Wednesday, September 18, 2002
Young Scientific Investigator Session

1

HLA-DRB5*0101 AND -DRB1*1501 EXPRESSION IN THE MULTIPLE SCLEROSIS-ASSOCIATED HLA-DR15Dw2 HAPLOTYP

Prat E1, Kwok W2, Kruse N3, Pujol-Borrell R4, Bettinotti MP5, McFarland HF6, Martin R7
1Neuroimmunology Branch, NINDS, National Institutes of Health, Bethesda, Maryland, USA; 2Virginia Mason Research Center, Seattle, Washington, USA; 3INSERM U 437, Nantes, France; 4Department of Neurology, Nantes, France; 5Medical Center, Amsterdam, Noord-Holland, Netherlands; 6Institut Virion/Serion GmbH, Wurzburg, Germany; 7Immunology Division, University Hospital “Germans Trias i Pujol”, Badalona, Spain; 8DTM/Clinical Center, National Institutes of Health, Bethesda, Maryland, USA

Background: The major histocompatibility complex region shows the strongest association to multiple sclerosis (MS) and in Caucasians, HLA-DR15Dw2 and -DQw6 are the closest associated to the disease. In the HLA-DR15 haplotype, two β-chain HLA-DRB1*1501 and -DRB5*0101 are co-expressed resulting in two different surface MHC class II αβ heterodimers. They both can serve as antigen presenting molecules for myelin basic protein-specific T cells and they are able to differentially influence T cell effector functions. These findings point out that it is important to dissect which of the two alleles is functionally relevant for MS.

Objectives: The purpose of this study is to analyze the mRNA and surface expression of HLA-DRB5*0101 and -DRB1*1501 in antigen-presenting cells (APC) obtained from healthy donors (HD) and MS patients.

Methods: Peripheral blood mononuclear cells, monocytes, B cells and dendritic cells were obtained from a total of 8 HD and 14 MS patients. Three samples of thymic tissue were also analyzed. The expression of HLA-DRB5*0101 and -DRB1*1501 was evaluated by FACS and by real-time quantitative RT-PCR, using specific oligonucleotides and plasmid DNA standard curves. In B cells and monocytes alleles expression was analyzed under basal conditions and after stimulation with IL-4 and IFNγ in time-course (T-C) experiments.

Results: In all APC, transcripts were a few times more abundant for HLA-DRB5*0101 than for HLA-DRB1*1501, while FACS experiments showed that surface expression for the two alleles was different in different APC. In T-C experiments they were similarly modulated. No major differences were observed between MS patients and HD.

Conclusions: HLA-DRB5*0101 expression was overall comparable to the one of -DRB1*1501, providing further evidence for a role of this allele that is usually not considered in the pathogenesis of MS. Different surface expression patterns of the two alleles in various APC are currently being evaluated as they might be important for shaping the T cell receptor repertoire.

Disclosure: E Prat has nothing to disclose.

Funding: Elisabetta Prat was supported by a postdoctoral fellowship from the National Multiple Sclerosis Society. The two antibodies anti-HLA-DRB1*1501 and anti-HLA-DRB5*0101 were kindly made available by Dr. Jarhow Lee from One Lambda Inc.

2

QUALITATIVE AND QUANTITATIVE ANALYSIS OF THE BLOOD TCR β-CHAIN TRANSCRIPTOME AT DIFFERENT TIME POINTS OF MULTIPLE SCLEROSIS COURSE.

Laplaud DA1, Wiertlewski S2, Guillet M3, Ruiz C3, Melchior B4, Eden G5, Damier P6, Souilillou J7
1INSERM U 437, Nantes, France; 2Department of Neurology, Nantes, France; 3Department of Neurology, Rennes, France

Background: MS is an autoimmune demyelinating disease of the CNS associated with T-cells autoreactive for myelin components. The (auto)antigenic peptide is recognized by the T-cell through its hypervariable region on the TCR (β chain).

