 0.001), 0.3 in Rebiif-treated patients (p < 0.05) and remain stable in Avonex arm. **Conclusion:** Treatment with Betalferon, Avonex and Rebiif significantly reduce relapse rate and EDSS score in patients ith RRMS.

**P703**

**Effect of interferon-beta-1b therapy on nitric oxide (nitrite/nitrate) serum level in patients with relapsing-remitting multiple sclerosis**

A. Ilic, S. Vojinovic, I. Stanjanovic, L. Zvezdanovic, V. Djordjevic, M. Zivkovic; Clinical Center (Nis, CS)

**Background:** Interferon-beta (IFNbeta) reduces exacerbations of the relapsing-remitting (RR) form of multiple sclerosis (MS), but the exact mechanisms by which it exerts its beneficial effects are unknown. The therapeutic effect of IFNbeta in MS may be partly due to suppression of pathogenic nitric oxid (NO) production, molecules that have one of the most important roles in the pathogenesis of MS. **Purpose:** The aim of this study was to investigate the effect of IFNbeta on nitrite/nitrate serum levels in patients with RRMS. **Method:** We have evaluated 26 RRMS patients, diagnosed by McDonald’s criteria, subdivided in two groups: I group-11 RRMS patients on IFNbeta therapy, during last 18 months, 8 million units on alternate day by subcutaneous injection and without relapses, aged 38.63 ± 8.15 (rang 22 –52); eight females (72.72%) and two male (18.18%); EDSS 2.7 ± 1.01; and K group – 15 RRMS patients that have never had any IFNbeta therapy, without relapses in last 18 months, aged 37.06 ± 8.87 (rang 22 –51); 11 females (73.33%) and four male (26.66%); EDSS 2.03 ± 0.80. Disease course was clinically assessed by the means of the Expanded Disability Status Scale (EDSS) score. NO (nitrite/nitrate) levels were analysed in the peripheral blood serum as well as biochemical profile to observe general state of patients and to exclude other ongoing inflammation. NO (nitrite/nitrate) levels were determined according to the method of Navarro-Gonzalez, based on nitrate-nitrite determination using Griess reaction. **Results:** Nitrite and nitrate concentrations in peripheral blood serum in 1 group was 73.65 ± 11.93 micromol/l while in K group, without therapy, was 87.98 ± 20.41 micromol/l which was statistically significant difference, p = 0.03, among groups. **Conclusion:** IFNbeta therapy significantly influence on NO (nitrite/nitrate) levels in patient with RRMS. Disease control achieved by IFN beta could be explained by immunomodulation of NO (nitrite/nitrate) synthesis in patients with RRMS.
P704
Neutralising antibodies to interferon-beta in multiple sclerosis: technical report and validation of a cytopathic effect assay
C. Massart, J. Oger, S.E. Grossberg, J. Gbissah, E. Le Page, G. Eddan; CHU Pontchaillou (Rennes, F); University of British Colombia (Vancouver, CAN); Medical College of Wisconsin (Milwaukee, USA)

Introduction: Neutralising antibodies (NAB) to interferon beta (IFN-beta) reduce treatment efficacy in patients with multiple sclerosis (MS). Objective: In an attempt to validate a NAB assay, we report the analytical clinical performances of this bioassay based on antiviral cytopathic effect (CPE) according to the guidelines recently reported by the European Federation of Neurological Societies (EFNS).

Patients/Methods: The study included 63 sera from MS patients treated with IFN-beta 1a (Rebif®) or IFN-beta 1b (Betaferon®). Binding antibodies (BABs) were measured using a capture ELISA as a screening test for NABs. NABs were measured in all the sera using the WISH cell line infected by the stomatitis vesicular virus. NAB titres expressed in ten-fold reduction (TRU/ml) were then compared with those obtained with the validated CPE gold standard method, using A549 cells and the encephalomyocarditis virus. The National Institute of Health reference anti-IFN-beta antisemur (G038-S01 572) was included for NAB inter-assay precision. Results: No false-negative BAB result was obtained with the capture ELISA. The sensitivity of the NAB assay was 20 TRU/ml. The between-run coefficients of variation (CVs) determined with log10 NAB titres yielded good results (≤ 10.4%). Within-run variability was excellent with CV ≤ 2%. The log10 NAB titres were highly correlated with those obtained with the CPE reference method (r = 0.963, p < 0.0001 and r = 0.870, p < 0.0001 for anti-IFN-beta 1a and anti-IFN-beta 1b, respectively). The same NAB-positive patients with high titres ≥ 100 TRU/ml was the cut-off value for EINS to stop IFN-beta treatment were found with both CPE methods.

Conclusion: Our CPE assay for NAB measurement showed high sensitivity, good precision, and strong correlation with the reference gold-standard CPE assay. Further studies in larger numbers of IFN-beta-treated MS patients are planned to confirm our preliminary results.

P705
Incidence and effects of neutralising antibodies in patients with multiple sclerosis treated with Avonex® or Rebif® in PROOF
T.J. Murray, A. Minagar for the PROOF Study Investigators

Sustained benefits from interferon beta (IFN beta) products used to treat relapsing multiple sclerosis (RMS) require adherence (affected by tolerability and convenience) and clinical efficacy, which can be reduced when neutralizing antibodies (NAbs) are present. PROOF, a multi-centre (Finland, Denmark, Norway, Sverige), open label, non-comparative trial investigating the recovering of IFN-beta efficacy in relapsing-remitting MS (RRMS) patients included in this study. Fourteen of them participated also in the RECOVER trial, a multi-centre (Finland, Denmark, Norway, Sverige), open label, non-comparative trial investigating the recovering of IFN-beta efficacy in relapsing-remitting MS patients with neutralizing IFN-beta antibodies. These patients had been tested to be NAb positive in a cytopathic effect (CPE) assay. All 53 patients were tested for MxA protein induction by using both MxA ELISA assay and flow cytometric analysis. Results: There was a positive but weak correlation between ELISA and flow cytometry. The range of measured responses was higher in the ELISA. The presence of NABs associated with low MxA levels in both assays, but the number of patients was too small to quantitatively correlate MxA levels and NABs. Conclusions: Since MxA protein is produced for at least 12 – 24 hours after IFN-beta injection, whereas MxA messenger RNA is induced only for 9 – 12 hours, MxA protein seems to be the preferable measure for the bioefficacy of IFN-beta therapy. It has lower risk of false negative results and more practical timing of collection of blood samples. In addition, the simplified MxA ELISA assay seems to be a reliable method to measure MxA protein induction and the bioavailability of IFN-beta treatment in MS patients.

P706
MxA protein assay for optimal monitoring of IFN-beta bioefficacy in the treatment of multiple sclerosis patients
A.-M. Vallittu, P.-P. Eriluoma, A. Salmi, M. Waris; University of Turku (Turku, FIN)

Background: Myxovirus resistance protein A (MxA) seems to be one of the most appropriate markers of the biological activity of IFN-beta therapy. Because of neutralizing antibodies (NAbs), 5% to 45% of MS patients fail to respond to IFN-beta therapy leading to attenuation of MxA protein induction. Recently published guidelines on use of anti-IFN-beta antibody measurements in MS recommend that tests for the presence of NAbs should be performed in all patients at 12 and 24 months of therapy. Objectives: The aim of the study was to evaluate a simple MxA ELISA assay by comparing it with flow cytometric analysis of MxA and measurements of NAbs. Methods. A total of 53 relapsing-remitting MS (RRMS) patients were included in this study. Fourteen of them participated also in the RECOVER trial, a multi-centre centre (Finland, Denmark, Norway, Sverige), open label, non-comparative trial investigating the recovering of IFN-beta efficacy in relapsing-remitting MS patients with neutralizing IFN-beta antibodies. These patients had been tested to be NAb positive in a cytopathic effect (CPE) assay. All 53 patients were tested for MxA protein induction by using both MxA ELISA assay and flow cytometric analysis. Results: There was a positive but weak correlation between ELISA and flow cytometry. The range of measured responses was higher in the ELISA. The presence of NAbs associated with low MxA levels in both assays, but the number of patients was too small to quantitatively correlate MxA levels and NAbs. Conclusions: Since MxA protein is produced for at least 12 – 24 hours after IFN-beta injection, whereas MxA messenger RNA is induced only for 9 – 12 hours, MxA protein seems to be the preferable measure for the bioefficacy of IFN-beta therapy. It has lower risk of false negative results and more practical timing of collection of blood samples. In addition, the simplified MxA ELISA assay seems to be a reliable method to measure MxA protein induction and the bioavailability of IFN-beta treatment in MS patients.

P707
A longitudinal 36 monthly imaging study on the effect of NAB in patients with multiple sclerosis
A.W. Chiu, M. Ehrmanntraut, N. Richert, J. Ohtayan, F. Cantor, J. Frank, H. McFarland, F. Bagnato; NINDS-NIH (Bethesda, USA)

The clinical impact of neutralizing antibodies (NAB) in Multiple Sclerosis (MS) patients undergoing therapy with interferon beta (IFNbeta) remains controversial. To address this issue, both cross-sectional and longitudinal studies should be performed in the same cohort of patients. To our knowledge, monthly longitudinal assessment of NAB and its effect in MS patients has yet to be examined. Fifteen consecutive relapsing remitting MS patients were treated with IFNbeta (1b (250 micro g, subcutaneous, every other day) for 3 years. Patients were followed with monthly (6-month pretherapy phase [PTP] and 36-month therapy phase [TP]) magnetic resonance imaging (MRI) and clinical exams as well as bimonthly NAB evaluations. MRIs were performed on a 1.5 Tesla magnet. T2-weighted spin echo (SE) and...
T1-W images SE before and within 15 minutes of a 0.1 mmol/kg injection of gadopentetate dimeglumine were obtained at each session. NAb evaluation was performed using the Myxovirus-A protein inhibition assay. Patients with NAb titres ≥1:20 in at least 2 consecutive samples were considered NAb+. The relationship between NAB and contrast-enhancing lesion (CEL) activity is presently analyzed. Of the 15 patients enrolled, 5 developed NAB. Of those 5 NAB- patients, 2 developed NAB activity at low (e.g. ≤1:47) and transient titres, present only for no more than 6 measurements. However, both patients had consistent CEL activity throughout the entire TP. One of these patients progressed into secondary progressive MS. The remaining 3 patients exhibited NAB titres between 1:24 –1:2703 during the first 2 years of therapy that was coincident with the presence of CELs. Although all 3 patients showed waxing and waning of NAB activity throughout TP, the NAB activity of 1 patient waned completely by month 19 while the NAB titre of the remaining 2 patients only began to decrease at month 20. CEL levels were concomitant with NAB titres in the former patient whereas CEL activity remained high in the latter 2 individuals. Notably, CEL activity was high in all three patients prior to the occurrence of NAB. While our cohort is small, the length of the longitudinal follow-up offers a unique dataset worthy of description. While reduction in therapeutic efficacy is observed in patients with high NAB titres, those patients seem to exhibit a predisposition to poor IFNβ-1b response prior to NAB occurrence. Further analyses in larger sample populations are warranted to confirm our preliminary investigation.

P708

Acute and steady state effects of interferon-beta in multiple sclerosis evaluated by gene expression profiling

F. Sellebjerg, I. Larsen, P. Datta, K. Rieneck, I. Alsing, A. Ottuari, A. Sveiggaard, P.S. Sørensen, L.P. Ryder; Copenhagen University Hospital Rigshospitalet (Copenhagen, DK)

Treatment of multiple sclerosis (MS) with interferon (IFN)-beta results in substantial changes in in vivo mRNA expression in blood mononuclear cells. The expression of mRNA encoding the antiviral molecule myxovirus resistance protein (MxA, encoded by the Mx1 gene) has been used as a marker of the in vivo activity of IFN-beta in studies showing that this molecule is not induced in patients with high titers of neutralizing anti-IFN-beta antibodies. MxA mRNA expression after administration of IFN-beta is, however, transient with maximum levels seen within the first twelve hours. We hypothesized that it might be possible to identify mRNAs which are more stably induced by treatment with IFN-beta, and used the Affymetrix GeneChip Focus array with more than 8,500 different genes displayed to study changes in gene expression in 10 patients treated with IFN-beta1a (30 μg intramuscularly once weekly). Blood samples for the study of mRNA expression in purified mononuclear cells (MNC) were drawn before the first injection of IFN-beta1a and 14 –20 hours after the first injection of IFN-beta1a (T1). After three months of treatment new blood samples were obtained before the injection of IFN-beta (T2) and after the injection of IFN-beta (T3). After the first injection of IFN-beta (T1) the expression of 283 genes differed significantly from baseline values (T0; p < 0.05 with Bonferroni correction). Among 135 up-regulated genes, 57 were induced at least two-fold. MxA mRNA was among the most induced genes, being 8-fold induced after the first injection of IFN-beta (p = 0.00000001). Among 148 down-regulated genes, 11 were at least 50% reduced. Gene expression at baseline and T2 did not differ significantly. After three months of treatment with IFN-beta, the injection of IFN-beta resulted in slightly less pronounced changes in gene expression, but there was no significant difference in the gene expression profile at T1 and T3. We conclude that treatment with IFN-beta results in acute changes but no major, persisting changes in blood MNC mRNA expression. As MxA/Mx1 is one of the poorly induced molecules, we also conclude that this molecule is well suited as a marker of the acute in vivo effect of IFN-beta in MS.

P709

IFN-beta 1A expands CD4+ and CD8+ regulatory T-lymphocytes in vivo. A longitudinal 1-year study

C. Aritstimuño, C. de Andrés, V. de las Heras, M. Martínez-Gines, M. Bartolome, R. Arroyo, J. Navarro, S. Giménez-Roldán, E. Fernández-Cruz, S. Sanchez-Ramon; General University Hospital (Madrid, E); University Hospital Clinico San Carlos (Madrid, E)

Introduction: Interferon beta-1a (IFNb) has demonstrated clinical efficacy in multiple sclerosis (MS). However, the immunological effects at the cellular level remains only partially understood. CD8+ T-cells may contribute to lesion formation and axonal dysfunction in MS. Dendritic cells (DCs) may have a role in the local activation and expansion of presumably pathogenic T cells. Regulatory T-cells (Treg; CD4+CD25+ and CD4+CD25hi), play an important role in preventing autoimmune diseases such as MS. Recently, a new subset of CD8+ suppressor T-lymphocytes has been described. Patients and methods: We studied 23 patients with relapsing remitting MS subsequently treated with IFNb-1a (Rebiff 44 mcg tiw) therapy. We analysed by multiparametric flow-cytometry the ex-vivo changes on Treg and on the different subsets of CD8+ T-lymphocytes before IFNb-1a therapy and at 3, 6 and 12-mo; and in 18 healthy controls (HC). We also compared data of those patients that suffered relapses during follow-up versus asymptomatic patients (10 vs. 13). Results: IFNb-1a significantly increased the proportions of Treg (CD4+CD25+CD4+CD25hi+) after 12-mo of treatment in MS patients with respect to baseline (p = 0.004 and p = 0.0000 respec- tively). Furthermore, IFNb-1a increased proportions of regulatory CD8+CD25+ T-cells (p = 0.01) and CD8+CD25+CD28- T-cells (0.24 to 1:03). By contrast, a trend to decreased proportions of CD8+CD25+CD25+CD28- T-cells was observed at 12-mo. We purified CD8+CD25+CD28- T-cells and confirmed their suppressive function in vitro. Absolute numbers of CD8+CD25+ T-cells and CD8+ CD25+CD28- T-cells correlated with CD4+ Treg (r = 0.6, p = 0.01); CD8+CD25+ T-cells inversely correlated with plasmacytoid dendritic cells (pDCs CD123+) (r = −0.7, p = 0.001). A repeated measures analysis variance showed higher CD8+CD25+ and both subsets of CD4+ Treg levels while lower activated CD8+ T-cells in patients who suffered relapses with respect to asymptomatic patients. Conclusions: IFNb-1a significantly increases the proportions of CD4+ and CD8+ regulatory T-cells in MS patients after 1-year of treatment and decreases pathogenic CD8+ T-cells, such as CD8+TCRgd+ T-cells.

P710

Differential changes of circulating plasmacytoid dendritic cells and regulatory CD4+ T-cells on interferon-beta-1a therapy. An ex vivo 1-year observational longitudinal study in relapsing-remitting multiple sclerosis

C. de Andrés, C. Aritstimuño, V. de las Heras, M. Martinez-Gines, M. Bartolome, R. Arroyo, J. Navarro, S. Giménez-Roldán, E. Fernández-Cruz, S. Sanchez-Ramon; Hospital General Universitario Gregorio Marañon (Madrid, E); Hospital Clinico San Carlos (Madrid, E)

Background: Dendritic cells (DCs) have the unique ability to initiate and regulate immune responses, and thus play a pivotal role in multiple sclerosis (MS). CD4+CD25+ and CD4+CD25+high regulatory T-cells (Treg) actively control the activation, and effector function of both self-antigen-reactive CD4+ and CD8+ T-cells. DCs and Treg have been reported to be involved in MS pathophysiology. Interferon beta (IFNb) is effective in reducing clinical relapse rate and brain disease activity measured by MRI, but its cellular effects are to be defined. Objectives: Our main goal was to assess numbers and phenotypes of circulating myeloid DCs (mDC) and plasmacytoid DCs (pDCs) and CD4+CD25+ and CD4+CD25+high Treg cells in MS patients before the initiation of IFNb-1a therapy and after 3 – 6 and 12 mo of treatment. Methods: Clinical and haematological data were subsequently studied before initiation of IFNb-1a (Rebiff 44 mcg tiw)
therapy and at 3, 6 and 12 mo; and in 18 healthy controls (HC). We analysed lymphocyte subsets by multiparametric flow-cytometry. Functional suppression of TReg was measured by allogeneic and TCR-stimulation on mixed lymphocyte reaction. 

Results: Twenty-three patients with definite RR MS, 13 female and 10 men, with mean age 36.77 years, mean MS duration 3.97 years, mean EDSS score 2.17, were given IFNβ-1a therapy. There was a sustained trend to decrease in the %CD123 + pDCs after IFNβ-1a therapy at 3, 6 and 12-mo (p = 0.07, p = 0.04 and p = 0.08, respectively) respect to baseline. By contrast, significant increase in the %CD4 + CD25 + pDCs while increase in CD4 + CD25 high + TReg cells were observed at 12-mo respect to baseline (p = 0.004 and p = 0.000, respectively). 

Inverse correlation between %CD123 + pDCs and CD4 + CD25 +, %CD123 + pDCs and CD4 + CD25 high + TReg (r = -0.45 p = 0.06 and r = -0.5 p = 0.03) was observed. At 12-mo IFNβ-1a, suppressive TReg function was partially restored and both TReg subsets were higher in patients than in HC (p = 0.000 and p = 0.000). Longitudinal CD123 + pDCs and both subsets of TReg patterns were different in patients who suffered relapses with respect to asymptomatic patients. 

Conclusions: During the 1-year follow-up of IFNβ-1a therapy, we observed a decrease in the proportion of CD123 + pDCs while increase in numbers and function of TReg. Such changes may be relevant in the activity of MS. This study suggests that the differential changes of TReg and DCs on IFNB-1a therapy may be involved in its immunomodulatory and therapeutic effects.

P711 Interferon receptor expression in multiple sclerosis patients during the first year of treatment with IFN-beta

B. Oliver, C. Mayorga, V.E. Fernandez, L. Leyva, G. Luque, J. Tamayo, M.J. Pinto-Model, F. Diez de Baldeon, R. Bustamante, A. Alonso, O. Fernandez; Clinical Neurosciences Institute. Carlos Haya Hospital (Malaga, E)

Background: IFN-beta has shown to be an effective treatment for multiple sclerosis (MS), however, some patients fail to respond fully. The biological activity of IFN-beta is exerted through the IFN receptor (IFNAR), composed of two subunits, IFNAR1 and IFNAR2. In a previous work we have demonstrated that IFNAR expression decrease in MS patients treated with IFN-beta, especially in those patients considered as good responders to this treatment. Objective: To assess if the differences in IFNAR1 and IFNAR2 expression in MS patients treated with different IFN-beta molecules are maintained during all the treatment period in the same way in responder and non-responder patients. Methods: Sequential samples from MS patients were obtained: Before treatment and 1, 6 and 12 months after the starting of the IFN-beta treatment. The quantification of IFNAR1, IFNAR2 and MxA mRNA expression was carried out by real-time RT-PCR in peripheral blood mononuclear cells from MS patients. Patients treated with IFN-beta were classified as responders or non-responders according to annual relapse rate and EDSS.

Results: A total of 25 MS patients were followed during 12 months after the onset of the treatment with IFN-beta. Sequential samples obtained before, one, six and twelve months after the beginning of the IFN-beta treatment showed that there was a strong decrease in both IFNAR1 and IFNAR2 mRNA production right from the first month of treatment which were kept for the following months of the study. On the contrary, the MxA inducible protein was increased also from the first month of treatment. When we classified the patients as responders (17 MS patients) or non-responders (8 MS patients) the decrease on IFNAR expression and the increase in the MxA expression were only observed in those patients who had a good response to IFN-beta. The changes were equally detected from the first month of treatment. Conclusions: The influences produced by IFN-beta in the expression of its receptor determined by a down-regulation of IFNAR1 and IFNAR2 and an increase in MxA protein is observed just from the first month of treatment and maintained at least during the following twelve months. These changes are more important in patients who responded to IFN-beta treatment.
the first patients enrolled in RENeu. Thirteen patients (of 20 planned for RENeu) have now commenced treatment with monthly-pulsed prednisolone. Their NAb titres at study entry (CPE) ranged from 40 to >4000 (upper limit of assay). Of these, six patients have received methylprednisolone for up to 12 months, all starting with NAB titres > 200. NAb titres have decreased significantly in 4 of these 6 patients, reaching < 20 in one patient and 30 in three. The patient eligible for bioactivity testing (CPE < 20) was found to be responsive to injected IFN by the MxA mRNA test and has remained NAb- following subsequent treatment with AVONEX once weekly for 6 months. Most importantly, this patient continues to respond biologically to AVONEX. A recent report by Petersen et al in 2006 has demonstrated that in 18 patients with NAB titres > 200 by CPE, NAb titres remained high for extended periods after discontinuing IFN therapy. The NAb titre only decreased below 100 in one patient, and this did not occur until more than 3 years after ceasing therapy. In contrast, the initial results of the RENeu study show that monthly-pulsed methylprednisolone may be effective in reducing NAb titres. Bioactivity testing is useful for clarifying whether low titre NABs do impact on patient responsiveness to IFN.

P714 Neutralising antibodies against interferon in 379 patients with multiple sclerosis J.L. Frederiksen, N.H. Beyr; Glostrup University Hospital (Glostrup, DK)

Background: The development of neutralizing antibodies (NAB) against interferon-gamma (IFNg) has recently been shown to decrease the effect of IFNg in relapsing remitting multiple sclerosis (RRMS).

Materials: At the MS clinic at Glostrup University Hospital serving Copenhagen County 379 patients were treated with IFNg during the period from July 1996 to December 2004. They were divided into 2 groups: one group with minimum one value of NAB ≥ 80% (77 patients), and one group (control group) (302 patients) with no NABs or values of NAB always below 80%. The population based consecutively examined group of patients were followed prospectively.

Methods: Each patient course was registered systematically every sixth month with gender, age at onset of MS, age and date of onset of treatment with IFN, change of treatment, duration of treatment and actual treatment, number of relapses since last visit, EDSS-score, development of SPMS and development of NAB 80%. Results and Conclusion: Neither gender, age at onset of MS nor at onset of treatment, nor EDSS at onset of treatment was found to influence the development of NAB ≥ 80%. In the group of patients with NAB ≥ 80% patients treated with Betaferon and Rebif had a higher yearly attack rate than patients treated with Avonex. The time from onset of MS to development of NAB ≥ 80% was also influenced by the choice of treatment: Rebif as first used treatment resulted in a little higher frequency of NAB ≥ 80% than Betaferon and much higher than Avonex. The development of NAB ≥ 80% happened nearly only within 2 years from onset of treatment with Betaferon. Rebif resulted in a more smooth distribution with duration of treatment. Only 4 patients treated with Avonex developed NAB ≥ 80%. Fewer patients in the NAB ≥ 80% group continued the first treatment choice, and generally changed treatment more frequently than the controls, mainly due to NAB. Patients with Rebif as first choice treatment were overrepresented in this group. The NAB-values were not stable. A single NAB positive value was not predictive of NAB being present at continuous treatment with IFNg.

P715 Suggested limits for high and low neutralising antibody levels in interferon-beta treated multiple sclerosis patients E. Gibbs, J. Oger; University of British Columbia (Vancouver, CAN)

Objectives: To assess the incidence of NABs in a population of Interferon beta (IFNβ)-treated Multiple Sclerosis MS patients for whom antibody testing was requested by neurologists. To determine objectively cut-off points for High, Medium and Low levels of Neutralizing antibodies (NABs) in MS patients treated with Interferons. Background: The IFNbs have become integral in the clinical management of MS patients. Associated with IFNb therapy, is the development of antibodies, binding (BAbs) and neutralizing (NAb), the latter are linked to decreased bioavailability and efficacy of IFN. In our large experience of treating MS patients with Interferons and measuring neutralizing antibodies in an autologous system, we suspected that there are differences in the patterns of results according to the IFN the patient has received. Methods: Since the introduction of routine clinical testing for NABs, 1218 serum samples have been screened for BAbs by ELISA. Among those 675 were BAb+ and have been forwarded for NAb testing by the CPE method (Dr.S.Grossberg). All samples were blinded, except for the type of IFNb received, as this is needed for autologous assaying of anti-IFNbeta antibodies. Results: BAbs were positive by ELISA in 675 patients (35.4%); 283 of those were NAB positive. NAB positivity was 23% in Beta-1b-treated patients compared to 19% in Rebif®-treated patients. The NAB titres (mean ± SEM) in Beta-1a-treated patients (810 ± 2048), were significantly higher than in Beta-1b-treated patients (951 ± 151). When segregated into 3 groups equal in number of samples, the upper limits of low NABs, and the lower limit of high NABs were respectively 110TRU/mL and 492 for Beta-1b. For Beta-1as they were 352 and 2733 TRU/mL. Conclusion: NAB titres generally end up being higher in Beta-1a treated patients compared to Beta-1b treated patients. Consequently cutoff levels for high positivity should differ among the different IFNb preparations used. This can probably be explained because Beta-1b has a lower specific activity and NABs are measured using bio-assays. BAbs measures do not have this problem.

P716 The cross-reactivity of different interferon-beta is not 100% M. Hosseina, E. Gibbs, T. Aziz, J. Oger; University of British Columbia (Vancouver, CAN)

Objective: To determine cross-reactivity of serum binding antibodies (BAbs) in patients receiving each interferon-beta with the three commercially available interferon-beta. Background: An increasing number of MS patients are being treated with one IFN-beta, and then switched to an alternate IFN-beta. In such cases knowledge of cross-reactivity is of importance in deciding therapeutic strategies. Previous studies have found that BAbs to Betaseron (IFN-beta 1b) and Avonex or Rebif (IFN-beta 1a) are cross-reactive. Methods: Sera from six MS patients, two each treated with one of the three commercially available IFN-beta: Avonex®, Rebif®, or Betaseron®, who had previously tested positive for BAbs were assessed for cross-reactivity in direct and sandwich ELISA. Percent cross-reactivity for each sample was calculated as [Mean OD of heterologous antigen] / [Mean OD of homologous antigen]*100. Results: Direct ELISA: BAbs in sera of patients treated with Beta-1b cross reacted with Rebif and Avonex antigens in direct ELISA with only 34.2% and 42.3% cross reactivities respectively. BAbs in patients treated with any of the Beta-1a cross reacted with Beta-1b with 105.6% to 129.9% cross-reactivity while it reacted with the other Beta-1a with 95.3 to 105.6% cross reactivity. Sandwich ELISA: BAbs in sera of patients treated with Beta-1b cross reacted with Rebif and Avonex antigens in sandwich ELISA with 46.0% and 45.9% cross reactivities respectively. BAbs in sera of patients treated with Beta-1a cross reacted with Beta-1b with 105.6% to 129.9% cross-reactivity while it reacted with the other Beta-1a with 95.3 to 105.6% cross reactivity. Conclusion: Different cross-reactivity was found when sandwich ELISA and direct ELISA were used. Sandwich ELISA is thought to be closer to the in vivo situation (Brickelmaier et al.).
Betaseron than to Rebif and Avonex antigens. In contrast, anti-Rebif and anti-Avonex antibodies in sera of NAb positive patients had similar cross-reactivities with Betaseron, Rebif and Avonex antigens. In switching antibodies positive patients from one IFN-beta treatment to another, cross-reactivity as defined here should be taken in consideration.

P717
Persistent neutralising antibodies to interferon-beta and clinical outcomes in multiple sclerosis patients
L. Costelloe, K. O’Rourke, N. Tubridy, M. Hutchinson; St. Vincent’s University Hospital (Dublin, IRL)

Background: Neutralising antibody (Nab) positive patients have a relapse rate similar to placebo treated patients. The long-term effects of persistent NAb on disease progression and the clinical outcomes of those with persistent NAb remain unknown. Aims: To investigate the effects of persistent NAbS and Nab titre on disability as measured by the Expanded Disability Status Scale (EDSS) over a 3-year period. To examine the treatment outcomes of patients with persistent NAbS. Methods and Patients: 53 Nab positive patients with a disability range of EDSS 0–6.5 were followed for up to three years with yearly EDSS assessment. 9 patients had one positive test, 18 had 2 positive tests, and 26 had 3 positive tests. 40 were on Betaseron and 13 were on Rebif. Serum: Serum was tested for NAbS using a cytopathic effect assay. High titre NAbS were >295 NU, low titre NAbS were <295 NU and >10 NU. Results: 21 patients had unchanged EDSS scores during follow-up. 32 patients experienced a significant change during the 3-year period. The median Nab titre decreased during the follow-up period in both patients with stable and changed EDSS scores. Influence of Nab titre: We divided patients into 2 groups, those who had high titres at first testing, and those who had high titres at any time during follow up. Of the patients who had high titre NAbS initially, the mean change in EDSS during follow-up was higher than in those who initially had low titre NAbS (0.96 points Vs 0.71 points). Similarly, of patients who had high titre NAbS at any stage during follow-up, mean change in EDSS was higher than in those who never had high titre NAbS (0.94 points Vs 0.74 points). 16 low titre, Nab positive patients converted to Nab negative status during follow up. Change of ImI: 13/53 patients had a change of treatment during follow up. Of these 5 were switched to Copaxone, 3 to Mitoxantrone, 1 to an alternative interferonb preparation, and 4 were taken off all immunomodulators. Conclusion: In patients with persistent NAbS the median Nab titre decreases over time. However, high titre NAbS at any stage during treatment is associated with a worsening of disability as measured by the EDSS over time. Persistent Nab positivity often necessitates treatment change. Patients with high titre NAbS never converted to Nab negative status whereas those with low titre NabS often did. Therefore, interferon-beta treatment may have a continued efficacy in this subgroup of Nab positive patients.

P718
Measuring anti-interferon beta neutralising antibodies – A novel cell-based assay
R.A. Farrell, G. Giovannoni; Institute of Neurology-University College London (London, UK)

Background: Interferon-beta is well established as first line therapy in Relapsing Multiple Sclerosis. Neutralising (NabS) and binding (BabS) antibodies to IFN-beta have been widely reported. Subjects with NAbS have reduced response to treatment with IFN-beta, higher relapse rates, increased MRI activity and risk of disease progression. Measurement of Neutralising Antibodies is technically difficult. Existing assays utilise either the anti-viral effect of IFN-beta (the cytopathic effect assay) or measure the IFN-beta induced gene products (Myxovirus resistance protein or its mRNA). These assays are time consuming and expensive. Objectives: To develop and validate a bioassay which is easy to perform and reliable and utilises commercially available products. Methods: A Human fibrosarcoma cell line was stably transfected with the firefly luciferase gene. Transcription of the gene is controlled by the Interferon Stimulated Response Element (ISRE). When interferon binds to its receptor, transcription is activated by the JAK/STAT intracellular signalling mechanism and Luciferase is expressed within a few hours. Serum samples from subjects treated with IFN-beta are pre-incubated with a known amount of IFN-beta. This was added in serial dilutions to the cells and incubated for 5 hours. Luciferase expression was quantified by measuring luminescence in a Wallac Luminometer. Nab titre is calculated by the Kavade technique. Results: Luciferase expression was linear in response to stimulation with 0–50 U/ml IFN-beta. The percentage inhibition of luciferase expression, in response to stimulation by 10 U/ml of commercial IFN-beta, by subjects’ serum was calculated. The assay was shown to be robust in relation to cell density and incubation time. The intra-assay variability ranged from 0 to 20% depending on titre. Titres were comparable to those obtained with the Biogen Idec MxA assay with inter-assay variability of 0–10% and correlation coefficients r = 0.98. Results were also comparable when tested in two other European laboratories. Further validation of this assay is ongoing. Conclusion: This new assay has significant advantages over existing assays. It is simple, time efficient and could be used in the clinical setting to monitor patients on IFN-beta therapy for the presence of Nabs. Acknowledgements: Collaborators, Dr. A Bertolotto, Orbassano, Italy. Dr. F. Deisenhammer, Innsbruck, Austria. Dr. S. Goelz – Biogen Idec. Dr. Gilles Uze-Cern.

P719
Neutralising antibodies to interferon-beta-1b have no impact on clinical response in multiple sclerosis
D.S. Goodin, A. Nonnhe, B.J. Hurwitz; University of California (San Francisco, USA); University of Chicago (Chicago, USA); Duke University Medical Centre (Durham, USA)

Background: The impact of neutralising antibodies (Nabs) to interferon beta (IFNB) treatment in multiple sclerosis (MS) remains controversial, partly because most studies investigating the effects of NAbS in MS have involved small sample sizes. The rates of Nab-positive titres were investigated in three large cohorts of patients with MS, two of which largely comprised patients with a poor clinical response to IFNB-1b therapy, and one of patients unslected for response to therapy. Methods: This study included 6697 patients receiving IFNB-1b (Betaferon®/Betaseron®) in three geographical regions (North America, Europe, and Australia). In North America and Europe, patients were largely selected for Nab testing because of a poor clinical response to IFNB-1b according to the treating physician. In contrast, Nab testing was mandatory in Australia and undertaken irrespective of therapeutic response. For the purpose of this study, Nab-positivity was based on a single positive titre of ≥20 NU/ml. Results: Of the 6697 patients tested, 28.9% (1936) had at least one Nab-titre ≥20 NU/ml. 14.4% of patients (967) had Nab-titres ≥100 NU/ml and 7.7% (517) had Nab-titres ≥400 NU/ml. However, the Nab-positive rate in the Australian (unslected) cohort was significantly greater at 37.0% (840/2271) than the 21.3% (429/2010) in the North American and the 27.6% (667/2416) in the European largely worsening cohorts (p < 0.001). Moreover, lower Nab-positive rates in the North American and European groups were observed at every Nab-titre studied. Conclusion: In two large independent cohorts of patients with MS, largely selected because of a poor clinical response to IFNB-1b, the prevalence of NAbS was significantly lower than in an unslected cohort. This observation seems incompatible with the notion that NAbS are responsible, even partially, for the poor clinical response to IFNB in these patients.
P720

Binding properties of antibodies against interferon-beta mutants
C. Gneiss, M. Reimdl, T. Berger, F. Deisenhammer; Innsbruck Medical University (Innsbruck, A)

Background: Interferon-beta (IFN-b) therapy in multiple is associated with the occurrence of anti-IFN-beta antibodies. There is some evidence that the N-terminus of the IFN-b molecule is a specific domain for the binding of neutralizing antibodies (NAB). Substitutions or deletion of amino acids on the N-terminus of IFN-beta might reduce the immunogenicity of IFN-b. In this study we evaluated the binding behaviour of interferon antibodies to commercially available IFN-b preparations and two different IFN-b mutants with substitutions on the N-terminus. Methods: Six different IFN-b antigens were used in the test-systems: three commercially available IFN-b preparations, an unformulated IFN-b1a wildtyp and two mutants of IFN-b. Mutant A1 had 5 substitutions to alanine in residues 1 to 11 and mutant A2 had 6 substitutions to alanine in residues 15-23 of the molecule. The same protein weight of antigens was used. NAB titers were determined by a MaXa-bioassay. BAB titers were determined by a direct binding ELISA. Results: BAB titers to the 6 antigens (30 samples) differed significantly, with higher titers against the commercially available IFN-b preparations compared to the mutants and the unformulated wildtyp as compared to the commercial IFN-b preparations. Conclusion: This preliminary study shows a higher binding capacity of the commercial IFN-b preparations compared to the mutants and unformulated IFN-b indicating that the formulation of IFN-b might influence antibody binding strength. The elevated NAB titers support this observation because-owing to the properties of Kawadde's NAB titer calculation low binding capacity result in high titers.

P721

Contribution of NAbs status to the interferon beta clinical response in multiple sclerosis
E. Shardella, V. Tomassini, F. Belloni, C. Scaglonari, C. Gasperini, V. Brescia Morra, G.L. Leuzzi, G. Antonelli, C. Pozzilli; University La Sapienza (Rome, I); San Camillo Hospital (Rome, I); University Federico II (Naples, I)

Background: Several studies suggested that the presence of neutralizing antibodies (NAbs) against Interferon beta (IFN-beta) hampers the IFN-beta efficacy in Multiple Sclerosis (MS) patients. However, it’s not yet clear whether NAbs play a major role in determining a lack of treatment efficacy or they are just contributing factors with a marginal role in the response to IFN-beta. Objective: Aim of the study was to define the contribution of NAbs to IFN-beta clinical response in MS treated patients. Methods: In MS patients treated with one of the available IFN-beta formulations for at least one year the presence of NAbs was tested by using a cytotoxic effect (CPE) assay against IFN beta 1a. NAbs + was defined as a titre ≥ 20 TRU; high NAbs titres were arbitrarily defined as ≥ 100. Patients were divided into three groups according to the basis of the clinical response over the treatment period: group A (n = 45), patients developing ≥ 1 relapse after the first 6 months of therapy; group B (n = 46), patients having a confirmed disability progression with or without superimposed relapses during the study period; group C (n = 44), patients with a stable disease course. Results: We recruited 135 patients [86 women, mean age 39 years, mean disease duration 11 years, median EDSS score 2.0] receiving IFN-beta treatment [Rebif 22, n = 47, Rebif 44, n = 42, Betaseron, n = 34, Avonex, n = 12]. The median treatment period was 4 years (range 1–10). On the basis of the clinical response, patients were grouped as follows: 45 patients in group A, 46 in group B, 44 in group C. Seventeen out of 135 (12.6%) patients were NAbs +; 14 of them presented relatively high NAbs titres. The prevalence of NAbs was 8.3% (n = 1) in the group of patients receiving Avonex, 14.7% (n = 5) in the Betaseron group, 12.8% (n = 6) in the Rebif 22 and 11.9% (n = 5) in the Rebif 44 group. When considering the relationship between the response to treatment and NAbs prevalence, 8 (17.8%) patients in group A, 8 patients (17.4%) in group B and 1 patient (2.3%) in group C (p = 0.042 by Anova) had NAbs +. Conclusion: Treated patients differing for clinical response to therapy show different prevalence of NAbs +. However, NAbs + seems to account for a small percentage of non-responders (group A and B), whereas the majority of non-responders were antibody negative. Therefore, this study suggests that NAbs contribute to the lack of IFN-beta efficacy, but they only partially explain the poor clinical response to IFN-beta treatment.

P722

Use of MxA mRNA and neutralising antibodies detection as predictive factors of Interferon beta clinical efficacy in multiple sclerosis patients
S. Malucchi, A. Sala, M. Capobianco, A. di Sapio, F. Marnetto, M. Caldano, P. Valentino, F. Gilli, A. Bertolotto; Regional Multiple Sclerosis Centre (Orbassano, I)

Objective: to analyse the prognostic value of Mxa mRNA and neutralising antibodies (NAbs) on the risk of having a new relapse in interferon beta (IFNb) treated patients. Introduction: Mxa is a marker of IFNb biological activity; NAbs have been demonstrated to abolish IFNb efficacy and biological activity and to have a prognostic meaning. Correlation between Mxa and clinical efficacy of IFNb has never been investigated. Patients and Methods: 391 multiple sclerosis (MS) patients treated with the four different IFNb preparations underwent planned serum samples every 3 months for NAbs detection and random serum samples 12 hours after IFNb injection for Mxa mRNA quantification in peripheral blood mononuclear cells. Patients were classified in two ways: 1) according to Mxa mRNA level and 2) according to NAbs status. Mxa mRNA expression ≥ 99 fg/pgGAPDH means biological activity is present; NAbs titer < 20 LU means patient is NAbs negative. Time between serum sample and the following relapse was calculated and the risk of a new relapse according to Mxa expression and NAbs status was analysed. In order to avoid bias, analysis were performed in 150 out of 391 patients, in whom serum for Mxa mRNA quantification was taken between 9 and 15 months after IFNb treatment start. Results: 109 out of 150 patients had IFNb biological activity, while 41 out 150 had not (Mxa expression < 99); 124 out of 146 patients were NAbs negative, while 22 were NAbs positive (NAbs test missing in 4 out of 150 patients). According to Mxa, median time to a new relapse was statistically longer in patients with IFNb biological activity than in patients without it: 50 vs. 21 months (p 0.046; HR 1.6); according to NAbs, the difference was greater, as median time to a new relapse was 50 and 9 months in NAbs negative and NAbs positive patients respectively (p 0.0005, HR 3.2). Classifying patients as Mxa positive/NAbs negative (n = 104) and Mxa negative/NAbs positive (n = 18), a stronger difference between the two groups was found: median time to relapse was 50 and 8 months respectively (p = 0.0006, HR 3.5). Discussion: one single test for Mxa or NAbs predicts the risk of a new relapse; NAbs have a stronger prognostic value than Mxa; the two parameters together further increase the prognostic value. Conclusion: NAbs and Mxa test should be performed in each treated patient after one year of treatment, in order to discover IFNb non responders as soon as possible.

P723

Three ranges of titres of neutralising antibodies present different risk of loss of interferon-beta biological activity in vivo in multiple sclerosis patients
A. Sala, M. Capobianco, S. Malucchi, A. di Sapio, M. Caldano, F. Marnetto, P. Valentino, F. Gilli, A. Bertolotto; Regional Multiple Sclerosis Centre (Orbassano, I)

Objective: to establish the most useful neutralising antibodies (NAbs) positivity threshold in interferon-beta (IFNb) treated patients

Mult Scler 2006; 12: S1 – S228
The first 6 pivotal studies of the three different IFN-beta preparations that is concordant evidence from the present Danish study and the We collected clinical data and plasma samples for methods:

Neutralizing antibodies (NAbs) against interferon beta in MS have suggested that patients, who were tested NAb-negative (n = 15), and from 18 untreated control RRMS patients (13 female, 5 male, mean age 34). We analyzed transcription of MxA and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as housekeeping gene by TaqMan PCR. NAB analysis was performed at BioMonitor, Kopenhagen, using a cytopathic effect assay. Results: We detected high levels of NAB (>$80% neutralizing capacity) in 7, moderate NAB (20–80%) in 6, low NAB (<20%) in 25, and no NAB activity in 29 patients. In this cohort, high NAB were distributed as follows: Avonex 0/11, Betaseron 2/14, Rebif 6/40. High levels of NAB were associated with very low MxA expression, similar to untreated controls. Patients with negative, low, and moderate NAB activity had about 15-fold higher median MxA levels than untreated controls. In these patients MxA levels were clearly above the MxA levels of untreated controls, even when measured 24–48 hours after the last injection of IFN-beta. In patients who did not develop high NAB activity, different IFN-beta preparations induced similar MxA transcription levels.

Conclusion: Measuring MxA transcription in whole blood offers a robust and practical method for monitoring the bioactivity of all available IFN-beta preparations. The method allows reliable identification of patients who have high NAB activity. Partly supported by Biogen-Idec and “Therapieforschung für Multiple Sklerose e.V.”.