Objectives: The aim of this study was to explore the possible presence of a peripheral immune component in MS. We analysed the β chain transcriptome at a qualitative and quantitative level in the blood of MS patients using a new method designed in our laboratory (TeLand). This method allows to detect alterations in the T-cell repertoire and to estimate the T-cell pool size expressing a given altered β chain. No further cell manipulation is performed during this procedure avoiding selection biases due to cell culture.

Methods: Two groups of patients were analyzed. The first group (n=10) was composed of patients at early onset of MS. The second group (n=10) was composed of patients examined for a second or a third relapse of the disease. A group of healthy volunteers was also analyzed as a reference. First, peripheral blood mononuclear cells were isolated, the RNA was extracted and reverse transcribed to obtain single strand cDNA. Specific primers for 26 β chain families were used to carry out the combined qualitative and quantitative analysis of the TCR. The results were integrated to give colored coded vision of the whole T-cell repertoire referred as T-cell Landscapes (TeLand). After identification of the altered β chain family(ies) for each patient, the cells bearing the altered TCR can be separated with β chain-specific antibodies. DNA was then extracted and mRNA cytokine production can be analysed in a quantitative manner (TaMan).

Results: Our data suggest that there are more alterations in the MS group than in the healthy volunteer group. The alterations seem to be more frequent in MS patients at the second or third relapse than at the early onset of the disease. In addition, in preliminary experiments, we show that sorting of the cells bearing the altered TCR for transcriptional analysis is possible.

Conclusions: We suggest that this approach may open new opportunities for understanding the magnitude and the type of the immune component in MS.

Disclosure: D Laplaud has nothing to disclose.

Funding: Supported by ARSEP (Association pour la Recherche sur la Sclerose en Plaques), supported by le College des Enseignants de Neurologie.

3

EXPRESSION OF METABOTROPHIC GLUTAMATE RECEPTORS IN MULTIPLE SCLEROSIS BRAIN: UPREGULATION IN AXONS AND REACTIVE ASTROCYTES.

Geurts JP1, Kamphorst W2, van der Valk P3, Aronica EM4
1Radiology, MR Center for MS Research, VU Medical Center, Amsterdam, Noord-Holland, Netherlands; 2Pathology, MR Center for MS Research, VU Medical Center, Amsterdam, Noord-Holland, Netherlands; 3(Neuro)Pathology, Academic Medical Center, Amsterdam, Noord-Holland, Netherlands

Background: Metabotropic glutamate receptors (mGluRs) have been implicated in the regulation of synaptic plasticity, cell proliferation and cell death. Moreover, mGluRs seem to play a role in pathological processes like glutamate excitotoxicity and neurodegeneration. They are also involved in the regulation of amyloid precursor protein (APP) metabolism, a protein that is now widely regarded as a marker for (early) neuronal dysfunction in multiple sclerosis.

Objectives: to investigate the expression of group I (mGluR1 and mGluR5) and group II (mGluR2/3) mGluRs in multiple sclerosis brain.

Methods: We used immunohistochemistry in a total of 10 MS cases and 7 non-neurological controls. We also compared the MS cases to other conditions exhibiting neurodegeneration.

Results: Upregulation of both group I and II mGluRs was found in the MS cases, relative to healthy controls. A diffuse increase in expression of mGluR5 and mGluR2/3 was observed in reactive astrocytes. mGluR1α was found in a subpopulation of vimentin-positive reactive astrocytes and was strongly expressed in axons of the subcortical white matter. However, no distinct differences between MS lesion types were observed. Axonal immunopositivity was also observed in normal appearing white matter (NAWM) of MS tissue as well as in other neuropathological conditions like cerebral infarction and diffuse


axonal injury. Moreover, in cortical neurons located in the proximity of a lesion, atypical clusters of mGlur1a immunoreactivity were observed. 

Conclusions: group I and II mGlur1a are upregulated in MS brain. This may underlie the importance of glutamate excitotoxicity as a part of the pathophysiological process of multiple sclerosis and possibly also of other conditions showing neurodegeneration. 

Disclosure: J Geurts has nothing to disclose. 
Funding: Stichting Vrienden MS Research The Netherlands Brain Bank.