Approved therapy for multiple sclerosis (MS) includes 3 recombinant interferon-beta (IFN-B) products: Avonex® (IFN-B1a; Biogen Idec, Cambridge, MA, USA) 30 mcg intramuscularly once weekly; Rebif® (IFN-B1a; Serono, Geneva, Switzerland) 22 or 44 mcg subcutaneously 3 × weekly; and Betaseron® (IFN-B1b; Berlex, Emeryville, CA, USA) 250 mcg subcutaneously every other day. Such recombinant products can induce antibodies in patients. To determine the distribution and frequency of IFN-B neutralising antibody (NAb) levels in a large population of unselected, treated MS patients, the coded sera of MS patients distributed across all the provinces of Canada were tested over a 3-year period, involving 73 neurologists in 19 clinical sites. NAB levels were determined in 3124 sera from 2720 patients in a single independent laboratory, utilizing an objective, viral cytopathic, quantitative dye-uptake bioassay, from which dye absorbance data were processed by a customised software program that provides analyses of the dose-response curves and calculation of titres as Ten-fold Reduction Units (TRU)/ml, as recommended. NABs were measured against the same IFN-B product each patient was currently receiving. 740 patients of the 2720 tested had detectable NABs.
Background and aims: Recombinant human interferon-beta (IFN-beta) is a well-established treatment for Multiple Sclerosis (MS). The regulatory marketing authorisation process for “biosimilars” is currently on debate. Recent guidelines from the European Medicine Agency have addressed this issue, stating that the use of genomic microarray deserves consideration for the assessment of pharmacodynamic actions of biological products because of its capacity to detect minor changes in biological response to active substances. The aims of the study were to compare the pharmacodynamic genomic effects of two IFN-beta 1a preparations manufactured by two pharmaceutical companies, in order to assess the similarity between them and to characterise the genomic effects of IFN-beta in the treatment of MS using an ex vivo whole genome microarray assay.

Methods: We performed an ex vivo whole genome expression profiling of the effect of two IFN-beta 1a preparations on non-adherent mononuclears from S Relapsing-Remitting MS patients analysing microarrays (CodeLink Human Whole Genome). Patients blood was drawn, PBMCs isolated, non-adherent cells (mostly lymphocytes) were cultured 24 hs in 3 different conditions: IFN-beta vehicle (control), 1000 U/ml of IFN-beta 1 a (BLASTOFERON™, Bio Sidor S.A.) and 1000 U/ml of IFN-beta 1 a (REBIF™, SERONO S.A.). Raw Data was generated by array proprietary software. Data normalisation, quality control and analysis of differential gene expression between treatments were done using LIMMA (www.bioconductor.org). Functional annotation analysis of treatment transcriptome was done using DAVID (www.david.abcc.ncifcrf.gov).

Results: We did not find differences in the genomic effects of both IFN-beta preparations. There were not any gene of about 35000 examined differentially regulated between both treatments. (BLASTOFERON and REBIF) (p \(=0.999\)). The IFN-beta effect differentially expressed about 1000 genes, including standard markers such as Neopterin, MxA, OASA and OASB. The functional analysis of the pharmacodynamic transcriptome showed an over-representation of particular biological process GO terms such as Interferon response, stress response, inflammatory response, signal transduction, chemotaxis and cellular communication.

Conclusion: Under these conditions, the genomic pharmacodynamic action of both IFN-beta 1a biotechnological preparations (BLASTOFERON and REBIF) was similar. The IFN-beta 1 a MS treatment transcriptome was characterised.

P729

Baseline demographics of a randomised study comparing interferon beta-1a with glatiramer acetate in treating relapsing-remitting multiple sclerosis, using McDonald criteria for inclusion: the REGARD study

D. Mikol, F. Barkhof, P. Coyle, D. Jeffery, B. Uitdehaag, R. Kim, S. Rodriguez, S. Schiwit on behalf of REGARD Study Group

Objective: To evaluate baseline characteristics in a randomized, phase IV study (REGARD; Rebif vs. Glatiramer Acetate in Relapsing MS Disease) comparing the therapeutic effect of subcutaneous (sc) interferon (IFN) beta-1a (44 mcg tiw) versus sc glatiramer acetate (GA, 20 mg qd) for relapsing-remitting multiple sclerosis (RRMS), using McDonald criteria for enrollment, compared with previous studies using Poser criteria. Background: IFN-beta-1a and GA are commonly prescribed therapies for treatment of RRMS. Previously, clinical trials with IFN-beta-1a and GA utilized Poser criteria to select MS patients. In this head-to-head study, McDonald criteria were employed. Method: The primary endpoint for this multinational, open-label, randomized clinical trial is time to first relapse during a 96-week (2-year) treatment period. Additional clinical, radiographic, and safety endpoints will also be analyzed. Baseline characteristics of enrolled patients were compared with patients in the EVIDENCE (Neurology 2002; 59: 1496–1506) and PRISMS (Lancet 1998; 352: 1498–504) trials and a phase III GA study (Neurology 1995; 45: 1268–276), which relied on Poser criteria for patient inclusion. Results: A total of 765 patients were enrolled; 764 were randomized 1:1 to IFN-beta-1a or GA. Study participants’ mean age was 36.7 years, with 29.3% males and 70.7% females. At baseline, the average time since MS diagnosis was 6.2 years and a mean of 5 months had elapsed since patients’ most
Methods: emotional state, fatigue and quality of life in patients with MS. Fatigue and quality of life has not been widely investigated yet. The cognitive functions. However, the influence on emotional state, R. Kizlaitiene, G. Kaubrys, R. Parnarauskiene, L. Aleknaite, B. Viesulaite; Pose criteria were used, the patients enrolled had similar baseline characteristics compared with previous studies. The eventual outcome study will provide insight into the comparative therapeutic effectiveness of these agents and may allow for better comparisons between studies using either McDonald or Poser criteria. Study supported by Serono, Inc. and Pfizer, Inc.

P730

Menstrual disorders in multiple sclerosis patients receiving interferon-beta
J. Lotfi, P. Zohrevand, A. Heshmat, M. Sahravan, H. Parsi, M. Bagheri; Tehran University of Medical Sciences (Tehran, IR); Iranian Multiple Sclerosis Society (Tehran, IR)

Introduction: Multiple Sclerosis (MS) is an immuno-mediated demyelinating disease of the CNS. Interferons-beta (IFN-beta) (avonex, Betalferon, Rebif) are used as disease modifying drug for Multiple Sclerosis. Menstrual disorders of mild-to-moderate severity have been reported during clinical trials with IFN-betas. Materials and Methods: Among the MS patients who visited the Iranian MS Society, 57 female patients receiving different types of IFN-beta (avonex, Betalferon, Rebif) were selected. Seven of them were excluded from the study because their menses before initiating IFN-beta treatment were not regular. They were asked about the kind of their IFN-beta treatment and the characteristics of their menses before and after receiving IFNs-beta. Result: Menstrual disorders were seen in 12 (23.5%) patients after using IFN-betas and the mean interval from starting the treatment to initiating the menstrual irregularity was 2.2 ± 1.7 month. Five of 25 patients receiving IFN-beta 1a (Avonex), 4 of 15 patients receiving IFN-beta 1a (Rebif) and 3 of 10 patients receiving IFN-beta 1b (Betalferon) had menstrual irregularity. There was no significant difference in menstrual irregularity prevalence in three groups of patients (P=0.78). The most common forms of menstrual irregularity were hypermenorrhea (33.3%) and oligomenorrhea (33.3%). Conclusion: Menstrual disorders associated with recombinant IFN-betas are seen in some patients who are receiving the drugs and clinicians should be aware that this complication can occur. Other studies with large samples should be conducted to compare the prevalence of menstrual disorders in patients who are receiving different IFN-betas.

P731

The influence of interferon beta therapy on emotional state, fatigue and quality of life in patients with multiple sclerosis R. Kizlaitiene, G. Kaubrys, P. Parnarauskiene, L. Aleknaite, B. Viesulaite, V. Budrys; Vilnius University (Vilnius, LT)

Background: Multiple sclerosis (MS) is an immune-mediated neurologic disease. Studies have indicated that interferons beta (IFN beta) are effective in preventing loss of axons and decline in neurologic and cognitive functions. However, the influence on emotional state, fatigue and quality of life has not been widely investigated yet. The aim of this research was to assess the influence of IFN beta on emotional state, fatigue and quality of life in patients with MS. Methods: 60 patients with relapsing remitting MS (RRMS) were included. One RRMS group (29 patients) were treated with IFN beta(IFN+ group) and the other RRMS group (31 patient) had not been treated with IFN beta (IFN- group). The third group of 30 healthy controls (control group) was questioned. Participants were interviewed and fulfilled Hospital Anxiety and Depression scale (HAD), Fatigue Descriptive Scale (FDS), 36-item Short-Form Health Survey (SF-36) in order to assess their emotional state, fatigue and quality of life. Expanded Disability Status Scale (EDSS) was evaluated in MS patients. Participants with EDSS≤6.0 and more were excluded from the further research. Results: The average scores of Anxiety subscale (HAD scale) were 8.3 (IFN-), 6.1 (IFN+) and 5.6 (controls) with significant difference (p<0.05) between IFN- group and IFN+ group, IFN− group and control group. The average scores of Depression subscale (HAD scale) were 5.1 (IFN−), 3.7(IFN+and) 3.1 (controls) and the difference between IFN- group and controls was significant. The average FDS scores were 6.7 (IFN−) and 3.7 (IFN+) with better rates in patients treated with IFN. The average scores of SF-36 Physical Health scale (PCS) were 39.7 (IFN−), 45.6(IFN+) and 51.0 (controls) and these scores differed among groups. The average scores of SF-36 Mental Health scale (MCS) were 40.5 (IFN−), 47.6 (IFN+) and 47.9 (controls). The difference was significant comparing IFN− and IFN+ groups, as well as IFN− and controls. Conclusion: Anxiety, fatigue and mental health related quality of life in RRMS patients receiving IFN beta become similar to whole population. IFN beta has a positive effect on physical health quality, which however does not revert to the normal level. The influence of IFN beta to the level of depression in treated RRMS patients was not found to be significant, suggesting an idea, that special psychological scales are more sensitive to reveal emotional state of MS patients and argues, that IFN beta can cause depression itself.

P732

Integration of whole genome SNP and gene expression profiling studies in multiple sclerosis patients responders and non-responders to treatment with interferon-beta M. Comabella, D. Craig, J. Río, M. Canniha, A. Sánchez, M. Tintore, N. Tellez, C. López, X. Montalban, R. Martin; Vall d’Hebron University Hospital (Barcelona, E); TGen (Phoenix, USA); Universitat de Barcelona (Barcelona, E)

Background: Treatment with interferon-beta (IFN-b) in patients with RR-MS has been shown to decrease clinical relapses, reduce brain MRI activity, and possibly slow progression of disability. Although several studies have described the effects of IFN-b on gene expression, no definite treatment-response profile has been identified in MS. In addition, the cost of IFN-b is significant, the drug is associated with a number of adverse reactions, and there is a relatively large proportion of patients that do not respond to therapy. Objective: To identify biomarkers (single nucleotide polymorphisms, SNPs; differentially expressed genes) associated with the responder and non-responder status in relapsing-remitting MS (RR-MS) patients. Methods: Two independent cohorts of RR-MS patients were classified by stringent clinical criteria as IFN-b responders or non-responders after 2 years follow-up. DNA and RNA samples were isolated from peripheral blood mononuclear cells before and after treatment. SNP polymorphism mapping was performed on pooled DNA samples using high-density SNP arrays (Affymetrix GeneChip Mapping 500 K). Differential gene expression was examined from individual RNA samples using on-gene-nucleotide microarray (Affymetrix Human Genome U133 Plus 2.0). In the first round of analysis 53 non-responders and 53 responders were compared, and in a second confirmatory experiment 25 responders with 25 non-responders. Several biostatistical methods were applied to compare data between groups at the level of SNP mapping and gene expression profiling, and subsequently the two data sets were cross-related. Results and Conclusions: Broad screening for biomarkers associated with the clinical response to IFN-b lead to a number of SNPs and differentially expressed genes that are related to and predictive of the responder status. The use of genomics and gene expression platforms at the level of the entire genome offers unique opportunities for biomarker research. Cross-validation of genomic data by information from gene expression profiling strengthens the search for candidate biomarkers.
P733

Effect of early interferon-beta-1a therapy on conversion to clinically definite multiple sclerosis in Iranian patients with a first demyelinating event

H. Pakdaman, A. Fallah, R. Pakkaman, A. Shirani, M. Sahravan; Shahid Beheshti University of Medical Science (Tehran, IR); Tehran University of Medical science (Tehran, IR)

Background: Interferon beta has been shown to reduce clinically assessed and MRI-measured disease activity in multiple sclerosis (MS). Two recent studies have demonstrated a delay in conversion of patients to MS using interferon beta-1a. The goal of this study was to assess the effect of early treatment with interferon beta-1a (Avonex) on the risk of conversion to clinically definite MS in Iranian patients.

Methods: This was a multi-centre double-blind placebo-controlled randomized trial. Eligible patients had presented with a first episode of neurological dysfunction suggesting MS within the previous 3 months and had positive brain MRI Scan. Patients were randomly assigned interferon beta-1a (Avonex) 30 μg or placebo intramuscularly once weekly for 3 years. Neurological and clinical assessment and brain MRI scan was done on a regular basis. The primary outcome measure was conversion to clinically definite MS. Results: 200 completed the study. Fewer patients converted to clinically definite MS in the Avonex group than in the placebo group during the 3-year study (36% vs. 57%, p <0.003). The number of new or enlarging T2-weighted MRI lesions and T1-weighted enhancing lesions were significantly lower in Avonex group. Conclusion: Avonex treatment at an early stage of MS delays conversion to clinically definite MS and is recommended in Iranian patients as well. It has also positive effects on clinical and MRI outcomes.

P734

Idiopathic thrombocytopenic purpura in patients with multiple sclerosis

E. Munteis, N. Segura, J. Martinez, E. Cuadrado, A. Galvez, J. Roquer; Hospital del Mar (Barcelona, E)

Background and objectives: Multiple sclerosis (MS) is a chronic multifocal demyelinating disease of the central nervous system with a probable autoimmune etiology. MS has been associated with other autoimmune disorders, such as rheumatoid arthritis, psoriasis, thyroid autoimmune disorders and myasthenia gravis. Although idiopathic thrombocytopenic purpura (ITP) is another autoimmune disease that has been scarcely reported in MS patients, its association to beta-interferon treatment is not described. We report the clinical and laboratory characteristics of four patients with MS who developed ITP in the setting of beta-interferon treatment.

Patients and results: We retrospectively reviewed 4 patients that developed ITP during the clinical course of the MS from a total of 289 patients with definite MS based on Mc Donald criteria. They were four women with an age ranging from 27 to 59 years, 2 patients had relapsing-remitting MS and 2 secondary progressive MS. The EDSS in 3 patients was 6, and 1 patient had EDSS of 0. ITP appeared 17 years after MS onset. Patients had been treated with beta interferon for more than one year (range 1–5 years) before the development of ITP. All patients showed a platelet level < 50000, 3 patients presented ecchymosis in legs, and in 1 patient the diagnosis was made by routine blood test. Serum antiplatelet antibodies were detected in three cases. Serologic and immunologic tests excluded other secondary causes of thrombocytopenia. Patients had neither clinical nor laboratory findings of other autoimmune disorders. The immunomodulatory treatment with beta-interferon was stopped in all cases, ITP was controlled with steroids in autoimmune disorders. The immunomodulatory treatment with beta-interferon was stopped in all cases, ITP was controlled with steroids in one patient, immunoglobulin endovenous in two, and splenectomy in one patient, immunoglobulin endovenous in two, and splenectomy in one patient.

Conclusion: We found in our serie the prevalence of ITP of 1.3% and all patients that developed ITP have been treated with beta-interferon. However although beta-interferon treatment has been related with exacerbations of some autoimmune disorders such as myasthenia gravis, demyelinating polyneuropathy and lupus erythematosus, the development of ITP in our patients can not be considered as an adverse effect of beta-interferon treatment, since it did not occurred in the next six months after beta-interferon treatment onset.

Immunosuppression

P735

Mitoxantrone in the treatment of progressive multiple sclerosis

J. Scott, T. Lynch; Mater Misericordiae University Hospital (Dublin, IRL)

Mitoxantrone is a cytotoxic antineoplastic agent, which was approved for the treatment of progressive forms of Multiple Sclerosis (MS). However the drug should be used with caution due to it’s immunosuppressive nature and side effect profile. With this in mind we set up a Neurological protocol in order to improve the quality of service and reduce the incidence of neutropenia and consequences of this amongst this patient group. Granocyte (GCSF) is given to reduce the duration of neutropenia and hence reduce the increased risk of sepsis. Furthermore the aspect of possible induced infertility and/or induced secondary ovarian failure was addressed and Decapeptyl 3 mgs IM is given monthly to induce ovarian suppression. Mitoxantrone is administered as a pulsed intravenous infusion over six months 12 mgs/m². We examined the effectiveness, side effects, tolerability of the drug in forty patients. We reviewed all patients who were treated from 2001 – 2005. 10 patients had relapsing MS (RRMS), 18 has secondary MS (SPMS), 8 had primary progressive MS (PPMS), and 4 had relapsing progressive MS (RPMS). Duration of diagnosis ranged between 2– 17 years, aged from 23– 64. Disability was assessed at commencement of treatment using the Expanded Disability Status Scale (EDSS) (7.5 – 4.0). All patients are required to have an Echocardiogram at commencement of therapy, at three months and on completion of therapy. Full blood counts, renal and liver profiles prior to each treatment and 7–10 days post infusion. Relapses were recorded during the treatment and in the follow up period. Adverse effects were reported and recorded. There was no observed benefit in the PPMs group, although one patient reported subjective improvement. A reduction in disability was seen in almost all of the SPMS, RRMS and RPMS groups specifically in relation to ambulation. One person fulfilled the criteria for relapse during the treatment and there was an observed reduction in relapse rates and prolonged the time to first relapse following treatment. Two patients failed to complete therapy. We suggest that Mitoxantrone within our protocol is well tolerated and useful in the treatment of progressive relapsing MS’s.

P736

Azathioprine effect in patients with multiple sclerosis

L. Bolokadze; Kharkov Medical University (Kharkov, UKR)

Objective: Azathioprine is an immunosuppressive agent that reduces relapse rates in patients with multiple sclerosis (MS), but its efficacy in suppressing new brain lesions has never been evaluated. To evaluate the efficacy of azathioprine therapy on new brain lesion suppression in MS patients with relapsing-remitting MS of short duration and at least 3 gadolinium-enhancing (Gd+) brain lesions observed within 12 months before treatment. Azathioprine, up to 3 mg/kg daily, individually adjusted according to blood lymphocyte number and the occurrence of adverse events. Brain Gd+ lesions evaluated by monthly magnetic resonance imaging for 6 months before and 6 months during treatment and new T2 lesions evaluated during the same periods and after an additional 12 months.

Results: The treatment reduced to 0 the median Gd+ lesion number and volume per magnetic resonance image (p<0.002 for both), resulting...
Clinical effect of mitoxantrone in patients with multiple sclerosis
E. Kkokou, J. Toufexis, E. Gaglia, M. Pantzaris; The Cyprus Institute of Neurology (Nicosia, CY)

Purpose: To assess the efficacy of Mitoxantrone (MITO) in progressive Multiple Sclerosis (MS). Methods: 75 patients with progressive MS were included. 34 patients (45.3%) had worsening relapsing remitting (RR) MS, 24 patients (32%) secondary progressive (SP) MS and 17 patients (22.7%) primary progressive (PP) MS. Mean age of onset of the disease was 34 years. Mean duration of the disease was 13 years. The mean annual relapse rate was 2.17 and the mean worsening of the EDSS score was 0.76 for the worsening RR group for the 12 months before introducing MITO. The mean worsening of the EDSS score was 0.71 for the SP group and 0.78 for the PP group for the 12 months before introducing MITO. All patients received 12 mg/m² MITO intravenously every 3 months for a total of 12 months. All patients were evaluated by cardiac echo at baseline and every 6 months. Results: 43 patients (57.3%) discontinued the study. Main causes of discontinuation were: patient’s decision/no drug effect (17.3%), worsening (10.7%), cardiovascular side effects (8%), psychological side-effects (6.7%), infections (4%), white blood cell dyscrasias (4%), urogenital side-effects (2.7%), and liver dysfunction (2.7%). Worsening relapsing remitting patients concluding study period (N=20): The mean annual relapse rate was 1.12, a 48.4% reduction from baseline. 6 patients (30%) were relapse-free. 5 patients (25%) had a relapse reduction of 50% or greater. 9 patients (45%) did not show any significant change from baseline or had an increase in the number of relapses. Two patients (10%) showed improvement on the EDSS by 0.5 point. Nine patients (45%) remained stable. The EDSS score of two patients (10%) worsened by 0.5 points, and in another two patients (10%) worsened by 1 point. Secondary progressive patients concluding study period (N=7): The EDSS score of four patients (57.1%) worsened by 0.5 points, and in another three patients (42.9%) worsened by 1 point. Primary progressive patients concluding study period (N=5): One patient (20%) showed improvement on the EDSS by 1.5 point. One patient (20%) remained stable. The EDSS score of two patients (40%) worsened by 0.5 points, and in one patient (20%) worsened by 1.5 point. Conclusion: Mitoxantrone is beneficial in reducing the number of relapses as well as improving or stabilizing the EDSS score in some patients with worsening RR and PP multiple sclerosis. Mitoxantrone is also beneficial to patients with SP Multiple Sclerosis delaying the progression of the disease.

Long-term safety profile of mitoxantrone in a French cohort of 802 multiple sclerosis patients: final report
E. Le Page, E. Leray, B. Brochet, M. Clamet, P. Clavelou, C. Confavreux, M. Debuveure, C. Lebrun, C. Lubetzki, M. Madigand, J. Pelletier, E. Roulet, L. Rambach, D. Brassat, G. Edan; CHU Pontchaillou (Rennes, F); CHU Bordeaux, F; CHU Toulouse, F; CHU Clermont-Ferrand, F; CHU Lyon, F; CHU Nancy, F; CHU Nice, F; CHU La Pitié Salpêtrière (Paris, F); CHU Saint Brice, F; CHU Marseille, F; CHU Thieron (Paris, F); CHU Besançon, F

Objective: To determine the long term incidence of drug related adverse events in a cohort of multiple sclerosis (MS) patients treated with Mitoxantrone (MITOX). Background: MITOX is approved for the treatment of MS in the US and several European countries. In 2001, a French multicenter study was set up to determine the long-term safety profile of MITOX in a large cohort of MS patients. Since then, data were up-dated yearly. Every patient has currently a minimal follow-up duration of 5 years after MITOX start. Design/Patients/Methods: 802 MS patients (308 Relapsing-Remitting MS, 352 Secondary Progressive MS, and 142 Primary Progressive MS) were treated with MITOX in 12 French MS centers. MITOX was administered either monthly over six months in 87% or every 3 months in 13% of the cohort. Patients underwent clinical and hematological evaluations before every MITOX infusion and every 6 months after MITOX treatment end, up to 5 years. Echocardiograms were performed at MITOX treatment start and end and thereafter every 6 months. Results: The cohort had a follow-up duration of 5361 patient-years. 1/802 patients presented with acute congestive heart failure (0.1%). 39/794 patients (4.9%) experienced at least once an asymptomatic LVEF reduction under 50%; persisting in 10 patients, transitory in 26 patients and to be followed in 3 patients. There were also 2 cases of therapy-related leukemia (0.25%), detected 20 and 22 months after initiating MITOX treatment (1 death and 1 remission). 17.3% of 317 women treated before 45 years old developed a persistent amenorrhea (5.4% treated before and 30.7% treated after age of 35 years). Final updates concerning cardiac and hematological tolerance, reproduc- tion function will be presented. Conclusion: This large cohort with at least 5 years of follow-up gave good insights into the long-term safety profile of Mitoxantrone in MS.

Running a mitoxantrone service for multiple sclerosis

Mitoxantrone has been shown to reduce relapse rate and disability in patients with relapsing remitting and secondary progressive MS. However, the drug regimes used in studies have varied in dosage, interval between the treatment pulses and the number of treatments given in total. In clinical practice many centres have reduced the dosage further with or without follow on therapy in the hope of reducing the side effect profile. Subsequent long-term monitoring has revealed additional adverse events that require updated patient counselling and monitoring protocols. In Oxford we have set up a strict mitoxantrone protocol which has been adapted over the last 5 years. At present we give mitoxantrone 12 mg/m² monthly × 3, and then a reduced dose of 6 mg/m² 3 monthly × 2, with Copaxone follow on. We have continuously updated our treatment and monitoring forms and information sheets. In response to a) a recent audit of our mitoxantrone service b) updated information on the long-term cardiotoxicity and leukaemia risks c) FDA guidance, we have made further revisions. We hope by demonstrating the care pathways we have developed along with our protocol, monitoring, and information sheets we may provide a useful tool for other centres setting up similar mitoxantrone services.

Mitoxantrone leads to a persistent selective decrease of the B cell count in patients with multiple sclerosis
N. Putzki, M. Kumar, E. Kranefelder, V. Limmroth; University Clinic Essen (Essen, D)

Background: Most studies in multiple sclerosis focused on the role of T cells mediating CNS damage, but an expansion of B lymphocytes in the CNS could recently be demonstrated. The immunosuppressive agent mitoxantrone has a potent effect on relapses and disease progression in worsening relapsing remitting (RR) and secondary progressive (SP) MS. Its long term immunological effects are not fully understood. Objective: To investigate the course of the B lymphocyte cell counts by flow cytometry before treatment initiation with mitoxantrone (10 mg/m² i.v.) and 3 monthly up to one year.
Effects of mitoxantrone on the suppressive function of regulatory T cells in multiple sclerosis patients

M. Kumar, N. Putzki, E. Kreuzfelder, V. Limmroth; University Clinic Essen (Eisen, D)

Background: Multiple sclerosis (MS) is regarded as an inflammatory demyelinating disorder including autoimmune responses to self-antigens in a genetically susceptible host. CD4+ T cells, granulocytes, monocytes and natural Killer Cells were neither different at baseline (compared to age and gender matched healthy controls) nor were these parameters significantly changed at months 3, 9 or 12. Conclusion: Mitoxantrone has a selective long-term effect on B lymphocyte subsets while the frequency of T lymphocytes is not persistently reduced. The current data extends previous findings of short term effects of mitoxantrone. We propose that the persistent suppression of B lymphocytes and B lymphocyte function is the most important aspect as to the mode of action of mitoxantrone. Further studies should correlate the effect on B cells with clinical data to elucidate if B cell suppression is a suitable immunological response marker.

Clinical follow-up of 304 patients with multiple sclerosis

M. Sauver, S. Pfitzen-Vouyovitch, M. Debouverie, L. Gaullandier on behalf of the Lorsep Group

Objective: To assess the benefits of (1) mitoxantrone after three years of follow-up and (2) disease-modifying treatment (DMT) after stopping mitoxantrone. Method: A retrospective analysis was performed on 304 patients with active relapsing remitting, or progressive MS who were treated with one of two different regimens of mitoxantrone, either a monthly intravenous infusion of mitoxantrone (20 mg) for 6 months (relapsing-remitting MS-RMS), or an intravenous bolus (12 mg/m²) every 3 months for 2 years (progressive MS-PRMS). After mitoxantrone therapy, some patients received DMT (interferon-beta or glatiramer acetate) while others did not. The disease course of the two groups was evaluated by the Expanded Disability Status Scale (EDSS) before and after mitoxantrone and then every year for three years. Results: The mean EDSS at starting mitoxantrone and three years after stopping mitoxantrone respectively, were: 3.3 (1.3) and 3.2 (1.7) for the RMS patients and 5.9 (1.2) and 6.4 (1.4) for the PRMS patients. At one, two and three years after the end of the mitoxantrone therapy, disability was no significant difference between patients who were treated with DMT and those who were not. Before starting mitoxantrone, demographic and clinical parameters of predictive disability were not significantly different between patients who received DMT or not. The variation of EDSS between time of stopping mitoxantrone and three years later was significantly different (-0.9 vs. +0.3; p=0.03) for patients with RRMS. Conclusion: We found a clinical benefit of mitoxantrone for RRMS patients 3 years after stopping treatment. Overall, there was no significant difference between the group of patients treated with DMT and those who were not. A moderate benefit of DMT was found for patients with RRMS during the 3rd year after the end of mitoxantrone therapy.
its classification as a subtype of multiple sclerosis (MS), but it has several unique features. 70% of patients or more develop a relapsing course. There are few studies addressing NMO treatment. Interferons and immunosuppressive drugs efficacy have not yet been proved to be effective in preventing new attacks. We report four cases of NMO – relapsing type, who were treated with mitoxantrone, 12 mg/m², one perfusion monthly, 6 months. The patients were evaluated clinically each month, and with MRI before, at 3, 6 and 9 months after the beginning of treatment. Treatment was well tolerated. Three of four patients were relapse free, the fourth had one relapse in the third month of therapy. Three patients experienced a degree of recovery of neurological function and one patient was stable at 9 months of follow-up. The pretreatment median Expanded Disability Status Scale score was 7, and at follow-up examination was 5.5. The MRI activity of the spinal cord lesions decreased in average after the first three perfusions, the remaining deficits being in relation with the necrotic lesions. The best recovery rate was obtained for the two patients with newer active lesions of the spinal cord, hypertensive in T2 and isointense on T1 weighted sections. Mitoxantrone appears to be an efficient therapy in patients with relapsing NMO, although further studies are needed.

P745
Safety and tolerability of interferon-beta-1b in combination with tacrolimus in the treatment of relapsing-remitting and secondary progressive multiple sclerosis
F. Jacques, S. Christie, F. Grand'Maison, I. Gaboury, D. Halle, M.J. Carignan; CSSG-Hull (Gatineau, CAN); Ottawa Hospital-General (Ottawa, CAN); Charlemoyne (Montreal, CAN); CHEO (Ottawa, CAN)

Background: Tacrolimus is a calcineurin inhibitor. It binds to calcineurin via the FK binding protein (FKBP) thus preventing further cytokine transcription and lymphocyte activation. Combining the immunomodulator interferon Beta-1b (Betaseron) with the immunosuppressant tacrolimus (Prograf) has the potential of greater therapeutic effect than with either used alone. In this randomized, double-blind, placebo-controlled clinical trial, patients with aggressive relapsing-remitting or secondary progressive multiple sclerosis (SPMS) patients who have failed one or more immunomodulatory therapies. Methods: Patients (n = 25) with RRMS (n = 19) and SPMS (n = 9) received a combination of interferon Beta-1b (8 MUI) subcutaneously every other day and oral tacrolimus for a period of 38 weeks. Patients were randomized into two groups; low (1–5 ng/ml) or high (5–10 ng/ml) tacrolimus blood levels. The primary endpoint was safety and tolerability as measured by the incidence of adverse events and laboratory abnormalities. Secondary endpoints included efficacy parameters such as Multiple Sclerosis Functional Composite Scale (MSFCS), relapse frequency, disability progression, MRI defined disease burden and quality of life using the visual analogue scale (VAS). Results: 20/25 patients as of abstract submission have completed the study. There has not been any unexpected adverse event or therapy related serious adverse event. The most common adverse event was headache. No evidence of neurotoxicity was seen. 3 patients discontinued the study because of tacrolimus related adverse events (hyperglycemia, tremor and headache) and 2 because of interferon Beta-1b related injection site reactions. Four of the five were in the tacrolimus high blood level treatment arm. No other significant difference in tolerability or efficacy was found between the two study arms. Using an intention to treat analysis all clinical and MRI parameters have improved or remained unchanged. Final results will be presented at the meeting. Conclusion: As of abstract submission the combination of interferon Beta-1b and tacrolimus is safe. Though the tolerability was significantly reduced in the high tacrolimus blood level arm the combination therapy appeared to stabilize the clinical and MRI evolution of RRMS and SPMS patients who had previously failed one or more disease modifying therapies.

P746
Effects of mitoxantrone on CD4+CD25 high regulatory T cells
C. Pullankavumkal, Z. Mangal, V.S. Manda, J.E. Martinez-Rodriguez, D. Cadavid, L. Wolansky, S.D. Cook; University of Medicine and Dentistry of New Jersey (Newark, USA)

Background: Mitoxantrone is an immunosuppressive drug approved for treatment of active forms of MS. Further immunomodulatory properties of mitoxantrone have been suggested. Methods: For in vitro analyses, mitogen-activated peripheral blood lymphocytes (PBL) obtained from untreated MS patients or from healthy donors were treated with mitoxantrone. The presence of CD4+CD25high regulatory T cells was assessed by flow cytometry. The expression of FoxF3 was assessed by intracellular flow cytometry. Results: Mitoxantrone induced expression of CD4+CD25high T lymphocytes among activated PBL at concentrations between 0.02 and 0.2 ng/ml, whereas at higher concentrations the percentage of CD4+CD25high cells was reduced as compared to mitoxantrone-untreated activated PBL. The CD4+CD25high cells expressed FoxF3. The transcription of FoxF3 is currently being investigated. In addition, the functional properties of mitoxantrone-treated regulatory T cells as well as the presence of CD4+CD25high cells in mitoxantrone-treated MS patients (ex vivo analyses) are being assessed. Conclusion: Our results support the hypothesis that mitoxantrone may exhibit immunomodulatory properties by inducing regulatory T cells at lower doses.

P747
Cladribine in aggressive forms of multiple sclerosis
J.E. Martinez-Rodriguez, D. Cadavid, L. Wolansky, S.D. Cook; University of Medicine and Dentistry of New Jersey (Newark, USA)

Background: Aggressive or malignant forms of multiple sclerosis (MS) represent a small group of patients with a severe and rapid accumulation of disability with or without frequent relapses. Intense immunosuppression, plasmapheresis and autologous stem cell transplantation are the most common treatments applied to MS patients with aggressive forms, although with limited efficacy and considerable risk of severe side effects. Cladribine (2-chlorodeoxyadenosine) is a lymphocytotoxic drug that has been previously evaluated in relapsing-remitting and progressive forms of MS. We report a small series of patients with aggressive MS who failed to respond to other therapies had a favorable clinical evolution after cladribine. Patients and Results: We retrospectively evaluated a series of six patients (4 women and 2 men) with aggressive relapsing-remitting MS and active MRI lesions who were treated with cladribine due to a rapid increase in the number and severity of relapses in the previous 6–18 months leading to a progressive accumulation of disability. At the time of cladribine onset, mean age was 28.2 years (SD: 8.45, range 15–38) and EDSS ranged from 5.5 to 7.0. The mean disease duration prior to cladribine treatment was 54.83 months (SD: 43.65, range 15–133), with a mean relapse rate per year in the previous 2 years of 2.67 (SD: 0.75). The EDSS range in the previous year and 6 months respectively was 1.0–5.5 and 4.0–5.5. Cladribine was given at 0.07 mg/kg/day for 5 days monthly in 2–4 courses. After 6 months from first dose, EDSS was reduced from 5.5–7.0 to 1.5–4.5, and after 12 month (5 patients) to 1.5–5.0. The mean relapse rate per year after cladribine was reduced to 0.71 (SD: 0.55). MRIs showed decreased or suppression of the number of gadolinium enhancement lesions when compared with previous MRI. The mean period from first cladribine dose until last evaluation was 49.33 months (SD: 39.66, range 6–102). After 1 year, cladribine was again given in 4 patients due to new severe relapses. No significant side effects were found after the treatment. Conclusion: We found a favorable clinical evolution when cladribine was used for...
patients with aggressive clinical courses characterized by rapid accumulation of disability in the setting of relapses. Further clinical studies assessing the efficacy of this drug in aggressive forms should be performed.

P748

Acute hepatitis after high-dose intravenous methylprednisolone pulse therapy in a female relapsing-remitting multiple sclerosis patient

R. Reuß, K. Retzlaff, S. Vogel, P. Oschmann; University Hospital Giessen and Marburg (Giessen, D)

Introduction: A 42-year-old female patient suffering from relapsing-remitting multiple sclerosis (RR-MS) presented with a slight increase in leukocytes and lactate-dehydrogenase, alkaline phosphatase, amy- lase and c-reactive protein and a profound elevation of liver transaminases (GOT 485 U/l, GPT 1082 U/l, glutamyl transpeptidase 170 U/l). Eight months ago, the second relapse with paresis of the right leg in combination with typical results in MRI and oligoclonal bands in CSF led to the diagnosis of MS according to McDonald (2001). Laboratory values were normal. Ten weeks ago, an intravenous methylprednisolone therapy (MPT) brought about only a marginal recovery and three weeks ago, a second high-dose MPT produced a distinct remission (cumulative five grams, respectively).

Methods and Results: Clinical examination revealed a mild depression, bilaterally reduced visual acuity, accentuated (right) reflexes, ataxia in right leg and gait ataxia. Expanded disability status scale (EDSS) was 2.0. Virology for hepatitis A, B and C, HSV and CMV was negative. There was no acute EBV infection. One week later, abdominal sonography was normal except for confirmation of an intrauterine pregnancy with fetal length of 1.9 cm. Thyroid gland and abdominal MRI were normal. Pregnancy was terminated within five weeks after outpatient department visit. Symptomatic measures were taken. Ten weeks later, a liver biopsy revealed a profound, still active hepatitis with portal lymphocytic infiltration and fibrosis. During these ten weeks, GOT and GPT decreased to 100 or 140 U/l, while cholestasis parameters (alkaline phosphatase, glutamyl transpeptidase and bilirubin) increased. Conclusion: Comprehensive diagnostic investiga- tion didn’t yield another definite reason. Most likely, existing acute hepatitis was of autoimmune origin: autoimmune hepatitis might occur in patients with multiple autoimmunity as an immune rebound phenomenon after immunosuppressive therapy regimens as shown in two cases of patients with Hashimoto’s thyroiditis or Graves’ ophthalmopathy receiving MPT. Administration of corticosteroids could restore immunosuppression. Hitherto, there is only one single report on acute hepatitis related to high-dose methylprednisolone therapy in a 46-year-old female RR-MS patient that presented with repeated episodes of elevated liver enzymes. As for our patient, due to clinical situation and clear subclinical lesion load immunomodulatory therapy with glatiramer acetate was initiated.

P749

Comparison of multiple sclerosis patients with different progression course under mitoxantrone treatment

S. Vogel, K. Retzlaff, R. Reuß, P. Oschmann; Universitätsklinikum Giessen (Giessen, D)

Introduction: Mitoxantrone treatment is of well established value in the escalation therapy of multiple sclerosis (ms) patients with relapsing remitting (RRMS) and secondary progressive (SPMS) course who suffer a progress of disease (high relapse rate, increase in EDSS score) under immunomodulatory therapy. In addition to this we established the indication for a mitoxantrone treatment in patients with primary progressive (PPMS) course, in whom an immunosup- pressive treatment with methotrexat could not prevent a marked increase of disability over time. Despite basing on clinical charts (1999 – 2006). We evaluated the course of ms of 161 patients (EDSS two years before, at onset of, at the end of and two years after the end of treatment), mitoxantron dosing schemes, side effects and the drop-out quota. Results: At onset of treatment average age of the patients was 43.6 years. 69% of the patients were female. Mean EDSS-score was 5.7. After the end of treatment it was 5.5. At onset of treatment 135 patients (85% of the study group) had a SPMS with a mean EDSS of 5.8. After the end of treatment it was 5.9 (n = 59). Two years preceding treatment it was 4.6. The drop-out quota was 24.1% (n = 33). 13 patients suffered from a PPMS. Mean EDSS two years preceding treatment was 3.8 and 5.7 at baseline. It worsened to 6.6 (n = 11) after the end of treatment. The course of disease came to a halt in one patient. 38.5% aborted the treatment. 9 patients with relapsing remitting MS (RRMS) were treated. Mean EDSS scores improved from 3.9 at baseline to 3.7 after the end of treatment (n = 7). Two years before treatment it levelled at 2.6. One patient aborted the treatment (11.1%). Conclusion: Taking into account only the mean EDSS-score to measure therapeutical success of an immunosuppressive treatment with mitoxantron there was a good therapeutical effect in the RRMS and SPMS subgroups with stabilization or even improvement of the EDSS-score over time. In PPMS patients however the EDSS worsened indicating a poor therapeutical effect. The relatively high EDSS-scores in the PPMS subgroup at onset of therapy could account for the different outcome as could the underlying differences in this type of disease course (e.g. no relapse-activity, pathogenetical differences) compared to both other course types. Furthermore there was a relatively high drop-out quota. This might be partly due to an insufficient therapeutical effect of the mitoxantron treatment.

P750

Intravenous cyclophosphamide and mitoxantrone therapy in multiple sclerosis: a comparative study of efficacy and safety

V. Zipoli, E. Portaccio, B. Ikikiki, G. Srsacusa, S. Sorbh, M.P. Amato; University of Florence (Florence, I)

Background: Among immunosuppressive therapies considered to be efficacy in very active and rapidly progressive multiple sclerosis (MS), mitoxantrone (MTX) has been approved; however, its long-term use is limited due to its cardiotoxicity. Although intravenous (iv) cyclophosphamide (CTX) therapy may represent an alternative option, to date published studies have provided conflicting evidences on its efficacy and there are few studies comparing the two drugs.

Objectives: To compare the clinical efficacy and safety of CTX and MTX in a cohort of patients with very active relapsing-remitting (RR) or secondary progressive (SP) MS.

Methods: The study sample consisted of patients with very active RRMS (≥ 2 relapses in the last year) or SPMS (≥ 0.5 or 1.0 point deterioration on the EDSS over the last year). MTX was administered iv at a dosage of 8 mg/m² monthly for 3 months, then every 3 months, until a cumulative dosage of 100 mg/m² was reached. CTX was administered iv at the dosage of 700 mg/m² monthly for 12 months, then bimonthly for another 12 months. We evaluated the treatment efficacy in terms of time to the first relapse in RR and relapsing SP patients, and of time to disease progression on the EDSS in the subgroup of patients with purely SPMS, using the Kaplan-Meier curves. Moreover, we assessed the frequency of side effects and the overall tolerability by the patient perspective using a visual analogue scale (VAS).

Results: 75 patients received MTX (49 female; 31 RR, 44 SP) and 78 CTX (50 female;15 RR, 63 SP). The two groups did not significantly differ in terms of main demographic and clinical characteristics. The Kaplan-Meier curves did not demonstrate signifi- cant differences in terms of time to the first relapse (MTX 3.0 ± 0.4, versus CTX 3.0 ± 0.6 years; p = 0.50), and in terms of disease progres- sion (MTX 2.9 ± 0.3, CTX 2.5 ± 0.4 years; p = 0.17). On the whole, the safety profile of both therapy was acceptable. However, a significantly higher proportion of patients discontinued therapy due to side effects in the CTX group (22% versus 5%; p < 0.01). On the other hand, nausea was significantly more frequent in the MTX group than in the CTX one (37% versus 12%; p < 0.01). Finally, the overall tolerability assessed through the VAS score resulted to be comparable.
in the 2 groups. Conclusion: In our sample, MTX and CTX therapy showed a comparable clinical efficacy. Both the drugs showed acceptable safety and tolerability profiles.

P751

Blood and X-ray survey on multiple sclerosis patients treated with mitoxantrone in an Italian multiple sclerosis centre

I. Pesci, L. Manneschi, F. Ghisoni, E. Montanari; Civil Hospital (Fidenza, I)

Introduction: Mitoxantrone (MX), is an antineoplastic, immuno-suppressant agent used for improving disability and delaying progression in Multiple Sclerosis (MS) patients with worsening relapsing-remitting (RR) or secondary progressive (SP) disease. Severe infections (especially pulmonary), cardiac toxicity and leukaemias are more serious adverse events. A review of 1378 MX recipients in three MS studies showed that one only patient had acute leukaemia (AL), but on the other hand, by 2005, three cases of AL have been described. Objective: Examining the leukaemic and infection potential of MX in MS patients treated with 5 or 10 mg/m² every three months in a MS Centre of Fidenza (Parma-Italy) by a clinical survey based on RX chest and peripheral blood smear test (PBST) performed every 6 months.

Materials and Methods: By October 2005 until now we have been studying 42 patients (26 females, 16 males; mean EDSS = 4.5, mean age of 44 years old, mean cumulative MX dose of 65 mg/m²) with worsening RR or SPMS, treated with 5 or 10 mg/m² every three months, using a blood test (performed before and after 5, 10, 15 days each infusion), RX chest examination and PBST every 6 months. PBST has been sent to our Haematic Centre and analysed under the microscope, after having done a slide. The blood sample used for microscopic analysis was obtained before each infusion, at the same day.