4 

GROUP CONNECTIVITY MAPS OF OPTIC NERVES AFTER ISOLATED OPTIC NEURITIS 
Ciccarelli O^1, Hickman SP^1, Toosy AT^1, Parker GP^1, Wheeler-Kingshott CA^1, Barker GP^1, Miller DP^1, Thompson AP^1
^1UCU, Institute of Neurology, London, United Kingdom; ^2Imaging Science, University of Manchester, Manchester, UK, United Kingdom 

Background: Fast marching tractography (FMT) uses diffusion tensor (DT) imaging data to trace the white-matter tracts in vivo within the brain. Tractography group mapping allows the construction of anatomical connectivity maps of the optic radiation (OR) that compensate for the normal inter-subject variability. 

Objectives: The aim of this study was to investigate the changes in the OR of patients affected by isolated optic neuritis (ON) using tractography group mapping. 

Methods: Seven patients (mean age 37.3±9.4, all women) 1 year after isolated ON (4 R, 3 L) and 21 age-matched controls (mean age 33±7.9, 11 women and 10 men) were studied. Whole-brain DT imaging was performed and the fMT algorithm was used to trace the ORs in each individual. The OR fractional anisotropy (FA) and volume were calculated, and differences between patients and controls assessed using the t-test. Group connectivity maps of the ORs were created using SPM99, and VBM was employed to investigate the differences in the left and right OR connectivity between the two groups. A simple regression model was used to investigate whether the OR connectivity in patients are correlated with VEP amplitudes and latencies of the affected and unaffected eyes and with the number of lesions within the OR. 

Results: Patients had lower OR FA (mean 0.29±0.03) and volume (mean 4.5 cm³±1.2) than controls (FA: mean 0.31±0.03, volume: mean 5.3 cm³±1.3), but these differences were not statistically significant (FA: p=0.12; volume: p=0.13). VBM detected significant differences between patients and controls for the right and left OR connectivity maps (right OR: p=0.02, left OR: p=0.05, after SVC (small volume correction)). In patients, VEP amplitude and latency of the affected eye correlated with the left OR connectivity (p=0.007 corrected for whole brain and p=0.03 after SVC respectively). No correlations were found for the VEP of the unaffected eye or for the number of lesions within the ORs. 

Conclusions: Tractography group mapping detects differences in the ORs between normal subjects and patients affected by ON. The relationship between the clinical findings and the extent of OR connectivity may be secondary to the ON or due to pathological damage within the OR. 

Disclosure: O Ciccarelli has nothing to disclose. 
Funding: O Ciccarelli is supported by TEVA Ltd. S Hickman is supported by The Wellcome Trust. A. Toosy is supported by Action Research. The NMR Research Unit is supported by the Multiple Sclerosis Society of Great Britain and Northern Ireland.

5 

FUNCTIONAL DIVERSITY OF ANTIBODIES AGAINST MYELIN/OLIGODENDROCYTE GLYCOPROTEIN IN EXPERIMENTAL AUTOIMMUNE DEMYELINATION 
von Büdingen H^1, Hauser SI^1, Fuhrmann A^1, Nabavi CB^1, Genain CP^1 Neurology, University of California, San Francisco, San Francisco, California, USA 

Background: Disseminated CNS demyelination reminiscent of that seen in human multiple sclerosis (MS), is a hallmark feature of marmoset experimental allergic encephalomyelitis (EAE) following immunization with the recombinant form of myelin oligodendrocyte glycoprotein (MOG). Autoimmune antibodies against MOG play an important role in lesion pathogenesis in both this MS model and rodent EAE, however, little is known about the molecular and structural basis for pathogenicity. 

Objectives: To characterize the diversity of MOG-specific humoral immune responses in a primate model of MS. 

Methods: MOG-reactive antibody populations from animals immunized with MOGaa1-125 (EMOG), or 20mer MOG-derived peptides were fractionated by affinity chromatography according to specificity for linear or conformational MOG-epitopes. Monoclonal, MOG-reactive Fab fragments were selected from combinatorial IgG-Fab libraries generated from rMOG-immune marmosets, and analyzed for immunoglobulin gene usage and MOG-epitope recognition. 

CNS tissue was obtained at euthanasia and was stained with Luxol Fast Blue/Periodic Acid Schiff. 

Results: Animals immunized with the MOG-derived peptides displayed antibody reactivity exclusively directed against linear determinants of MOG. In contrast, animals immunized with rMOG developed a humoral repertoire directed against both conformational and linear epitopes. Neuropathologically, MOG peptide-immune animals developed very few lesions with scarce demyelination, in stark contrast to animals immunized with rMOG. A limited number of heavy and light chain variable region genes appear to be used by the marmoset immune system to target a number of different conformational epitopes of MOG. These epitopes are consistently found in MOG-immune antibody repertoires in this species, as demonstrated by competition experiments with monoclonal Fab fragments from the antibody libraries against native anti-MOG antibodies. 

Conclusions: In conclusion, these studies provide the first detailed description of MOG-specific antibody responses in an outbred primate, and suggest that conformation-dependent anti-MOG antibodies play a predominant role in the pathogenesis of CNS demyelination. 

Disclosure: H von Büdingen has nothing to disclose. 
Funding: Supported by the National Institutes of Health (NIADDK 53573 to SLH, NS1-996-02 to HCvB), the Nancy Davis Foundation, the New York Community Trust, the German Hertie Foundation, the German Multiple Sclerosis Society, and the National Multiple Sclerosis Society (JP2007-4-2 to CPG). 

6 

MRI EVIDENCE OF MORE EXTENSIVE TISSUE DAMAGE IN MS PATIENTS WITH THE ε4 ALLELE OF APOLOPROTEIN E: HIGHER PROPORTION OF LESIONS EVOLVING TO BLACK HOLES DURING TWO-YEAR FOLLOW-UP 
Eazingeon C^1, Ropele S^2, Strasser-Fuchs S^2, Kapeller Pa^3, Seifert T^4, Poltrum B^5, Schmidt H^5, Schmidt R^5, Fazekas F^6
^1Neurology, Karl-Franzens-University Graz, Graz, Styria, Austria; ^2MRI Centre, Karl-Franzens-University, Graz, Styria, Austria; ^3Institute of Biomed., Karl-Franzens-University, Graz, Styria, Austria 

Background: The Apolipoprotein E ε4-allele (ε4) has been associated with clinical worsening in multiple sclerosis (MS) and more pronounced tissue damage on magnetic resonance imaging (MRI) and proton MR spectroscopy. 

Objectives: We attempted to consolidate this assumption by using serial MRI of the brain to follow the evolution of black holes which are regarded as a putative marker of matrix destruction and axonal loss. 

Methods: 99 individuals with clinically definite relapsing-remitting MS (age 35.3±9.5 yrs, disease duration 6.6±7.2 yrs, Expanded Disability Status Scale score 1.5±1.2) underwent genotyping and clinical examination. T2- and T1-weighted axial MRI of the brain (1.5 T, TR/TE=2500/30 and 90; 600/15) for semi-automated lesion segmentation was performed at baseline and after 2.7±1.1 yrs. Black holes were defined as T1-lesions with a signal intensity between Black holes were defined as T1-lesions with a signal intensity between

Results: At baseline, T2- and T1-lesion loads (LL) were non-significantly higher in patients with ε4 (n=23; T2-LL: 11.8±/11.4; T1-LL: 1.2±/2.3 cm³) than in those without ε4 (n=76; T2-LL: 8.9±/9.5; T1-LL: 0.7±/1.8 cm³), despite a shorter disease duration (4.2±/5.2 vs. non-ε4: 7.4±/7.6 yrs, p=0.06) and the absence of significant differences in clinical variables between groups. During follow-up, T2-LL significantly enlarged in patients without ε4 (10.6+/+ 11.0 cm³; p=0.001), whereas it remained unchanged in ε4-carriers (11.3±/1.17 cm³; p=0.58).