Results: According to literature data, total white blood cell count is reduced at 10 days post-MX infusion but returned to normal levels by day 21. There is no pulmonary infections evaluated at RX chest examination. We have found 4 PBST alterations, in 4 females, two of them at mean cumulative MX dose (MCMD) of 45 mg/m² and two at MCMD of 75. Each alteration was no post-infusion correlated because found on a blood sample performed before the drug infusion; in all cases, alterations were represented by incomplete maturation (IM) of neutrophic cells, with or without neutrophic cell count reduction and with or without lymphocyte count cell reduction. A new PBST done after 2 months on these 4 females found one only alteration, with IM: this patient stopped therapy. Conclusion: Although these observations provide preliminary reassurance, extended follow-up of patients is required to define the long-term risk of therapy-related AL. In particular blood cell count must be closely monitored and already done at the beginning of MX cycle; nevertheless, as papers have shown, we must be careful even after three years by the last MX infusion.

P752

ABC-transporter gene-polymorphisms as potential predictors of therapeutic efficacy of mitoxantrone in multiple sclerosis

N. Krause, N. von Ahlsen, S. Cotte, R. Gold, A. Chan; Institute for MS Research (Göttingen, D); Universitätsklinikum Göttingen (Göttingen, D)

Objectives: To investigate the role of multi-drug resistance transporters of the ABC gene family as potential predictors of therapeutic efficacy/side effects of mitoxantrone (MX) in MS. Background: Escalation therapy with MX in highly-active MS-patients is limited by its cardiotoxicity, typically correlating with cumulative dosage. Predictors of therapeutic efficacy/side effects may result in individual titration of MX-dosages applied, thus potentially leading to longer therapeutic time windows. ABC-transporters (ABC2, ABCB1) are involved in the transport of MX in a variety of cells and organs.

Patients and Methods: RNA/DNA probes were obtained from 134 MS-patients after informed consent and with approval of the local ethics committee. Myocardial RNA-probes were obtained from heart transplants. Five functionally relevant single nucleotide polymorphisms (SNPs) in the ABCG2-gene as well as the C343 T-mutation in the ABCB1-gene were examined by allele-specific polymerase-chain reaction. Relative quantification of ABCG2 mRNA expression was done using pre-developed quantitative assays. For MX-efflux assays, intracellular MX was analyzed in peripheral blood leukocytes (PBL) by flow-cytometry. Retrospective clinical correlations were done in 20 genotyped, MX-treated patients using EDSS, MSFC or magnetic evoked potentials as outcome parameters defining MX-response.

Results: 9% of the analyzed MS-patients were wild type for both ABCG2 and ABCB1, 22.4% carried mutations in both genes. Hemolytic mutations were identified in 30.6% (ABCG2) and 50.7% (ABCB1). Relative quantification of ABCG2 mRNA-expression was higher in human myocard than in PBL. In vitro efflux-assays, intracellular PBL MX-concentration was higher in individuals with combined ABCG2/ABCB1-mutations as compared to wildtype. 15 of 20 patients were MX-responders on clinical criteria. 3 of 4 patients carrying mutations in both genes were responders, as well as all 4 ABCB1 homzygous mutation carriers. In contrast, 2 of 3 patients wildtype for ABCG2 and ABCB1 were non-responders.

Conclusion: Gene-polymorphisms of ABC-transporters involved in MX-metabolism may serve as surrogate parameters associated with response to MX-therapy.

P753

Mitoxantrone-related acute myeloid leukaemia, induced one year after the discontinuation of mitoxantrone therapy

M. Capobianco, S. Malucchi, M. Rosa, C. Fava, L. Avonto, A. Bertolotto; Regional Multiple Sclerosis Centre (Orbassano, I); Haematological Unit (Orbassano, I); Cardiological Unit (Orbassano, I)

Mitoxantrone is effective in aggressive relapsing-remitting and secondary progressive patients, but it has severe side effects. Acute leukaemia may be induced by mitoxantrone treatment in multiple sclerosis patients, but the exact risk is not yet known. A 35 years old woman who was affected by relapsing-remitting multiple sclerosis since 2000 was treated with intravenous mitoxantrone from July 2003 to September 2004 for the presence of a very aggressive course of the disease (at least 4 documented relapses under Betaferon treatment in the previous 7 months) with a total load of 130 mg (95 mg/m²). During mitoxantrone treatment she experienced only 1 relapse in July 2004. The brain magnetic resonance imaging (MRI) follow-up showed disease activity (as new T2 and enhancing lesions) in July 2004 but not in May 2005. In August 2005, eleven months after the discontinuation of mitoxantrone therapy, she was admitted for the presence of deep asthenia and she was diagnosed for the presence of treatment-related myeloid acute leukaemia (t-LAM) of the M4 type according to the FAB classification. A new brain MRI showed 1 new T2 lesion. She was treated with two courses of polychemotherapy with oncological disease remission, and then she was treated with autologous stem cells transplantation complicated only by severe mucositis that required parenteral feeding. No further clinical or magnetic resonance signs of MS disease activity were noted. As acute leukaemia may occur late after mitoxantrone therapy, periodic evaluation of blood cells count is mandatory also after the interruption of the treatment.

P754

Twelve months after suspension of natalizumab: experience at our multiple sclerosis centre

E. Moral, O. Carmona, V. Casado, L. Romero-Pinel, L. Gabieras, J. Niibo, C. Polo, S. Martinez-Yelamos, T. Arbizu; Bellvitge University Hospital (Hospitalet de Llobregat, E)

Background: In spite of promising results, clinical trials with natalizumab were suspended at the beginning of 2005 because 3

www.sagepub.co.uk

Multiple Sclerosis 2006; 12: 51–5228

Abstracts S215
patients treated with natalizumab and other immunosuppressants/immunomodulators developed progressive multifocal leukoencephalopathy (PML). **Goals:** To describe the 12-month clinical and analytical follow-up of 14 patients at our MS centre included in c-1802 double-blind and c-1808 open-label extension trials after drug suspension, with emphasis on relapse rate. **Methods:** Eight patients were initially treated with natalizumab (32 doses) plus Avonex and 6 with Avonex plus placebo (at least 4 natalizumab doses in c-1808). Avonex was continued in all patients after suspension of natalizumab, except 1 treated initially with placebo, switched to Rebif44. After suspension, we initiated a diagnostic protocol lasting 12 months with scheduled visits every 3 months and unscheduled visits whenever an adverse event or relapse occurred to detect possible signs of PML. Tests included physical and neurological exam, brain MRI and quantification of blood lymphocyte subpopulations. Qualitative PCR were used to test JC virus in urine and CSF of all patients who accepted lumbar puncture. **Results:** No patient showed any change suggestive of PML in neurological exam or brain MRI. No abnormalities were detected in blood lymphocyte subpopulations. All 10 CSF samples were negative for JC virus whereas 3/14 urine samples were positive. No adverse effects were reported during follow-up. During initial treatment with natalizumab, 3/7 patients had remained relapse free and the rest had a 50% reduction in their previous annualised relapse rate (ARR medians, from 1.0 to 0.5). After suspension, 6/7 presented new relapses and ARR was higher (1.71) than pre-natalizumab relapse rate. One natalizumab-treated patient was excluded from this analysis because of persistent high titres of anti-natalizumab antibodies and a poor clinical outcome. Half the patients initially on placebo remained relapse free after drug suspension.

**Conclusion:**

No patients in our centre presented signs suggestive of PML despite treatment.

An increase in the annualised relapse rate was observed during the follow up period, when they did not received natalizumab, compared with treatment period. This indicates that the biological effects of natalizumab wane after stopping therapy.

The presence of anti-natalizumab antibodies neutralised the effect of natalizumab, in line with results for the full cohort.

**P755**

Measurement of plasma CD31+ endothelial micro particles during mitoxantrone treatment of progressive multiple sclerosis

W. Sherenmata, W. Jy, S. Delgado, Y. Ahm; Miller School of Medicine (Miami, USA)

**Background:** Plasma CD31+ Endothelial Micro Particles (CD31+EMP) are elevated in untreated relapsing-remitting MS (RR MS). This is hypothesized to result from activated mononuclear cell adhesion to cerebral endothelium and transmigration into the central nervous system (CNS). We have found that levels of CD31+EMP decrease with interferon-beta (IFN) treatment but progressive MS has not been studied. **Objectives:** To measure plasma CD31+EMP in a pilot study of patients with progressive MS prior to and during treatment with mitoxantrone and the antinecaspase-3 enzyme mitoxantrone (MTX) and compare the findings with results from RRMS and controls. The drug is highly effective in MS and impacts both T-cell and B-cell function. **Methods:** Patients received an average of 8 treatments of MTX, 12 mg/M² intravenously, every 4 weeks. We measured CD31+EMP by flow cytometry at different times over one year in 25 MS patients with progressive (20 secondary progressive (SPMS), 3 relapsing progressive (RPMs), and 2 worsening relapsing-remitting (WRMMS). These results were contrasted with the findings in 30 RRMS and 67 controls.

**Results:** Prior to MTX a mean ± SD of 1253 ± 2497/ml was obtained in 12; compared with 585 ± 366/ml in 15 at 12 weeks; 327 ± 204/ml in 12 at 24 weeks; and 623 ± 810/ml in 11, 52 weeks after their first infusion. These findings contrast with 3866 ± 1900 for 30 untreated RRMS and 3192 ± 2154; 2841 ± 1845; and 2491 ± 1391 at weeks 12, 24, and 52 of IFN treatment and values of 697 ± 403/ml in 67 normals. RRMS are statistically higher than controls but our cohort of progressive MS does not differ from normal. Pretreatment values in all but 2 patients may be lower, in part, because they continued IFN treatment until and through MTX treatment, but values in untreated patients were similar. Two SPMS patients with frequent severe relapses and accumulating disability had high pretreatment values that dropped to normal after MTX. **Conclusion:** Findings suggest there are differences in the pathogenesis of progressive MS, who progress despite IFN as compared with RRMS. The finding of lower CD31+ EMP values may indicate that T-cells and macrophages are less important in progressive illness but B-cell activation and antibody to myelin components may be more relevant factors. Further study is warranted.

**P756**

Accelerated axonal degeneration after bone marrow transplantation for aggressive multiple sclerosis?

A. Petzold, T. Mondura, G. Keir, E. Linn, P. te Boekhorst, G. Giovannoni, R.O. Huitgen; Institute of Neurology (London, UK); MS Centre Erasus MC (Rotterdam, NL)

**Background:** A recent treatment trial using a severe immunosuppressive regimen (including high dose cyclophosphamide and CNS irradiation) followed by bone marrow transplant (BMT) was not effective in preventing clinical disease progression in severely disabled MS patients and was associated with serious side effect (NNIP 2006; 77: 46–50). Here we investigated whether axonal degeneration was, at least transiently halted by this treatment trial. **Methods:** Serum neurofilament heavy chain (NfH) levels were measured serially in all 14 MS patients participating in this trial and 14 healthy control subjects. Serum samples were taken at baseline and following BMT at months 1, 2, 3, 6, 9, 12. The clinical assessment included the EDSS and an ambulation index (AI) at baseline and following BMT at month 6, 12, 24, 36. **Results:** At baseline serum NfH levels were comparable in control (median 0 ng/mL, range 0 – 0.01 ng/mL) and MS patients (median 0 ng/mL, range 2.97 ng/mL, p = 0.49, not significant). Following BMT a significant increase of serum NfH levels was observed (F(88,94) = 3.43, p < 0.01). Median serum NfH levels were highest at one month following BMT (0.88 ng/mL) and decreased subsequently to 0.34 ng/mL (month 2), 0.12 ng/mL (month 3), 0.07 ng/mL (month 6), 0.01 ng/mL (month 9), with a small increase at month 12 to 0.03 ng/mL. Serum NfH levels at month two correlated with the EDSS at month 6 (R = 0.76, p = 0.002), month 12 (R = 0.79, p = 0.001), month 24 (R = 0.69, p = 0.009) and month 36 (R = 0.64, p = 0.017). Serum NfH levels also correlated with the followup AI (R = 0.66, p = 0.014). **Conclusion:** These results, based on serum NfH levels as a biomarker for axonal degeneration suggest that axonal degeneration may have been an unwanted complication of this treatment trial.

**P757**

Glatiramer acetate after mitoxantrone in non-responders to interferon-beta

N. Téllez, J. Río, M. Tintore, A. Rovira, C. Nos, Í. Galán, R. Pelayo, H. Perkal, S. Muntalban; Vall d’Hebron University Hospital (Barcelona, E)

**Introduction:** Mitoxantrone has been aproved to treat MS patients not respondering to interferon beta. Nonetheless, the recommended maximum cumulative dose limits the duration of treatment up to two years. After this period, no new strategies have been established. **Objective:** We aimed to analyse the clinical and radiological response to glatiramer acetate (GA) after one year of mitoxantrone in MS patients refractory to interferon beta. **Methods:** Since 2000 eighty relapsing remitting or secondary progressive patients non responders to interferon beta started mitoxantrone. Sixty of these completed six years of mitoxantrone, seen every three to six months, and 60 of these completed six years of mitoxantrone, seen every three to six months, and were asked to start GA therapy. Patients have been followed as early as two years before mitoxantrone, seen every three to six months, and...
serial MRI scans were performed. **Results:** Forty-three patients started GA after mitoxantrone, 30 of these have reached one year of treatment. Relapses: considering the whole follow-up period, mean relapse rate was 1.4 (range 0–7) during the two years before mitoxantrone, 0.1 (range 0–1) during mitoxantrone and 0.4 (range 0–4) during GA. Disability: Patients included in this protocol experienced a mean increase of 1.1 (SD 1.1) EDSS points during the year before mitoxantrone. During immunosuppressive treatment, a mean decrease of 0.2 (SD 0.8) points was observed and after one year on GA patients had an increase of 0.4 (SD 0.8) EDSS points. Comparisons between these patients and those that did not receive GA after mitoxantrone was not possible since there were baseline differences between groups in terms of disability. Thus, patients who did not start GA had higher EDSS at the end of mitoxantrone and experienced a higher increase of disability during the immunosuppressive drug infusion (p = 0.015 and p = 0.014, respectively). MRI: A significant decrease of gadolinium enhancing lesions was observed from the baseline MRI before mitoxantrone (mean number of active lesions 3.6, range 0–24) to the end of mitoxantrone (0.3, range 0–3), that persisted after one year on GA (mean number of active lesions 0.3, range 0–3) (p < 0.0001). **Conclusion:** Clinical and MRI activity seems to be stable after one year of mitoxantrone in patients under glatiramer acetate.

**P758**

**Prevention of persistent ovarian failure after mitoxantrone in women with MS. Design of a treatment protocol with triptorelin**

N. Téllez, J. Gris, M. Tintoré, J. Rio, C. Nos, I. Galtán, R. Pelayo, X. Montalban; Vall d’Hebron University Hospital (Barcelona, E)

**Introduction:** Mitoxantrone is an immunosuppressive agent currently used in MS patients non responders to immunomodulation. Many adverse events have been attributed to the drug. Leukaemia and cardiopathy are the most serious although the less frequent. Other such as nausea and vomiting, hair loss and amenorrhoea are more common. The known odds ratio to develop persistent amenorrhoea after mitoxantrone is about 8.5 and consequences are double: infertility and early menopause. Proposed options to protect the ovarian tissue in women at risk are cryopreservation of mature oocytes, embryos or ovarian tissue, although these options are not fully accepted yet. Alternatives such as gonadotropin-releasing hormone analogue (GnRH-a) have been used in oncohematological disorders showing a capacity to prevent gonadal damage after chemotherapy with no major side effects. **Objective:** Our aim was to study the ovarian function after a GnRH-a treatment, the triptorelin, in MS women on mitoxantrone. **Protocol design:** A protocol has been designed with the following inclusion criteria: women with relapsing remitting or secondary progressive MS, non responders to interferon beta, with an age between 18–45 that are invited to start mitoxantrone. Patients are then planned to be on treatment with a monthly intramuscular injection of triptorelin for12 months. Serial gynaecological evaluations are performed at baseline visit, day 15, month 2, 12, 13 (end of study) and 14 (follow-up visit). Before starting mitoxantrone the baseline visit include: 1. Plasma levels of luteinising hormone, and follicle-stimulating hormone, estradiol, prolactin, thyroid profile and inhibine, 2. Echography to assess the ovarian size, number of follicles >5 mm and endometrium characteristics, 3. Doppler of the uterin and ovarian arteries and 4. Bone densitometry. At each visit time the hormonal analysis, the echography and the Doppler are repeated. Densitometry is repeated at the end of the study. Mitoxantrone is started after the first dose of triptorelin, and its efficacy and side effects are assessed every three months by the by neurologist. **Current status:** Since 2004 we have started mitoxantrone in 10 women. Six of them were younger than 45 and were referred to gynaecology to start triptorelin. No dropouts or relevant side effects have been identified since then. At present only two women have finished the protocol. New results will be presented.

**P759**

**Ongoing evaluation of the safety and tolerability of Novantrone® (mitoxantrone) worsening multiple sclerosis: the RENEW study**

E. Fox, A. Al-Sabbagh, R. Bennett, P. Coyle, D. Mikol, H. Pantich, V. Rivera, L. Rolak, W. Sheremata, S.B. Elias on behalf of the RENEW Study Group

**Background:** Novantrone® (mitoxantrone) therapy is currently being evaluated for long-term safety and tolerability in patients with worsening relapsing-remitting MS (WRRMS), progressive relapsing MS (PRMS) and secondary progressive MS (SPMS) as part of the ongoing multicenter, open-label RENEW (Registry to Evaluate Novantrone Effects in Worsening MS) study. **Objectives:** To evaluate the continuing safety and tolerability of mitoxantrone in MS patients using the dosing and monitoring guidelines specified in the Novantrone package insert. **Methods:** 509 patients with WRRMS, PRMS, or SPMS who initiated Novantrone(12 mg/m²) were included. Patients were excluded for: primary progressive MS (PPMS); history of congestive heart failure (CHF); left ventricular ejection fraction (LVEF) <50%; and previous treatment with mitoxantrone, other antithrominonies or antracyclines. Patients were assessed every 3 months during treatment (3 years) and are being followed for an additional 2 years for safety evaluation (total:5 years). **Results:** Data presented include data collected from April 2001 to Jan 2006 for 509 patients. Mean cumulative dose is 67.5 mg/m² (8.0–144.6 mg/m²) and mean treatment duration is 1.4 years (0.0–4.0). 16 patients have reached the recommended maximum cumulative dose (140 mg/m²). 355 (70.0%) patients have received concomitant medications for MS. Treatment discontinuation occurred in 404/509 (80%) subjects with validated data. Follow-up data are being collected for 361 patients regardless of treatment status (those receiving mitoxantrone [104] plus patients who discontinued mitoxantrone [257 of the 404]). 301 relapses were reported during treatment in 221 patients. Median time to first relapse was 155 days (range: 3–1215). 95 patients experienced 158 AEs. The most frequently reported AEs were infectious (54/158 [34%]) and cardiac events (36/158 [23%]). CHF was reported in 8 patients, and LVEF <50% was observed in 24/340 (7.0%) patients with post-baseline LVEF tests. 9/46 patients with serious infections were severely neutropenic (ANC <500). There have been 7 deaths: 5 unrelated and 2 possibly related to treatment. One therapy-related leukemia case has been reported. **Conclusion:** These results, reflecting patient treatment at higher cumulative mitoxantrone doses (mean: 67.5 mg/m²), appear consistent with the known safety profile. Continued patient observation will provide important longer-term safety and tolerability data for mitoxantrone use in clinical practice.

**P760**

**Is Campath 1-h an effective rescue treatment in patients with aggressive relapsing-remitting multiple sclerosis?**

C. Hirst, A. Pace, T. Pickersgill, R. Jones, J. Zajicek, N. Scolding, N. Robertson; University Hospital of Wales (Cardiff, UK); Derriford Hospital (Plymouth, UK); Frenchay Hospital (Bristol, UK)

Alemtuzumab (Campath-1H) is a humanized anti CD52 monoclonal antibody which causes rapid and prolonged peripheral T lymphocyte depletion and has demonstrated some efficacy in open label trials of relapsing and secondary progressive multiple sclerosis (MS). Initial reports of its use in small cohorts of patients has demonstrated a reduction in relapse rates and new enhancing lesions on T2 MR and also a possible reduction in brain atrophy in certain patient subsets. We report 32 patients (19 female) with aggressive relapsing MS with rapidly accumulating disability treated with Alemtuzumab as an immunomodulatory rescue therapy in 3 regional MS treatment centres. All patients were assessed and followed up according to a predetermined protocol and routine clinical, biochemical and disability data collected prospectively. Patients received either 120 mg or 60 mg of Alemtuzumab over 5 days concurrently with 1 g of intravenous methylprednisolone.
for the first 3 days. Patients were retreated annually with an attenuated regime of 36 or 60 mg of Alemtuzumab and methylprednisolone over 3 days where appropriate. Frequently occurring side effects included rashes(12), opportunistic infections (5), transient worsening of preexisting neurological deficits (2) and thyroid disease(4). No serious side effects were observed. Mean disease duration at time of first treatment was 3.62 years (SD 2.86, Range 0.16–10.93), mean age 34.3 years (SD 9.23, Range 18.1– 52.3) and mean EDSS 4.0 (SD 1.76, Range 0.0–6.5). 20 patients were treated with one course of Alemtuzumab, 11 with 2 courses and 1 with three courses. Annualized relapse rates fell from 2.55 to 0.24 in the first 12 months following treatment and to 0.18 overall (p < 0.001) (54.9 patient years follow up). In those patients with stable baseline EDSS scores 4 patients had deteriorated by ≥1 EDSS point twelve months following treatment, 17 remained stable and 0 had improved by ≥1 EDSS point. Six patients with an unstable baseline EDSS had improved by ≥1 EDSS point. Analysis of this group of patients suggests that Alemtuzumab is safe and effective as a rescue therapy in aggressive relapsing MS, reducing relapse rates both in the short and medium term and may help to stabilize disability.