Disclosure: O Ciccarelli has nothing to disclose.
In contrast, T1-LL significantly increased in the ε4-subgroup (1.7±2.7 vs. non-ε4: 0.8±1.5 ccm, p=0.039). Moreover, the proportion of black-holes (T1ILL/T2LL)x100) increased significantly from 5.5±7.7 % to 12.4±13.9 % (p=0.005) in ε4-patients whereas it did not change significantly in non-ε4 patients (baseline: 5.0±7.9 %, follow-up: 5.7±7.3 %, p=0.37).

Conclusions: The observed higher proportion of MRI brain lesions that develops into black holes in MS patients with ε4 provides further support for a more aggressive disease course in ε4 carriers.

Disclosure: C Enzinger has nothing to disclose.

7  
EVIDENCE FOR AXONAL PATHOLOGY AND ADAPTIVE CORTICAL REORGANIZATION IN PATIENTS AT PRESENTATION WITH CLINICALLY ISOLATED SYMPTOMS SUGGESTIVE OF MULTIPLE SCLEROSIS

*Neuroimaging Research Unit, Department of Neuroscience, Scientific Institute and University HSR, Milan, Italy;  
Department of Neuroradiology, University Hospital Basel, Basel, Basel Stadt, Switzerland;  
Department of Neurology, Scientific Institute and University HSR, Milan, Italy;  
Clinical Trials Unit, University of Basel, Basel, Basel Stadt, Switzerland;  
Department of Physiology, University of Basel, Basel, Basel Stadt, Switzerland.

Background: Recent MRI and pathologic studies have demonstrated the presence of axonal loss and dysfunction even in the early stages of MS.  
Objectives: To assess, using IMRI, the brain pattern of movement-associated corticospinal activations in patients at presentation with clinically isolated syndromes (CIS) suggestive of MS. To investigate the correlation between the extent of functional corticospinal activations and the extent of MS-related pathology, measured using conventional MRI and whole brain N-acetylaspartate (WBNAAs) 1-HMRS.  
Methods: From 16 right-handed patients at presentation with CIS and 15 controls, we obtained: a) fMRI (repetitive flexion-extension of the last four fingers of the right hand), b) dual-echo scans, c) 1H-MRS to assess WBNAAs levels. FMRI data were analyzed using SPM99.  
Results: Compared to controls, patients with CIS had decreased whole brain NAA levels (p=0.001). They also had more significant activation of the contralateral primary somatomotor cortex (SMC), secondary somatosensory cortex and inferior frontal gyrus. Relative activation of the contralateral primary SMC was correlated with whole brain NAA levels (r=−0.78, p=0.001).  
Conclusions: This study demonstrates that functional cortical changes can be detected in patients at presentation with CIS. These changes might have a favorable role in limiting the impact of axonal pathology on subsequent disease evolution.  
Disclosure: M Rocca has nothing to disclose.

8  
A 36-MONTH LONGITUDINAL STUDY ON THE EVALUATION OF THE EFFECT OF INTERFERON BETA IN THE DURATION OF BLACK HOLES IN MULTIPLE SCLEROSIS

Cognitive Psychology, University of Basel, Basel, Basel Stadt, Switzerland;  
Neurology, University Hospital Basel, Basel, Basel Stadt, Switzerland;  
Novartis Pharma AG, Central Technologies, Basel, Basel Stadt, Switzerland;  
Neuroimmunology Branch NINDS, NIH, Bethesda, Maryland, USA;  
Biostatistics Branch NINDS, NIH, Bethesda, Maryland, USA;  
Neuroimmunology Branch NINDS, NIH, Bethesda, Maryland, USA;  
Laboratory of Diagnostic Radiology Research, NIH, Bethesda, Maryland, USA;  
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Objectives: To assess, using IMRI, the brain pattern of movement-associated corticospinal activations in patients at presentation with clinically isolated syndromes (CIS) suggestive of MS. To investigate the correlation between the extent of functional corticospinal activations and the extent of MS-related pathology, measured using conventional MRI and whole brain N-acetylaspartate (WBNAAs) 1-HMRS.  
Methods: From 16 right-handed patients at presentation with CIS and 15 controls, we obtained: a) fMRI (repetitive flexion-extension of the last four fingers of the right hand), b) dual-echo scans, c) 1H-MRS to assess WBNAAs levels. FMRI data were analyzed using SPM99.  
Results: Compared to controls, patients with CIS had decreased whole brain NAA levels (p=0.001). They also had more significant activation of the contralateral primary somatomotor cortex (SMC), secondary somatosensory cortex and inferior frontal gyrus. Relative activation of the contralateral primary SMC was correlated with whole brain NAA levels (r=−0.78, p=0.001).  
Conclusions: This study demonstrates that functional cortical changes can be detected in patients at presentation with CIS. These changes might have a favorable role in limiting the impact of axonal pathology on subsequent disease evolution.  
Disclosure: M Rocca has nothing to disclose.