Immunology-Part II

P761

Circadian rhythmity of cytokines and cytokine receptors in the serum of multiple sclerosis patients

J. Kraus, A. Kreiling, S. Mannes-Keil, K. Retzlaff, P. Oschmann; Paracelsus Private Medical University (Salzburg, A); University Hospital of Giessen and Marburg (Giessen, D)

Objective: To obtain changes in the circadian rhythmicity of immunological markers in the serum of patients with multiple sclerosis (MS). Background: Changes in the serum concentrations of cytokines and cytokine receptors have been found in numerous studies. However, some of the results are contradictory. Consequently, no conclusions can be drawn yet for these different results might be diurnal changes. Methods: We included 34 untreated patients with relapsing-remitting MS and 34 age- and sex-matched healthy controls. 12 MS patients showed acute disease activity in corresponding MRI scans. Blood samples were obtained at five time points between 7.00 a.m. and 9.30 p.m within one day. We determined the serum concentration levels of cortisol, tumor necrosis factor-beta (TNF-beta), TNF-Receptor-1 (TNFR-1) and -2 as well as Interleukin-4-Receptor (IL-4-R) by ELISA. Results: The serum concentration levels of cortisol showed almost the same course for the MS patients and healthy donors with a significant (p < 0.001) decrease from 22 microg/dl at 7.30 a.m. to 3.6 microg/dl at 9.30 p.m. However, MS active patients had significantly (p < 0.05) increased cortisol serum levels at all five time points as compared to MS patients in remission. TNFR-1 and TNFR-2 levels followed a less marked (p < 0.05) descending course over the day in all groups. MS patients with acute disease activity had significantly (p < 0.05) elevated levels for TNFR-1 as compared to healthy donors. In contrast, IL-4-R and TNF-beta serum concentrations were relatively stable over the day. Both MS groups had significantly (p < 0.05) elevated TNF-beta serum levels at any time point as compared with the control group. Conclusion: Our data show that the diurnal rhythmity of immunological markers must be considered in at least some of the investigated immunological markers. We could not observe a substantial difference in the circadian rhythmity between MS patients and healthy donors.

P762

The cytokine and nitric oxide levels in the sera of the relapsing-remitting multiple sclerosis patients and its correlation with clinical and MRI findings

M. Isik, M. Mirza, K. Köse, N. Koç, A. Coskun; Erciyes University (Kayseri, TR)

Objectives: The aim of this study was to evaluate the serum levels of cytokines and nitric oxide (NO), which is considered to play a role in immunopathogenesis of multiple sclerosis (MS), in relapsing-remitting multiple sclerosis (RRMS) patients both during relaps and remission periods, and these measurements were correlated with neurologic disability and MRI findings. Materials and Methods: The study included 30 patients with definite MS according to McDonald and Poser diagnostic criteria. Blood samples were collected twice, in relaps period before steroid treatment and approximately two months later after remission. These findings were compared to 30 healthy subjects, having no neurologic complaint. Several cytokines (TNF-alpha, IL-1, IL-10, IL-12, IL-13, TGFBeta) and Vascular Cellular Adhesion Molecule-1 (VCAM-1) were measured using the ELISA method and NO levels were measured using the Colorimetric Assay method. Expanded Disability Status Scale (EDSS) was used for clinical evaluation. MRI were evaluated by radiologist, who does not know patients clinically. The correlation between the cytokines and NO levels with EDSS and MRI findings is investigated. Mann-Whitney U test, Wilcoxon test, student t tests, Pearson and Spearman’s correlation analysis were used in statistical studies. Results: NO levels in relaps period were found to be significantly higher than those in remission (p < 0.05). There was no elevation on TNF-alpha, IL-12 and VCAM-1 levels in relaps period. IL-12 levels were higher in MS patients during the relaps period when compared to control subjects, but the difference was not statistically significant (p > 0.05). TNF-alpha, VCAM-1 and NO levels were similar both in MS and control groups. IL-1, IL-10, IL-13 and TGFBeta levels were not measurable by the ELISA method both in MS and control groups. The 48 percent of MS patients showed cortical atrophy on cranial MRI. There was no correlation between the cytokine levels, EDSS and MRI findings and also cytokine levels and EDSS. Conclusion: In this study, it is observed that NO levels were elevated during the relaps period. Therefore, it is concluded that the treatment options which prevent NO releasing may be beneficial. Additionally, the cortical atrophy of MS patients which is demonstrated by MRI may be the evidence of axonal degeneration. However, in order to comment absolutely on this issue, more detailed studies must be performed.

P763

Increased serum level of CXCL11 (I-TAC) in relapsing-remitting multiple sclerosis patients

A. Sazownicki, A. Kalinowska, J. Losy; University of Medical Sciences (Poznan, PL)

Introduction: Chemokines may play an important role in the pathogenesis of MS, facilitating the trafficking of immune cells across the blood-brain barrier and mediating their transfer to lesion sites. Interferon-inducible T cell alpha chemoattractant (I-TAC, CXCL11) is a CXC chemokine, which binds with high affinity to the CXCR3 receptor. Interestingly, the increased percentage of CD4+ /CXCR3+ cells in the blood of MS patients was associated with MS relapses, and correlated with the number of Gadolinium enhancing lesions on MRI images. The goal of this study was to estimate the level of CXCL11 in sera of relapsing-remitting MS (RRMS) patients during relapse both before and after methylprednisolone treatment and to compare the results with the serum level of CXCL11 in healthy blood donors.

Methods: The studied groups consisted of thirty patients with relapsing-remitting MS during relapse, and 20 healthy blood donors as controls. In the group of RRMS, the blood samples were obtained both before steroid therapy and after a five-day treatment with methylprednisolone in a dose of 1 g i.v. once daily. The levels of CXCL11 were measured in sera by ELISA method. Results: CXCL11 levels were significantly higher in sera of RRMS patients both before (mean ±SD, 55.4 ±63.1 pg/mL) and after steroid therapy (40.7 ± 43.2 pg/mL) in comparison with the control group (17.1 ±18.3 pg/mL, p = 0.002). Serum levels of CXCL11 before and after methylprednisolone treatment in RRMS patients did not differ significantly. Serum concentrations of CXCL11 were detectable in 23 RRMS patients and in none of 20 controls. The minimum detectable dose was 12.8 pg/mL. The Mann-Whitney and Wilcoxon tests were used for statistical analysis. Conclusion: We found the increased level of CXCL11 in sera of...
RMS patients during relapse in comparison with results of the control group. Our results suggest that CXCL11 chemokine may play an important role in the pathogenesis of MS.

P764
Expression of fractalkine in plasma of patients with relapsing-remitting multiple sclerosis
S. Pujari, O. Salinan, N. Woodroffe, B. Sharrack; Royal Hallamshire Hospital (Sheffield, UK); Sheffield Hallam University (Sheffield, UK)

Fractalkine (CX3CL1), a chemokine first described in 1997, is the only chemokine described to date with a C-X3-C motif. It is expressed constitutively in the central nervous system by neurons, astrocytes and endothelial cells. Its receptor (CX3CR1) is expressed mainly by microglia. Unlike the majority of chemokines, fractalkine has a mucin-like stalk and is expressed in a membrane anchored form as well as in a soluble form. In the former case it acts as an adhesion molecule whereas in the latter it acts as a chemoattractant. In vitro studies have shown that it serves an important function in neuronal-microglia interaction, microglia proliferation and neuroprotection. A previous in vivo study of 15 patients with multiple sclerosis (MS) showed increased serum levels of fractalkine in patients compared to controls. The aim of this present longitudinal study was to assess plasma fractalkine levels in MS patients over a 12 month period to determine whether there were any changes in fractalkine levels associated with relapse and whether fractalkine levels were significantly different in MS patients when compared with healthy controls. Thirty patients with diagnosed relapsing remitting MS and 10 healthy volunteers were recruited into the study. After obtaining consent, blood samples were collected in all subjects at 2 monthly intervals. Additional samples were obtained when clinical relapses were reported during the study period. The plasma was analysed for fractalkine levels by Enzyme Linked Immunosorbent Assay (ELISA). Preliminary analysis of the data from 23 MS patients and 7 controls has shown that there were no significant fluctuations in plasma fractalkine levels in MS patients and controls during the study period including the relapse time points. However there was a significant difference in the mean level of fractalkine in the plasma of MS patients (1504.4 ± 538.4 pg/ml) compared to controls (412.9 ± 71.1 pg/ml) (p = 0.037 using Mann Whitney test). Over the 12 month study period, 7 out of 24 MS patients (29.2%) had fractalkine values higher than mean (+3 SD) of controls whereas only one MS patient had levels of fractalkine below the detection limit of the assay. Our results suggest that fractalkine may play a role in the pathogenesis of MS. The absence of any significant fluctuation in fractalkine levels measured during relapses suggests that fractalkine does not mediate acute inflammatory events in MS.

P765
High motility of T lymphocytes in chronic neuroinflammatory diseases is associated with the overexpression of CRMP2
P. Giraudon, K. Martinier, C. Vaillat, N. Davoust, S. Cavagna, J. Sagaidou, M. Varrin-Doyer, C. Confavreux; INSERM (Lyon, F); Hopital Neurologique (Lyon, F)

High motility of activated T lymphocytes is a hallmark of neuroinflammation and favor T cell recruitment in the central nervous system (CNS). Although T cell accumulation in the CNS correlates with neurodegeneration, little is known on the mechanisms that support such a high T cell motility. We have identified the phosphoprotein collapsin response mediator protein 2 (CRMP2) as a modulator of T cell motility (Vincent et al., 2005) and observed its elevated expression in activated T lymphocytes of patients suffering multiple sclerosis (MS). We therefore performed a series of experiments aiming to associate CRMP2 expression and motility of MS patients’ T cells, and to establish the signaling transduction pathway involving CRMP2 and implicated in elevated motility. We first examined CRMP2 expression in VLA-4 positive cells of MS patients. High CRMP2 expression in these cells suggested their elevated ability of binding to endothelial cells and transmigration into the CNS. This hypothesis was first tested ex vivo, on activated T lymphocytes expressing high CRMP2 level (CD69 + CRMP2 hi) isolated from MS patients, focusing on their ability to migrate towards chemokines known to be expressed in inflamed brain. Association between CRMP2 level and migratory rate was observed. Secondly, study performed on experimental allergic encephalomyelitis (EAE) showed that elevated CRMP2 expression in CD3+ peripheral T lymphocytes correlated with accumulation of CRMP2 positive T cells in the mice brain during the chronic phase of neuroinflammation. These in vivo data confirmed the link between CRMP2 level expression in peripheral T lymphocytes and CNS infiltration during neuroinflammation. In order to define the intracellular signaling pathway supporting such elevated motility, gene expression of CD69 + CRMP2 hi cells was compared to that of CD69 + CRMP2 cells from healthy donors. CD69 + CRMP2 hi cells detected in patients suffering another chronic neuroinflammatory disease (HAMI/TSP) were also analyzed. Oligonucleotide microarrays identified genes that were only expressed in the highly motile activated cells CD69 + CRMP2 hi of neuroinflammatory patients and not in activated cells of healthy donors. These genes defined a signaling pathway that specifically functions in activated immune cells of patients suffering neuroinflammation. Altogether, our data point out CRMP2 as a possible biological marker of CNS infiltration and a potential therapeutic target in neuroinflammatory situation.

P766
IL-17 producing T cells and their induction in multiple sclerosis
J.M. Fletcher, L. Costelloe, O. O’Farrelly, N. Tubridy, K.H.G. Mills; Trinity College Dublin (Dublin, IRL); St Vincent’s University Hospital (Dublin, IRL); University College Dublin (Dublin, IRL)

Recent evidence has demonstrated a crucial role for interleukin (IL)-17 producing T cells in the pathogenesis of experimental autoimmune encephalitis (EAE). IL-17 promotes tissue inflammation via stimulation of pro-inflammatory cytokines and chemokines from a wide range of cell types. Murine IL-17 producing T cells develop via a lineage distinct from the T helper cell (Th)1 and Th2 subsets, and are regulated by cytokines including transforming growth factor beta (TGFbeta), IL-23 and IL-6. As yet, it has not been determined whether IL-17 also plays a role in multiple sclerosis (MS), and little is known about the generation and regulation of IL-17 producing T cells in humans. To investigate the potential role of IL-17 in MS we have measured IL-17, INFgamma and IL-10 levels in the serum of 200 MS patients and controls. We found elevated serum levels of IL-17 in a subset of patients compared to controls, and the levels of IL-17 showed a strong correlation with those of INFgamma. Using flow cytometry and intracellular cytokine staining we have demonstrated that IL-17 is produced by both CD4 and CD8 T cells, and that the IL-17 producing T cells are a subset distinct from that which produces INFgamma. Using these techniques, we are also measuring the antigen-specific induction of IL-17 in response to peptides corresponding to sequences from myelin antigens. We have measured the ability of different cytokines to induce IL-17 in human PBMC and have identified a role for IL-15 and IL-1beta.

P767
FOXp3, CTLA-4 and GITR expression levels in CD25+ CD4+ T-cells before and after corticosteroid treatment during relapse in interferon-beta-treated relapsing-remitting multiple sclerosis patients
N. Grigoriadis, I. Psapaltis, D. Tsiantoulas, G. Deretzis, A. Dimakopoulou, V. Giantzi, T. Sklavudis, I. Milonas; Aristotle University of Thessaloniki (Thessaloniki, GR); Ippokrateio Hospital of Thessaloniki (Thessaloniki, GR)

High motility of activated T lymphocytes is a hallmark of neuroinflammation and favors T cell recruitment in the central nervous system (CNS). Although T cell accumulation in the CNS correlates with neurodegeneration, little is known on the mechanisms that support such high T cell motility. We have identified the phosphoprotein collapsin response mediator protein 2 (CRMP2) as a modulator of T cell motility (Vincent et al., 2005) and observed its elevated expression in activated T lymphocytes of patients suffering multiple sclerosis (MS). We therefore performed a series of experiments aiming to associate CRMP2 expression and motility of MS patients’ T cells, and to establish the signaling transduction pathway involving CRMP2 and implicated in elevated motility. We first examined CRMP2 expression in VLA-4 positive cells of MS patients. High CRMP2 expression in these cells suggested their elevated ability of binding to endothelial cells and transmigration into the CNS. This hypothesis was first tested ex vivo, on activated T lymphocytes expressing high CRMP2 level (CD69 + CRMP2 hi) isolated from MS patients, focusing on their ability to migrate towards chemokines known to be expressed in inflamed brain. Association between CRMP2 level and migratory rate was observed. Secondly, study performed on experimental allergic encephalomyelitis (EAE) showed that elevated CRMP2 expression in CD3+ peripheral T lymphocytes correlated with accumulation of CRMP2 positive T cells in the mice brain during the chronic phase of neuroinflammation. These in vivo data confirmed the link between CRMP2 level expression in peripheral T lymphocytes and CNS infiltration during neuroinflammation. In order to define the intracellular signaling pathway supporting such elevated motility, gene expression of CD69 + CRMP2 hi cells was compared to that of CD69 + CRMP2 cells from healthy donors. CD69 + CRMP2 hi cells detected in patients suffering another chronic neuroinflammatory disease (HAMI/TSP) were also analyzed. Oligonucleotide microarrays identified genes that were only expressed in the highly motile activated cells CD69 + CRMP2 hi of neuroinflammatory patients and not in activated cells of healthy donors. These genes defined a signaling pathway that specifically functions in activated immune cells of patients suffering neuroinflammation. Altogether, our data point out CRMP2 as a possible biological marker of CNS infiltration and a potential therapeutic target in neuroinflammatory situation.
Abstracts

Introduction: CD25+ CD4+ T regulatory (TR) cells play an important role in the maintenance of immunological self-tolerance in autoimmune diseases such as multiple sclerosis (MS) through a cell-to-cell suppression. The master gene for the suppression phenotype is the Forkhead box P3 (FOXP3) that is highly expressed and required for homeostatic maintenance of natural CD25+ CD4+ TR cells. Cyto- toxic T lymphocyte antigen 4 (CTLA4) and glucocorticoid induced TNFRSF 18 (GITR) are also expressed in TR cells. Materials and Methods: In this study the mRNA and protein levels of FOXP3, CTLA-4 and GITR in TR cells were measured in interferon beta treated relapsing-remitting MS patients (10) on the day when a relapse was clinically confirmed (day 0) as well as on days 6 and 30. On day 0, all patients underwent a 5 day – long course with corticosteroid (COR) treatment (1000 mg/day methylprednisolone, IV). Sex- and age- matched healthy donors (10), served as controls. All patients were clinically evaluated at the same time points with expanded disability status score (EDSS). CD25+ CD4+ T cells were isolated from PBMC’s. Total RNA was extracted. The quantification of FOXP3, CTLA-4 and GITR mRNA level was performed by Real-time PCR. The protein level was quantified by Western blot followed by densitometry. In addition, all patients were tested by Mx1 gene expression assay for the detection of anti-interferon beta neutralizing antibodies. Results: All patients responded well to COR treatment with full recovery within a month. The possibility of reduced interferon beta bioavailability was ruled out since all patients were NABs – negative. Compared to pretreatment with COR, the levels FOXP3 and CTLA-4 mRNA expression were decreased by 37.82% and 17.54%, respectively on day 6 and increased to the initial pre-treatment levels on day 30. On the contrary, gene expression levels of GITR increased up to 42.96% on day 6 and remained at high levels on day 30. Compared to healthy controls, the expression levels of all three genes were higher at all time-points tested. Protein level expression followed the same pattern as indicated by western blot. Conclusion: Our results indicate a clear trend in which COR treatment results in an upregulation of GITR molecule, already by day 6 and remain high for at least a month, thereafter. On the contrary, FOXP3 and CTLA-4 expression profiles were shown to be downregulated on day six and increased at the initial, pre-treatment levels on day 30.

P768
IL-6 induces IL-17 production by human CD4+CD45RO+ cells
L.A. Minns, J. Channon, R. Dupont, L. Kasper; Dartmouth Medical School (Lebanon, USA)

Multiple sclerosis (MS) is a central nervous system disease in which activated autoreactive T-cells invade the blood brain barrier and initiate an inflammatory response that leads to myelin destruction and axonal loss. The IL-12 family of cytokines, including IL-12 (p35 p40) and IL-23 (p19 p40), are involved in T cell activation and the induction disease in the mouse model of MS, experimental autoimmune encephalitis (EAE). Recent studies in mice strongly suggest that both members of this family are important for CD4+ T cell responses; IL-12 induces IFN-gamma production by CD4+ Th1 cells, whereas, IL-23 maintains Th17-cells. Further studies showed that TGF-beta and IL-6 are essential for the differentiation of Th17-cells from CD4+ T cells; blocking IL-23 with an anti-p19 antibody reduced both IL-6 and IL-17 expression in the CNS of EAE mice. IL-6 is a unique cytokine that acts as a lymphocyte stimulatory factor. Previous studies demonstrated increased IL-6 in plasma, sera and CSF, higher IL-6 mRNA expression in PBMCs, and increased sIL-6R and its signaling cofactor, gp130, in MS patients compared to healthy controls, suggesting a role for IL-6 in MS. The hypothesis to be tested is that IL-6 is an essential cytokine involved in the induction of pathogenic Th17-cells in humans. Peripheral Blood Mononuclear Cells (PBMCs) and magnetically sorted CD4+ T cells from healthy donors were conditioned in vitro with various cytokines including IL-6, TGF-beta, and IL-23 for 4–10 days. After, in vitro, conditioning, analysis for cell surface and intracellular IFN-gamma and IL-17 as well as mRNA analysis of IL-17 was performed. IL-6 independent of TGF-beta resulted in the expansion of IL-17 producing CD4+ T cells. Negatively selected, CD25+ depleted CD4+ T cells skewed with IL-6, IL-23, and/or TGF-beta resulted in an expansion of IL-17-producing CD45RO+ T cells distinct from IFN-gamma producing CD4+ T cells; results were confirmed by real time quantitative PCR analysis. The highest induction of IFN-gamma producing Th1 cells and ThIL-17 cells was observed when cells were skewed only in the presence of IL-6; these cells were phenotypically distinct from IL-23 induced Th17 cells. Similar analysis of PBMCs from patients with clinically defined MS is underway. This data suggests that in humans, targeting IL-23 or IL-6 could be sufficient to reduce the population of both IFN-gamma Th1 and ThIL-17 cells in MS patients.

P769
Abnormally low surface CTLA4 expression on CD25hi CD4+ T cells correlates with Foxp3 mRNA expression and is normalised by treatment with interferon-beta
F. Sellebjerg, M. Krakauer, M. Khademi, T. Olsson, P.S. Sørensen; Danish MS Research Center (Copenhagen, DK); Karolinska Hospital (Stockholm, S)

Regulatory CD4+ T cells (Treg) have high expression of CD25 (CD25hi). The immunoregulatory activity of CD25hi CD4+ Treg cells is deficient in untreated multiple sclerosis (MS). We used flow cytometry to study CD25+, CD25lo, and CD25hi T cells from healthy controls and patients with MS. In healthy controls a higher percentage of CD25hi CD4+ T cells than CD25lo and CD25– CD4+ T cells had intracellular and surface expression of CTLA4, a molecule with inhibitory effects on T cell activation. In MS patients a higher percentage of CD25hi CD4+ T cells expressed intracellular CTLA4 than in healthy controls (p = 0.006). In contrast MS patients had a lower percentage of CD25hi CD4+ T cells that expressed CTLA4 on the cell surface than did healthy controls (p = 0.003). These changes in CTLA4 expression are similar to those previously reported in autoimmune diabetes. In untreated MS patients the percentage of CD25hi CD4+ T cells with surface expression of CTLA4 correlated with blood mononuclear cell expression of Foxp3 (which controls the regulatory phenotype of CD25 hi CD4+ T cells), Chl-b (a negative regulator of T cell activation), GATA3 (which controls Th2 differentiation of T cells), IL-4, TGF-beta and brain-derived neurotrophic factor mRNA (all p < 0.05), but not with expression of T-bet (which controls Th1 differentiation of T cells), IFN-gamma or TNF mRNA. In MS patients treated with IFN-beta, surface expression of CTLA4 was comparable to the expression in healthy controls, and the injection of IFN-beta resulted in a time-dependent increase in the percentage of CD25hi CD4+ T cells expressing surface CTLA4 (r = 0.8, p = 0.001). Intracellular expression of CTLA4 was, however, not normalized by treatment with IFN-beta. These findings indicate that surface expression of CTLA4 in CD25hi CD4+ T cells may reflect Treg activity, and we are currently addressing this hypothesis in functional studies.

P770
Expression of chemokine receptors by peripheral blood mononuclear cells in multiple sclerosis
O. Suliman, B. Sharrack, N. Woodroofe; Royal Hallamshire Hospital (Sheffield, UK); Sheffield Hallam University (Sheffield, UK)

Introduction: Chemokines are chemotactic cytokines. The interaction between chemokines and their receptors controls the recruitment of inflammatory cells, including peripheral blood mononuclear cells (PBMCs), into sites of inflammation as seen in the central nervous system (CNS) in multiple sclerosis (MS). Within the CNS in MS, increased expression of a number of key chemokines including CCL2, CCL5 and CXCL10, has been demonstrated. PBMCs express the corresponding receptors for these chemokines respectively CCR2, CCR5 and CXCR3. Currently, blockade of chemokines and chemokine receptors are under investigation in clinical trials, as a
Possible therapeutic targets in inflammatory diseases including MS.

**Aims:** To investigate the expression of chemokine receptors CCR2, CCR5 and CXCR3 by peripheral blood T-cells (CD4 and CD8) and monocytes (CD14) in patients with MS and healthy controls longitudinally over a 12 month period. To correlate the level of expression of these receptors to MS clinical disease activity. **Methods:** 30 patients with relapsing remitting MS and 10 healthy controls were recruited. Blood samples were collected every 2 months and at any time during an acute MS relapse for a total period of 12 month. Whole blood was stained using fluorescently labelled monoclonal antibodies. The level of expression of these chemokine receptors on each cell type was determined using 3 colour flow cytometry. **Results:** CD4 cells expressed mainly CXCR3 (>30%) and less than 3% were CCR2+ and CCR5+. CD8 cells expressed mainly CXCR3 (>55%) and less than 10% were CCR2+ and CCR5+. CD14 cells highly expressed CCR2 (>80%) and less than 10% were positive for CCR2 and CXCR3. 18 of the MS patients had at least one relapse during this study and they were grouped as active MS (total number of relapses were 27). There was no significant difference in the expression of the 3 receptors by CD4,CD8 and CD14 cells between the healthy subjects, the stable MS and the active MS patient groups. There was no significant difference in the expression of the 3 receptors by CD4,CD8 and CD14 between relapses and remissions. **Conclusion:** There is no association between MS clinical disease activity and the mean percentage expression of CCR2, CCR5 and CXCR3 by peripheral blood mononuclear cells in this MS patient population.

**P771**

**Alteration of chemokine receptor expression by CD4+CD25-high regulatory T cells in patients with multiple sclerosis**

L. Rinaldi, M. Calabrese, E. Del Giudice, A. Leon, P. Gallo; Multiple Sclerosis Centre (Padua, I); Research and Innovation (Padua, I)

**Object:** To characterise the expression of a number of surface antigens associated with activation, migration and antigen presentation on human regulatory CD4+CD25 high T cells (Tregs); to analyse the expression of chemokine receptors on Tregs from newly diagnosed untreated multiple sclerosis (MS) patients. **Methods:** Peripheral blood from twenty MS patients and twenty healthy donors (HC) was directly derived from whole blood of patients with stable RRMS or PPMS not on immunomodulatory treatment. Using real-time RT-PCR differential expression of candidate genes was quantified in individual MS patients of two independent patient cohorts containing both subtypes of MS. **Results:** Gene expression array analysis identified 51 genes differentially upregulated in RR-MS and their receptors play an important role in that process. **Conclusion:** The major goal of this study was to analyze the migratory activity of regulatory T lymphocytes from MS patients and healthy controls. This effect was dose dependent. We did not observe any significant changes in spontaneous and stimulated by CCL5 migratory activity of monocytes from MS patients. **Conclusion:** Obtained results suggest that in active MS CCL5-induced migratory activity of lymphocytes is increased and this activity may be significantly diminished by treatment of MS with methylprednisolone and mitoxantrone.

**P772**

**CCL5-induced in vitro chemotaxis of mononuclear leukocytes in multiple sclerosis**

M. Jalosinski, A. Glabinski; Medical University of Lodz (Lodz, PL)

**Background:** Active multiple sclerosis (MS) is characterized by the presence of perivascular inflammatory foci localized in the central nervous system (CNS). Inflammatory cells forming those foci migrate from the blood to the CNS. Several studies confirmed that chemokines and their receptors play an important role in that process. **Goals:** The major goal of this study was to analyze the migratory activity of subpopulations of peripheral blood mononuclear cells (PBMC) isolated from the blood of MS patients and stimulated by chemokine CCL5/RANTES. Moreover the impact of MS treatment with methylprednisolone and mitoxantrone on CCL5-induced chemotactic activity of PBMC subpopulations was analyzed. **Methods:** PBMC’s were isolated from blood of untreated MS patients, from MS patients in relapse treated with methylprednisolone, from MS patients with chronic progressive disease treated with mitoxantrone and from control groups (healthy volunteers and patients with other neurological diseases – OND). Chemotactic activity of mononuclear leukocytes was measured in vitro in Neuroprobe MBA96 chemotaxis chamber using fluorometric reader. Chemotactic activity of nonadherent PBMC’s – mostly lymphocytes was measured in lower well of chemotactic chamber. Chemotactic activity of adherent PBMC’s (mostly monocytes) was measured on the filter separating upper and lower well. **Results:** In active MS before any treatment in vitro chemotactic activity of lymphocytes after stimulation with CCL5 was significantly increased (p = 0.023). Spontaneous migration of lymphocytes was similar in all studied groups. Treatment of MS with methylprednisolone and mitoxantrone diminished this activity to lower well. **Conclusion:** Chemotactic activity of monocytes from MS patients. **Conclusion:** Obtained results suggest that in active MS CCL5-induced migratory activity of lymphocytes is increased and this activity may be significantly diminished by treatment of MS with methylprednisolone and mitoxantrone.
and 11 in PP-MS. Out of several candidate genes re-analysed individually by quantitative RT-PCR the death domain containing protein TRADD involved in tumor necrosis factor receptor (TNFR) superfamily signalling, was consistently expressed at higher levels in PP-MS patients compared to RR-MS and healthy controls. This result was confirmed in two independent cohorts. High expression of TRADD protein associated with infiltrates was detected mainly in the spinal cord of MS patients. Results of western blots and three-coloured FACS analysis for TRADD in different PBMC populations will be presented. Conclusion: Array analysis is a useful screening approach in pooled patient samples of MS to identify differentially expressed genes. TRADD, a member of TNFR superfamily, is one of these candidate genes and may serve as a biomarker to discriminate PPMS from RRMS patients. This suggests, distinct pathogenic processes in these MS subtypes.

P774

CD11c expression on peripheral blood NK cells as a biomarker reflecting disease activity of multiple sclerosis
T. Aranami, S. Miyake, T. Yamnamura; National Institute of Neuroscience, NCMF (Kodaira, JP)

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS), in which autoreactive CD4+ T cells are presumed to play a pathogenic role. Although a large majority of the patients show relapsing-remitting course of the disease, the clinical profile varies greatly among individuals. For better quality of management of the disease, establishment of appropriate biomarkers reflecting the disease activity is currently desired. Recently we have demonstrated that peripheral blood NK cells biased towards secreting IL-5 are associated with the remission state of MS and might function as regulatory cells on autoreactive CD4+ T cells (J. Clin. Invest, 2001, 107(S): R23-9). Brain, 2004, 127 (Pt 9): 1917–27). Here we report that MS patients in remission (MS-rem) differentially express CD11c on NK cell surface. MS-rem can be divided into CD11chigh and CD11clow according to NK cell phenotype. When we analyzed cytokine expression in NK cells, upregulation of IL-5 and GATA-3 was observed in CD11chigh but not in CD11clow patients. In contrast, the CD11chigh patients showed a higher expression of HLA-DR on NK cells. Since in vitro studies demonstrated that NK cell stimulatory cytokines such as IL-15 would upregulate CD11c on NK cells, we postulate that inflammatory signals may play a role in inducing the CD11chigh NK cell phenotype. Given regulatory function of NK cells in MS-rem on autoreactive CD4+ T cells, we hypothesized that these alterations of NK cells in CD11chigh MS may be correlated with exacerbation of autoreactive CD4+ T cells. Consistent with this idea, activation markers such as HLA-DR and CD25, were overexpressed on CD4+ T cells in CD11chigh MS. Namely, proportions of HLA-DR + cells or CD25+ Foxp3− non-regulatory T cells but not CD25− Foxp3+ regulatory T cells increased in CD11chigh compared to CD11clow MS. Importantly, 5 out of 10 CD11chigh MS patients developed a clinical relapse within 120 days after blood sampling, whereas only 1 out of 10 CD11clow developed exacerbated disease. These results suggest that CD11chigh patients may be in more unstable condition than CD11clow. Thus, a higher expression of CD11c on NK cells may reflect the disease activity of MS, which may allow us to use it as a potential biomarker to monitor the immunological status of MS-rem.

P775

Study of the migratory and survival potentials of circulating myeloid and plasmacytoid dendritic cells in multiple sclerosis patients
C. Lopez, M. Mintore, R. Martin, X. Montalban, M. Comabella; Vall d’Hebron University Hospital (Barcelona, E)

Dendritic cells (DC) are the most potent antigen presenting cells, capable to induce and regulate T-cell mediated immune responses. The two main peripheral blood DC populations are DC1 (CD11c+ myeloid DC) and DC2 (CD11c− plasmacytoid DC). DC1 and 2 respond to different stimuli and may drive Th1 or Th2 responses in T cells depending on maturation state. Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) supposedly mediated by Th1 autoreactive T cells. DC are detected in inflamed MS lesions and numbers of plasmacytoid DC are found increased in the cerebrospinal fluid (CSF) of MS patients. Recruitment of blood DC into the CNS relies on the expression of specific chemokine receptors and adhesion molecules. In addition, the balance in the expression between pro- and anti-apoptotic molecules may be critical in the survival of DC and hence, in the perpetuation of the inflammatory process that is taking place in the CNS of MS patients. Objectives: To compare the migratory and survival potentials of circulating blood DC1 and DC2 between untreated and interferon-beta (IFN-b)-treated MS patients and healthy controls. Methods: The expression of a panel of chemokine receptors (CCR5 and CXCR3), adhesion molecules (LFA-1 (CD11a) and VLA-4 (CD49d)), and apoptosis markers (Fas and Bcl-2) by circulating DC1 and DC2 was determined by flow cytometry on fresh whole blood samples from 31 MS patients (8 patients with relapsing-remitting (RR) MS, 6 with secondary progressive (SP) MS, 7 with primary progressive (PP) MS, and 12 with RRMS treated with IFN-b) and 15 healthy controls. Results: The expression of LFA-1 by DC1 was increased in untreated RRMS patients when compared to healthy controls and progressive forms of MS. The expression of Fas by DC2 was overall increased in MS patients when compared to healthy controls, especially in SPMS patients. The percentage of DC2 expressing CCR5 was higher in RRMS patients receiving IFN-b compared to untreated RRMS patients. Conclusion: The higher expression of LFA-1 in RRMS patients may be associated with increased migratory potential of DC1 to enter the CNS in this subset of patients. The increased expression of Fas in SPMS may reflect differences in the maturation state of DC2 in the clinical forms of MS. Finally, one mechanism by which IFN-7 exerts its beneficial effects could be the increase in CCR5 expression by DC2, thus favoring migration into the CNS and inducing Th2 type immune responses.

P776

Cytokines and cytotoxic markers in the CD4+ CD28− T cell subset in multiple sclerosis patients

Background: It has been shown that a subpopulation of CD4+ T cells that express cell surface markers characteristic of both NK cells and conventional T cells, and lack the expression of the costimulatory molecule CD28, is able to exert strong immunoregulatory and cytotoxic activity. We have previously shown that CD4+ CD28− T cell expression was significantly increased in multiple sclerosis (MS) patients compared to that in healthy controls, and that surface markers typical of NK cells, activation markers and adhesion molecules were preferentially expressed in this CD4+ CD28− T cell subpopulation. The aim of this study was to assess cytokine and cytotoxic markers (perforin and granzyme B) expression in CD28− and CD28+ T CD4+ cell subsets in MS patients. Granzyme B is a serine protease found primarily in NK cells and cytotoxic T lymphocytes and it acts by means of inducing apoptosis in target cells. Perforin facilitates the entry of serine proteases into the target cell and is also capable of lysing non-specifically a variety of target cells. Methods: Sixty patients with relapsing–remitting MS were studied and sampled during remissions. Evaluation of perforin, granzyme B, IL-2, IFN-g, TNF-a, IL-4 and IL-5 intracellular expression was performed by flow cytometry. Results: Expression of the cytotoxic markers, perforin and granzyme B, was significantly higher in the CD4+ CD28− T subset. Additionally, intracellular IFN-g and TNF-a expression was also significantly increased in the T CD4+ subset that lacks the expression of the costimulatory molecule. On the contrary, IL-2 and IL-4 expression was significantly enhanced in the CD4+
CD28+ T cell subset. **Conclusion:** The fact that cytotoxic markers as perforin and granzyme B as well as the proinflammatory cytokines, IFN-γ and TNF-α, were preferentially produced by CD4+CD28− T cell subset, in MS patients, suggest that these cells might have a potential cytotoxic effector role in the immune responses and might play an important part in the pathogenesis of MS. Assessment of the CD4+CD28− T cell subset phenotype could help us to evaluate the clinical and immunological status of individual MS patients.

P777

**The neuroprotective potential of immune cells: the role of gender, age and multiple sclerosis**

M. Caggiula, A.P. Batocchi, G. Frisullo, F. Angelucci, V. Nociti, A.K. Patanella, C. Sancricca, P.A. Tonali, M. Mirabella; Fondazione Don Carlo Gnocchi (Rome, I); Catholic University (Rome, I)

**Background:** Inflammatory reactions in the central nervous system are usually considered detrimental, but recent evidence suggests that they can also be beneficial and even have neuroprotective effects. Those effects are, at least in part, mediated by the release of neurotrophic factors. In multiple sclerosis (MS) the beneficial effect of inflammation seems to be greater in younger patients and in the ones with a shorter disease duration. Few data are, to date, available about neurotrophin production in healthy people. **Objective:** The aim of this study was to compare brain-derived neurotrophic factor (BDNF) production by peripheral blood mononuclear cells (PBMCs) in healthy subjects with its levels in a large group of relapsing-remitting (RR) MS patients. **Methods:** We determined BDNF production by unstimulated PBMCs derived from 43 healthy subjects (23 females and 20 males) and 77 patients with RR MS (53 females and 24 males). Mean age of controls was 43.9 ± 14.8 years (43.7 ± 14.7 as to females and 44.15 ± 15.4 as to males); nine females were postmenopausal. Mean age of RRMS patients was 31.3 ± 9.5 years; mean disease duration was 4.8 ± 5.9 years. All patients were in stable phase of disease. The last relapse or steroid administration had occurred at least 3 months before blood sample. None of our patients had ever been treated with any immunomodulatory drugs except corticosteroids. Spontaneous BDNF production was measured in duplicate by enzyme-linked immunosorbent assay (ELISA) in supernatants of PBMCs. **Results:** In healthy subjects we found that PBMCs derived from females produced higher levels of BDNF as compared with PBMCs from males. Besides, we observed an inverse correlation between age and BDNF production by PBMCs. Also among MS patients we detected higher BDNF levels in females than in males. Moreover, BDNF production seems to decline during the course of the disease. Relapse rate in the year before and EDSS score seemed not to influence BDNF production by PBMCs. We also found that PBMCs from healthy subjects produced higher levels of BDNF than PBMCs from MS patients in stable phase of disease. **Conclusion:** These data demonstrate that BDNF production by immune cells is more effective in females than in males both in MS patients and controls. It declines with age in healthy subjects and seems to be reduced in MS where it is inversely correlated with disease duration.

P778

**Dendritic cells are abundant in non-lesional grey matter in multiple sclerosis**

C. Cudrici, E. Zaffranyskaia, F. Niculescu, K. Mullen, S. Judge, P.A. Calabresi, H. Bac; University of Maryland (Baltimore, USA); Johns Hopkins University (Baltimore, USA)

It was previously demonstrated that a population of perivascular cells expressing dendritic cell (DC) markers are present in multiple sclerosis (MS) plaques. We have further analyzed DC markers expression and localization in acute and chronic active lesions and compared with expression in non-lesional white (NLWM) and gray matter (NLGM). Frozen brain tissue specimens were obtained at autopsy from eight patients with a definite diagnosis of MS. Immunohistochemistry was performed on cryostat sections for CD209, CD205, CD68, CD3, CCR7, CCR5, MHC class II, and Myelin/oligodendrocyte antigen. We found CD209 labeling of some of the perivascular cells in both acute and chronic lesions. Many, but not all, of these cells stained positively for CCR5. Serial sections through areas revealed an absence of CCR7 co-localization with CD209. Although less numerous than CD209+ cells, cells expressing mature DC marker CD205 were consistently detected in perivascular cuffs of most active lesions. Some of the CD209+ cells were in close proximity with CD3+ lymphocytes. Interestingly, sections of NLWM and NLGM also had areas with perivascular CD209+ positive cells. In NLGM parenchymal cells expressing CD209/CD205 were also found. Some of the parenchymal CD209+ cells had morphology suggestive of microglia and others of macrophages with ingested myelin. In double labeling experiments some but not all of the CD209 cells also expressed CD68 suggesting that these cells might be of macrophage/microglial origin. These data suggest that some microglia express DC-like markers and might play roles in the initial antigen presentation and promotion of T-cells entry in the brain.

P779

**The role of dendritic cells during the course of multiple sclerosis relapses associated with bacterial infections**

J. Correale, M. Farez; FLENI (Buenos Aires, RA)

Dendritic cells (DC) are potent antigen-presenting cells and are critical for onset of the immune responses generates against infections. Bacteria and viruses have been implicated in autoimmune disease pathogenesis. Indeed, Multiple Sclerosis (MS) relapses are frequently associated with infection, in some instances bacterial. The hypothesis of this study was to establish whether DC from bacteria-infected MS patients modified autoreactive T cells patterns of activation, thus triggering disease exacerbation. CD1+, CD11c+, CD80+, CD86+, CD14−, HLA-DR bright DC were separated from peripheral blood during 11 exacerbations linked to bacterial infections and 11 relapses without infections. DC antigen-presenting capacity was assessed with MBP, MOG, GM1, and GM3 specific autologous T cell clones (TCCs). TCCs stimulated with DC from infected MS patients showed maximal proliferation, and induced the secretion of IL-12, IL-17, and IFN-γ at 10 to 30 times less concentration than after incubation with DC isolated from uninfected individuals. Interestingly, CD1-restricted GM1 and GM3 TCCs incubated with DC from infected MS patients secreted IFN-γ, and IL-12 even in the absence of exogenous antigens. These activation patterns correlate with increased IFN T cell survival. DC from infected MS patients secreted more IL-12 and IL-18, and showed higher expression of the myeloid differentiation factor 88, as well as molecules CD1, B7−1, B7−2, B7−DC, CD40, CD83 and CCR7, as compared to DC from uninfected individuals. Overall, these results are consistent with the concept that during bacterial infections, DC play a critical role in MS relapse induction.

P780

**Pathological relevance of meningeal B-cell follicles in secondary progressive multiple sclerosis**

R. Magliozzi, A. Vora, B. Serafini, R. Nicholas, O. Howell, M. Puopolo, R. Reynolds, F. Aloisi; Istituto Superiore di Sanita’ (Rome, I); Imperial College of London (London, UK)

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system in which the humoral response is believed to play an important immunopathogenic role. We have previously shown that ectopic B-cell follicles with germinal centers develop in the meninges
of a subset of patients with secondary progressive (SP) MS (Serafini et al., Brain Pathol. 2004, 14: 164) and of mice with relapsing remitting experimental autoimmune encephalomyelitis (EAE) (Magliozzi et al., J Neuroim. 2004, 148: 11). The aims of this study were: 1) to determine the frequency of ectopic B-cell follicles in MS cases with different progressive disease courses; and 2) to examine whether there is an association of ectopic follicles with demographic, clinical and neuropathological features. A detailed immunohistochemical and morphometric analysis was performed on post-mortem cerebral tissue from 29 SP, 4 progressive relapsing (PR) and 7 primary progressive (PP) MS cases. Ectopic follicles were detected in the inflamed meninges of 41% of the SPMS cases, but in none of the PRMS and PPMS cases. Compared to the SPMS group without follicles, the SPMS group with follicles was characterized by a higher female preponderance and by a lower age at MS onset, first use of wheelchair and death. Pathologically, there was more pronounced demyelination, microglial activation and axonal loss in the cerebral cortex of SPMS cases with follicles. Cortical demyelination in the latter cases was also more severe than in the PRMS and PPMS cases. These data indicate that the presence of ectopic follicles identifies a clinically and neuropathologically distinct subset of MS patients and imply a role for these abnormal structures in the exacerbation of cortical pathology and rapid progression of the disease.

P781

The thymus and regulatory T-cell function in patients with multiple sclerosis

B. Wildemann, J. Haas, P. Trubswetter, B. Fritzsching, P.H. Krammer, E. Suri-Payer; University of Heidelberg (Heidelberg, D); DKFZ Heidelberg (Heidelberg, D)

Background: CD4+CD25+ regulatory T-cells (Treg) are defective in patients with multiple sclerosis (MS) as they inhibit myelin-specific and antigen-nonspecific T-cell proliferation less potently as compared to Treg from healthy donors while their numbers and elimination are unaltered. The mechanisms of this Treg defect are unclear. Treg mature in the thymus and the majority of cells circulating in the periphery rapidly adopts a memory phenotype. Since recent evidence suggests that the thymic output of T-cells is impaired in MS patients we hypothesized that a defective Treg generation may contribute to the functional Treg impairment associated with MS. We therefore determined the role of Treg which enter the circulation as recent thymic emigrants (RTEs) and, unlike their CD45RO+/memory Treg. Methods: Treg as well as CD4+CD25− effector T-cells (Teff) were immunomagnetically isolated from peripheral blood of 10 healthy individuals. Treg were further separated according to their CD31 expression in CD31+ RTE Treg and CD31− memory Treg. We used in vitro proliferation assays to test the inhibitory capacity of Treg subsets and of Treg obtained from MS patients (n=7) and healthy donors (n=9) before and after depletion of CD31+ RTE Treg. We also performed CDR3 spectratyping to determine the T-cell receptor V beta (TCR Vb) repertoire in patient and donor derived Treg. Results: CD31+ RTE Treg displayed a markedly superior suppressive ability compared to CD31− memory Treg. Parallel assessment of both patient and donor derived total Treg and Treg depleted of CD31+ cells revealed a less potent suppressive activity of donor CD31− memory Treg and neutralized the difference in inhibitory potencies of patient and donor Treg detectable when using total Treg in the co-culture experiments. Furthermore, MS Treg but not healthy Treg, exhibited a significantly contracted TCR Vb repertoire. Conclusion: The depletion of highly potent CD31+ RTE Treg downregulates the degree of suppression mediated by total Treg in healthy individuals and reverses the MS associated Treg defect. Since the TCR Vb repertoire is contracted in patient derived Treg our observations suggest that a shift in the homeostatic composition of peripheral Treg subsets related to a reduced de novo generation of thymic-derived Treg and compensatory expansion of less suppressive memory Treg may contribute to the impaired Treg function in MS patients.