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DOES FUNCTIONAL MRI ALLOW INFERENCES ABOUT COGNITIVE TRAINING EFFICACY IN MULTIPLE SCLEROSIS?

Peuer F, Kappos L, Rausch M, Opwis K, Radj E  
Cognitive Psychology, University of Basel, Basel, Basel Stadt, Switzerland;  
Neurology, University Hospital Basel, Basel, Basel Stadt, Switzerland;  
Novartis Pharma AG, Central Technologies, Basel, Basel Stadt, Switzerland;  
Neuroimmunology Branch NINDS, NIH, Bethesda, Maryland, USA.

Background: Little is known about the effects a cognitive training might induce on the brain organisation and its possible visualization by fMRI.  
Objectives: Our aim is to analyse the effects of a cognitive training on attention with functional MRI and to relate baseline and follow up fMRI findings with the result of training.  
Methods: We assessed different attention domains with a neuropsychological test battery (TAP) in twelve MS patients and seven healthy controls. According to the patients’ performance on the tests, they were classified as mildly and severely impaired. After the baseline examination, all patients underwent a cognitive training program for the most impaired attentional function. To determine the brain structures induced by the attention tasks before and after training, all subjects were investigated by fMRI.  
Results: Training facilitated functional activation of brain structures that were not responding initially. Those were located mainly in the frontal and parietal cortex. This effect was found in all tasks in mildly impaired patients who were able to compensate for their deficits even in the tasks with high complexity; in the more severely impaired patients additional activation and compensation was restricted to the simplest task. However, when improvement in performance in the attention tasks was chosen as dependent variable instead of absolute degree of dysfunction, the patients with improvements showed a decrease in brain activation after training while the patients without improvement showed a significant increase in activation.  
Conclusion: Effects induced by a cognitive training can be visualized by functional MRI. The amount of performance improvement after retraining however, seems to depend on the brain’s capacity to establish new interconnections. Successful training might be dependent on the individuals ability to focus on relevant brain structures while failure to improve after training might be
related to waste non-focused brain activation. Supported by the Swiss MS Society.

Disclosure: I Penner has nothing to disclose.

10

COMBINATION THERAPY OF MS PATIENTS WITH INCOMPLETE RESPONSE TO INTERFERON-BETA WITH HUMANIZED ANTIBODY AGAINST THE INTERLEUKIN-2 RECEPTOR ALPHA CHAIN

Bielekova B1, Reichert-Scrivner S1, Wuerfel J1, Ohayon J1, McCartin J1, Richert N1, Frank P1, Waldmann T1, McFarland H1, Martin R2

1Neurology, Institute of Neurology, Cambridge, United Kingdom; 2Laboratory of Diagnostic Radiology Research, NIH/NCI, Bethesda, Maryland, USA; 3Metabolism Branch, NIH/NCI, Bethesda, Maryland, USA

Background: MS is an immune-mediated demyelinating disorder of the CNS. Current treatments of MS are based on immunosuppressive or immunomodulatory strategies and are only partially effective in majority of patients. The experience with one of these therapies, IFN-beta, clearly demonstrated that the therapeutic effect is dose-dependent. It is therefore likely that, depending on the level of activation of the immune system, multimodal approaches will be required in some patients to reach optimal therapeutic effect.