P782

Using biological (MxA protein or IL-10 mRNA level) or MRI indicators for assessing treatment response to interferon-beta


Objective: Specificity, sensitivity, and predictivity of MxA protein or IL-10 mRNA or MRI activity as response indicators of interferon beta(IFNB) treatment. Background: The Optimization of Interferon for MS (OPTIMS) trial is a multicenter trial involving 20 MS centers designed to identify suboptimal responders (SR) to IFNB-1b and to test the efficacy of 375 mcg IFNB-1b. Design/methods: IL-10 mRNA level quantified by real time RT-PCR (Taqman, ABI PRISMTM 7900 Sequence detection system) in whole blood. MxA protein leukocyte production determined by a two-site chemiluminescent sandwich assay in whole blood. SR identified during a 6-month run-in phase where all patients were treated with 250 mcg eod IFNB-1b as patients with either a moderate increase of MxA protein or a decrease of IL-10 mRNA levels or an active scan (with at least a new T2 or gadolinium-enhancing lesion). Primary outcome: persistent SR, who had, during 18 months after run-in, clinical (either a relapse or confirmed EDSS progression) or MRI activity signs. ROC analysis used to assess sensitivity, specificity, and predictivity of the response indicators. Results: Subject of the main predictivity analysis were 144 patients continuously treated with 250 mcg IFNB-1b (36 received 375 mcg). A single active scan during the first 6 months of treatment predicted a clinical SR with a significant specificity and positive predictivity (p<0.05). MxA levels were available from 78 and IL-10 mRNA levels from 38 patients. An increase of MxA above 72 ng/mL during the first 6 months of IFNB-1b treatment significantly discriminated patients (free from MRI activity) during the same period. The increase of MxA level above the thresholds identified by ROC analysis was not, however, able to discriminate patients with a good clinical response over the subsequent 18 months of IFN beta treatment. IL-10 mRNA level decreased in SR during the first 6 months of treatment. Conclusion: Since MxA or mRNA tests require whole blood samples and more complex methodologies, they have been done only in patients of a single MS center. MRI scans were available from all 20 centers indicating that the use of MRI activity as treatment response indicator can be easily done by any MS center. In addition, the early changes biological indicators had no significant predictivity on the occurrence of clinical signs of disease activity over the 2-year follow-up of the study.

P783

Elevated levels of kappa free light chains in CSF support the diagnosis of multiple sclerosis

S. Presslauer, D. Miloslavijevic, W. Hüb, T. Brünic, P. Bayer; Wilhelminenspital (Vienna, A)

Background: Numerous studies have demonstrated kappa free light chains (KFLC) in cerebrospinal fluid (CSF) of multiple sclerosis (MS) patients but so far only small cohorts have been examined and in most studies the KFLC were simply qualitatively detected. However, using a recently developed particle-enhanced immunoassay protocol (FreeliteTM, The Binding Site, UK) it has become possible to make rapid and automated quantitative measurements of KFLC using nephelometry. Objective: To determine whether KFLC-levels in CSF correlate with the diagnosis of MS or possible MS and compare their diagnostic utility with measurement of IgG-index and detection of oligoclonal banding (O CB) by isoelectric focusing. Furthermore to establish a threshold value for pathologically elevated KFLC-levels in CSF. Methods: Between 2001 and 2006 we collected CSF and serum samples from 567 unselected patients including a group of 56 MS-patients (32 definite MS, 24 possible MS). The samples were analysed using the nephelometric KFLC assay, an IgG-assay and
isoelectric focusing. The results were then compared to the previously established diagnoses, the diagnosis of MS was made utilising the diagnostic criteria published by McDonald et al. (Ann. Neurol. 2001 Jul; 50 (1): 121–7) Results: Best diagnostic performance was achieved by constructing a KFLC-index comparable to the IgG-index: Concentration of KFLC in CSF was divided by the concentration of KFLC in serum; the result was then divided by the albumin quotient. In our study the threshold value for pathologically elevated KFLC-index was 5.5. Out of the 56 patients with the diagnosis of MS or possible MS, where intrathecal IgG synthesis would be expected, 54 had an elevated KFLC-index, in 50 patients OCB were detected and 42 had elevated IgG-indices (lgG > 0.6). The sensitivity of the KFLC-index was 0.96 compared to 0.89 for OCB and 0.75 for the IgG-index.

In two of the MS-patients none of these methods could detect an inflammatory process. Four of the 56 patients in the MS-group showed no OCB but significantly elevated KFLC-indices, two of these patients also had elevated IgG-indices. Most other intrafetal infections like meningitis (n = 7), neuroborreliosis (n = 7) etc. were also associated with elevated KFLC-indices. Therefore the specificity of KFLC-index > 5.5 for the MS group (0.87) is lower than that of OCB (0.91) but still distinctly higher than the specificity of the IgG-Index (0.83).

Conclusion: In this study an elevated KFLC-index represents the most sensitive and specific quantitative diagnostic parameter for MS, outperforming the widely used IgG-index. As it is measured by an automated, readily available laboratory method, the KFLC-index could provide an important early indication of an intrafetal immunological process for patients suspected of suffering from MS before isoelectric focusing is performed. Moreover, the determination of the KFLC-index could improve the diagnosis of MS-patients with no OCB.

Pathology

P784

Dirty-appearing white matter in multiple sclerosis: histological evidence of reduced myelin phospholipids

W. Moore, C. Laule, G. Zhao, A. MacKay, A. Traboulsi, D. Li; University of British Columbia (Vancouver, CAN); UBC MS/MRI Research Group (Vancouver, CAN)

Background: Dirty-appearing white matter (DWM) has been described in patients with multiple sclerosis (MS) as diffuse areas of slightly increased signal intensity in white matter with conventional MRI distinct from the typical focal high signal intensity MS lesion. It is evident in 25% of RRMS patients, however, the pathological basis of these abnormalities is poorly studied. Objective: To delineate the histopathology of DWM and correlation with MRI derived myelin content. Methods: Four formalin-fixed cerebral hemisphere slices from three MS patients with DWM were scanned with a 32-echo T2 relaxation sequence on a GE 1.5T Signa scanner. T2 relaxation distributions were calculated using a regularised non-negative least-squares algorithm. MWF was defined as the amplitude of the short (<30 ms) T2 component (myelin water) divided by total T2 distribution. Sections were stained for myelin using luxol fast blue (LFB) and myelin basic protein (MBP) and for axons using Bielschowsky. DWM regions of interest were compared to normal appearing white matter (NAWM) and quantified using the optical density of myelin. DWM regions of interest were compared to normal appearing (LFB) and myelin basic protein (MBP) and for axons using Bielschowsky. T2 distributions were calculated using a regularised non-negative least-squares algorithm. MWF was defined as the amplitude of the short (<30 ms) T2 component (myelin water) divided by total T2 distribution. Sections were stained for myelin using luxol fast blue (LFB) and myelin basic protein (MBP) and for axons using Bielschowsky.

DWM regions of interest were compared to normal appearing white matter (NAWM) and quantified using the optical density of myelin. DWM regions of interest were compared to normal appearing (LFB) and myelin basic protein (MBP) and for axons using Bielschowsky. T2 distributions were calculated using a regularised non-negative least-squares algorithm. MWF was defined as the amplitude of the short (<30 ms) T2 component (myelin water) divided by total T2 distribution. Sections were stained for myelin using luxol fast blue (LFB) and myelin basic protein (MBP) and for axons using Bielschowsky.

Results: DWM showed a mean reduction in MWF of 33% (p < 0.0001) compared to NAWM. LFB was reduced by 33% and Bielschowsky was reduced by 15% (p < 0.0001). DWM was poorly visualized with MBP and it was decreased by 11% (p < 0.0008). The correlation between LFB and MWF ranged from r2 0.53 to 0.92. LFB also correlated with Bielschowsky from r2 0.62 to 0.85. LFB did not correlate well with MBP. Conclusion: Since LFB stains for myelin phospholipids, the findings are consistent with DWM being the MRI equivalent of a region with selective reduction of myelin phospholipids detected by the MWF, but with a relative preservation of myelin proteins, as well as axons.

P785

Cortical pathology in multiple sclerosis: expression of glutamate transporter EAAT2

M. Vercellino, A. Merola, S. Masera, F. Plano, B. Votta, E. Capello, G.L. Mancardi, M.T. Giordana, R. Mutani, P. Cavalla; University of Turin (Turin, I); University of Genoa (Genoa, I)

Glutamate excitotoxicity has been described as a putative pathogenetic factor in Multiple Sclerosis (MS). Oligodendrocytes are highly vulnerable to excitotoxic damage, and blockade of glutamatergic receptors has been shown to ameliorate experimental allergic encephalomyelitis. A reduction of the expression of Excitatory Amino Acid Transporter 2 (EAAT2) has been observed in acute MS white matter lesions; an increase of the expression of EAAT2 has been observed in MS optic nerves. In our study we wished to assess the pattern of expression of EAAT2 in MS cortex, evaluating the possible relationship with cortical demyelination and with inflammation. Sections of 6 brains of MS patients (3 secondary progressive MS, 3 relapsing remitting MS) were selected, as well as 5 control brains. Immunohistochemistry was performed with antibodies for myelin basic protein, EAAT2, glial fibrillary acid protein, CD68, MHCII, beta amyloid precursor protein, neurofilaments. Demyelinating lesions were observed in all MS cases in the cerebral cortex, in the thalamus and in the basal ganglia. Most lesions were limited to the outer layers of the cerebral cortex (layer I to layer III – IV). Two cases of secondary progressive MS displayed a pattern of generalized subpial demyelination; in the remaining cases only focal cortical lesions were observed. A reduction of the number of EAAT2 expressing cells in the cortex was observed in the two cases with generalized subpial demyelination; a moderate reduction was observed also in other MS brains, if compared to control brains. According to our results, the expression of EAAT2 is decreased in MS cortex, particularly in cases showing diffuse cortical demyelination. EAAT2 pattern changes observed in MS cortex may suggest a role for alterations of glutamate homeostasis in MS cortical pathology.
Regional variation in the extent and pattern of grey matter demyelination in multiple sclerosis: a pathological comparison between the cerebral cortex, cerebellar cortex, thalamus and spinal cord

C. Gilmore, I. Donaldson, L. Bo, T. Owens, J. Lowe, N. Evangelou; Queen’s Medical Centre (Nottingham, UK); VU Medical Centre (Amsterdam, NL)

**Background:** Myelin protein immunohistochemistry staining techniques demonstrate substantial demyelination in both the cerebral cortex and the spinal cord in Multiple Sclerosis (MS). Grey matter (GM) demyelination has not been studied pathologically in both the brain and spinal cord of the same subjects. **Aim:** To examine the extent and pattern of GM demyelination in the cerebral cortex, cerebellum, thalamus and spinal cord in MS. **Method:** Post-mortem material was obtained from 14 pathologically confirmed MS cases and 3 controls. Sections were taken from 7 predetermined areas of the CNS (motor cortex, cingulate gyrus, cerebellar cortex, thalamus and spinal cord-cervical, thoracic and lumbar levels), irrespective of macroscopic appearance, and stained for Proteolipid Protein. In each region the proportion of GM that was demyelinated (PGMd) and the proportion of white matter (WM) that was demyelinated (PWMd) was evaluated. **Results:** Overall, 28.8% of the GM was demyelinated compared with 15.6% of the WM (p < 0.001). PGMd was greater than PWMd in all of the areas examined. This difference reached statistical significance in the motor cortex (p = 0.013), cerebellar cortex (p = 0.006), cervical cord (0.046) and thoracic cord (p = 0.008), but not in the lumbar cord (p = 0.069), cingulate (p = 0.119) or thalamus (p = 0.389). Multiple regression models demonstrate that PGMd was significantly higher in the spinal cord and cerebellum than in the motor cortex, cingulate and thalamus. PWMd was significantly higher in the spinal cord than in the other regions, including the cerebellum. Within the cerebral cortex PGMd was greater in the cingulate gyrus than in the motor cortex (p = 0.013). GM plaques in the cerebral cortex, spinal cord and thalamus showed a similar morphology as those described previously. A large number of plaques within the cerebellum show a distinct morphology characterised by a complete loss of myelin within the full thickness of the cerebellar cortex, with sparing of the subcortical WM. **Discussion:** There is substantial variation in the extent of both GM and WM demyelination between different regions of the CNS. Of the areas examined, GM demyelination was most extensive in the spinal cord and cerebellum, while WM demyelination was most prominent in the spinal cord. Demyelination was greater in the GM than in the WM at each of the anatomical sites.
normal appearing white matter (NAWM). Similarly the number of remyelinated fibres per OL was determined, after 15 days, in the centre of lysophosphatidyl choline (LPC)-induced lesions in the corpus callosum of adult rats and their littermates in which remyelination was accelerated by stereotaxic microinjection of neurotrophin-3 (NT-3). In addition pure rat OL cultures were submitted to unique (2.10–3 M, 24 h) or repeated (0.5. 10–5 M, 4 × 6 h) LPC toxicity and the effects of NT-3 and PDGF were recorded in these conditions. Results: In chronic MS lesions and in LPC-induced lesions the number of myelinated fibres per OL was significantly (p < 0.05) and strongly decreased compared to the NAWM (85%) and to animals treated with NT-3 (100%), respectively. In vitro OL showed a greater vulnerability to LPC-repeated attacks at low concentration than to a unique injury at high LPC concentration, and this was improved by NT-3 and PDGF. Conclusion: Our results suggest that the capacity of OL to remyelinate axons is impaired during MS, and that this defect is due to successive relapses. Therefore, limiting relapses could represent one way to improve remyelination. Experimentally growth factors like NT-3 and PDGF rescue myelination by OL, since these growth factors are also neurotrophic they might be considered as potentially useful for therapeutic prospects. Acknowledgments: Supported by a grant from the University Hospital of Angers (PHRC 21–01).

Neuroprotection

P791

Oral fingolimod maintains and restores neuronal function in demyelinating models of multiple sclerosis, as assessed by somatosensory and visual evoked potentials

B. Balatoni, M.K. Storch, R. Weissert, C.A. Foster; Novartis Inst for BioMedical Research (Vienna, Austria); University of Graz (Graz, Austria); Hertie Institute for Clinical Brain Research (Tubingen, Germany)

Background: Fingolimod (FTY720) is an orally-active sphingosine 1-phosphate (S1P) receptor modulator under development for the treatment of multiple sclerosis (MS). During a phase II trial in relapsing MS, it reduced clinical relapse rate by more than 50% and inflammatory lesion activity on magnetic resonance imaging by up to 80%. Phase III studies are ongoing. Aim: To elucidate how FTY720 exerts its beneficial effects in the central nervous system (CNS), we compared functional parameters of nerve conductance with morphological features in the DA rat model of experimental autoimmunedencephalomyelitis (EAE) under prophylactic and therapeutic settings. Methods: EAE was induced with myelin oligodendrocyte glycoprotein (MOG) or syngeneic neuroantigen. Prophylactic oral treatment with FTY720 (0.3 to 0.4 mg/kg) or repeated (0.5. 10–5 M, 6 h) LPC was started 15 or 4 days post-infection. Clinical scores were assessed daily; visual and somatosensory evoked potentials (VEP, SEP) were recorded prior to perfusion-fixation; histological and immunocytochemical analyses were performed on the brain, optic nerves and spinal cord to assess inflammation, demyelination, axonal loss and blood-brain-barrier (BBB) integrity. Results: FTY720 prophylaxis completely protected against the emergence of EAE symptoms, electrophysiological disturbances and pathology in the CNS. Therapeutic treatment reversed severe neurological deficits in established EAE induced by syngeneic myelin proteins as well as MOG. In parallel to these clinical benefits, FTY720 restored and normalized the functional responses to SEP and VEP stimulation. Neuronal function and clinical efficacy correlated with a reversal of BBB leakage and a reduction of inflammatory infiltrates, actively demyelinating lesions and axonal loss. Conclusion: The effectiveness of FTY720 in reversing clinical disease and restoring nerve conductance is likely due to several contributing factors. Evidence thus far support its role in the reduction of inflammation and preservation of BBB integrity. FTY720 may also act via S1P receptors expressed by glial cells and/ or neurons in the CNS to promote endogenous repair mechanisms that complement its immunomodulatory action.

P792

The sphingosine-1-phosphate receptor subtype-1 stimulates ERK phosphorylation in astrocytes

M. Osinde, K.K. Dev; Novartis AG (Basel, Switzerland)

The five sphingosine-1-phosphate receptors (S1P1-5) are activated by the endogenous ligand, sphingosine-1-phosphate (S1P) and are expressed in varying degrees on neurons, oligodendrocytes, astrocytes and microglia. In astrocytes, activation of S1P receptor ligands, the aim of our study was to determine which receptor(s) is/are involved in ERK phosphorylation in astrocytes. We find the pan-receptor agonists, S1P or AML629 (a phosphorylated version of FTY720), stimulate ERK phosphorylation in cortical cultures prepared from E18 rat brains. This process can be blocked by the ERK kinase inhibitor U0126 or by pertussis toxin treatment, confirming the involvement of both the ERK signaling cascade and a Gi-coupled receptor, respectively. Immunocytochemistry revealed co-localisation of anti-phospho-ERK staining with GFAP positive astrocytes but not NeuN stained neurons or CNPase positive oligodendrocytes, indicating ERK phosphorylation takes place in astrocytes. Finally, the S1P1 receptor selective agonist SEW2871 showed a U0126 and pertussis toxin sensitive stimulation of ERK phosphorylation that was similar to S1P and AML629. Collectively, these results demonstrate that the S1P receptor subtype-1 mediates ERK phosphorylation in astrocytes.

P793

Interferon-beta 1a-induced expression of brain-derived neurotrophic factor in human T lymphocytes

Z. Mangal, C. Pullakavumkal, V.S. Manda, H.P. Hartung, O. Neuhaus; Heinrich Heine University (Dusseldorf, Germany)

Objective: To investigate the effects of interferon (IFN)-beta 1a (Rebif) on the expression of brain-derived neurotrophic factor (BDNF) and other neurotrophic factors in human T lymphocytes. Background: Neurodegeneration correlates with progression of disability in multiple sclerosis (MS). IFN-beta 1a reduces progression of sustained disability in MS, suggesting neuroprotective properties. BDNF and other neurotrophic factors capable of promoting neural cell survival may contribute to “neuroprotective immunity” in MS. Methods: For in vitro analyses, mitogen-activated peripheral blood lymphocytes (PBL) obtained from untreated MS patients or from healthy donors were treated with IFN-beta 1a. Their proliferative activity was compared to the secretion and transcription of BDNF as assessed by standard ELISA and by real-time PCR. Results: Proliferative activity of mitogen-activated PBL was inhibited by IFN-beta 1a in a dose-dependent manner. In contrast, IFN-beta 1a induced BDNF expression both in activated and in non-activated PBL. Highest BDNF levels were induced at IFN-beta 1a concentrations between 1,000 to 5,000 U/ml, whereas higher or lower concentrations induced lower BDNF levels. BDNF mRNA levels, other neurotrophic factors and pro- and anti-inflammatory cytokines are currently being investigated. In addition, for ex vivo analyses, serum levels and mRNA levels of BDNF are being assessed in blood obtained from untreated or Rebif-treated MS patients. Conclusion: Our results support the hypothesis that IFN-beta 1a may promote neuroprotection by induction of BDNF expression.
**P794**

*A randomised controlled trial of neuroprotection with lamotrigine in secondary progressive multiple sclerosis*

J. Furby, T. Hayton, K.J. Smith, D. Altmann, R. Brenner, J. Chataway, N.C. Fox, R.A.C. Hughes, D.H. Miller, R. Kapoor; Institute of Neurology (London, UK); Kings College (London, UK); School of Hygiene and Tropical Medicine (London, UK); Royal Free Hospital (London, UK); National Hospital for Neurology and Neurosurgery (London, UK); GKT School of Medicine (London, UK)

**Background:** There is good evidence that the primary cause of disability is neuro-axonal degeneration within the CNS. Experimental work by members of our group has established that axons may degenerate upon exposure to the inflammatory mediator nitric oxide. The mechanism of the damage implies that protection might be afforded by the novel approach of partially blocking sodium channels. Our group and others have demonstrated that drugs including flecainide, phenytoin and lamotrigine can reduce axonal degeneration when optic nerves or spinal roots are exposed to nitric oxide, and in experimental autoimmune encephalomyelitis. Consequently, we have initiated a clinical trial to assess whether the sodium channel blocker lamotrigine has a neuroprotective, disease modifying effect on a) the rate of axonal degeneration and b) the accumulation of disability in patients with secondary progressive multiple sclerosis (SPMS). **Methodology:** We are recruiting 120 people with SPMS, in whom progression rather than relapse is the major cause of increasing disability, into a double-blind, parallel group, controlled trial lasting two years. Random allocation will be made to receive treatment with either extended release lamotrigine (GlaxoSmithKline) or placebo. The primary endpoint will be an effect of treatment on cerebral atrophy, which correlates with other MR markers of axonal loss, and which can be measured reliably and sensitively using recently developed MR techniques. The trial is powered to detect a 60% beneficial effect on the rate of development of cerebral atrophy. Secondary endpoints include effects of treatment on spinal cord atrophy and on clinical measurements of impairment/disability. MR measures of brain volume and scores of clinical impairment/disability will be determined at entry, and after 6, 12, 18 and 24 months. Cervical spinal cord cross-sectional area will be measured at entry, and after 12 and 24 months. **Utilization of results:** A phase II trial of sodium channel blockade in SPMS is timely, given recent advances arising from experimental and imaging work. Our results are likely to help with the design of future trials of neuroprotection in MS, and a successful outcome would demonstrate a novel, safe neuroprotective strategy to reduce long-term disability. Supported by the MS Society of GB&NI.