Objectives: We tested the hypothesis, whether in patients with only partial therapeutic response to IFN-beta addition of humanized antibody against Interleukin-2 receptor alpha chain (Zenapax) would result in further decrease in contrast-enhancing brain MRI lesions.

Methods: This was an unblinded, baseline (IFN-beta alone)-versus add-on treatment (IFN-beta + Zenapax) crossover phase II trial, with total of 10 MS patients. Inclusion criteria included clinical and/or MRI definitions for partial response to IFN-beta. Patients have been followed by monthly clinical-, MRI-and immunological measures for 4 months of baseline and 9 months of treatment. Primary outcome measure was the change in number of contrast-enhancing MRI lesions. Number of additional MRI, clinical and immunological parameters served as secondary outcome measures.

Results: Zenapax add-on therapy was tolerated extremely well and led to over 50% additional reduction in MRI contrast-enhancing lesions in the majority of patients. Zenapax did not cause general immunosuppression, nor did it significantly decrease the proliferation of peripheral blood T-lymphocytes to strong polyclonal stimuli or recall antigens.

Conclusions: We anticipate that Zenapax will become a treatment alternative in MS, however, it needs to be studied whether Zenapax acts in concert with IFN-beta or by itself.

Disclosure: Dr. Thomas Waldman holds a patent for the use of an antibody to the IL-2 receptor for treating malignancy and autoimmune disorders. Other authors have nothing to disclose.

Thursday, September 19, 2002

Keynote Address

11

INFLAMMATION, DEMYELINATION AND AXONAL LOSS: UNRAVELING THE RELATIONSHIPS

Ludwin SK

Department of Pathology, Queens University and Kingston General Hospital, Kingston, Ontario, Canada

Abstract Body: Multiple Sclerosis is characterized by demyelination, inflammation, gliosis and axonal loss. In recent years it has become obvious that axonal loss accounts for a major amount of the symptomatology and disability, especially in progressive cases. Etiopathogenesis of the axonal loss has become of prime importance, as prevention and regeneration strategies will become part of the Multiple Sclerosis physician’s armamentarium. Although axonal damage in acute cases can be well accounted for by the marked cellular infiltration, together with its resultant chemical toxic factors, continuing and progressive axonal damage in chronic lesions is less easy to understand. Possible mechanisms include susceptibility of demyelinated axons to continual insult from ongoing inflammation, axonal degeneration following lack of trophic support from oligodendroglia and myelin, and primary damage and degeneration to the neurons. It is possible that one or more of these mechanisms may result in axonal damage. The relationship between these factors and axon loss may vary from case to case, and also during varying timepoints within each case. Experimental models where the elements such as inflammation, demyelination and gliosis can be dissected and examined in isolation may assist in elucidating possible mechanisms for this axonal loss. The axon may be differentially affected in animal models which are predominantly either inflammatory or demyelinating as in toxic disorders; models of ischemia and edema may under different conditions damage the axon and at times the myelin sheath or both. These relationships can also be studied in human diseases where the same elements are prominent, such as hereditary leukodystrophies, toxic diseases such as carbon monoxide, and ischemic lesions. Each of these groups of diseases may have varying combinations of these elements. Careful examination of the distribution of lesions may also help to determine the role of neuronal dysfunction in causing axonal loss. Patterns with a distal-proximal gradient along tracts may suggest neuronal dysfunction with a dying-back picture, as opposed to Wallerian distribution. The role of gliosis as an alternative neuroprotectant in various experimental and clinical conditions may also render clues as to glial trophic protection.

Disclosure: S Ludwin has nothing to disclose. Funding: CHRM/RMC MS Society of Canada.

Session I

Inflammation, Demyelination and Axonal Loss: Insights From Pathology

12

MECHANISMS OF AXONAL LOSS

Trapp BD1, Bjartmar C2, Peterson J3, Chang A1, Rudick R4

1Department of Neurosciences, Cleveland Clinic Foundation, Cleveland, Ohio, USA; 2Department of Neurology, Cleveland Clinic Foundation, Cleveland, Ohio, USA; 3Neuroscience Graduate Studies Program, Ohio State University, Columbus, Ohio, USA

Abstract Body: This presentation will summarize pathological studies from our laboratory that describe axonal pathology and axonal degeneration in post-mortem MS brains. Three mechanisms of axonal loss will be discussed. The first is axonal transaction in the setting of inflammatory demyelination. This mechanism of axonal loss begins at disease onset, predominates in early stages of relapsing-remitting MS and is often clinically silent because the brain compensates for axonal loss. The topological relationship between axonal transection and inflammatory demyelination suggests that demyelinated axons are vulnerable to components of the inflammatory environment. Proteolytic enzymes, cytokines, oxidative products and free radicals produced by activated immune and glial cells may cause axonal transection by reducing energy metabolism, ATP synthesis and Ca2+ homeostasis. The second mechanism is progressive degeneration of chronically demyelinated axons. This process results in the inexorable progression of neurological disability that so commonly complicates the secondary progressive stages of MS, when brain inflammation often subsides. Myelin or myelin-forming cells provide trophic support to the axon and chronic loss of myelin can result in degeneration. The possibility has also been raised that the redistribution and abnormal expression of Na+ channel subunits on demyelinated axolemma may render axons vulnerable to degeneration. An acquired chameleonopathy, therefore, may also contribute to
degeneration of chronically demyelinated axons. The third mechanism of axonal loss occurs in the setting of demyelination of the cerebral cortex. Transected axons and dendrites are abundant in cortical lesions and they correlate with the degree of microglia activation. Infiltration of hematogenous leukocytes and perivascular cuffs are not features of cortical lesions. Recent studies indicate that significant areas (over 25%) of cerebral cortex are demyelinated in many MS patients. A better understanding of the dynamics of cortical lesion formation is needed to fully appreciate their role in the pathogenesis of neurological disability in MS.

Disclosure: B Trapp has nothing to disclose.
Funding: Supported by National Institutes of Health (NS8667) National Institutes of Health (NS35058)

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RELATIONSHIP BETWEEN INFLAMMATION AND AXONAL LOSS

Brueck W
Institutfur Neuropathologie, Berlin, Berlin, Germany

Abstract Body: Multiple sclerosis (MS) is characterized morphologically by the key features demyelination, inflammation, gliosis, and axonal damage. In recent years it has become more evident that axonal damage is the major morphological substrate of permanent clinical disability. In our study we investigated the occurrence of acute axonal damage determined by immunocytochemistry for amyloid precursor protein (APP) which is produced in neurons and accumulates at sites of recent axon transaction or damage. The extent of acute axonal damage was correlated with the stage of demyelinating activity, disease duration and course as well as numbers and components of the inflammatory infiltrate. Most APP positive axons were detected within the first year after disease onset, but acute axonal damage was also detected to a minor degree in lesions of patients with a disease duration of 10 years and more. This effect was not due to the lack of active demyelinating lesions in the chronic disease stage. Late remyelinated lesions (so-called shadow plaques) did not show signs of axon destruction. The number of inflammatory cells showed a similar decrease over time like the number of APP positive axons. There was a significant correlation between the extent of axon damage and the numbers of CD8-positive cytotoxic T cells and macrophages/microglia. Our results indicate that a putative axon-protective treatment should start as early as possible and include strategies preventing T cell/macrophage mediated axon destruction and leading to remyelination of axons.

Disclosure: W Brueck has nothing to disclose.
Funding: Supported by: Gemeinnützige Hertie-Stiftung

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DIFFERENTIAL GENE EXPRESSION ANALYSIS OF MULTIPLE SCLEROSIS TISSUE: COMPARISON OF ACTIVE AND INACTIVE LESIONS.

Mycko MP, Papoian R, Boschert U, Raine CS, Selmi KW
a Department of Neurology, Medical University of Lodz; Lodz, Poland; b Serono Research Institute, Serono, Geneva; c Albert Einstein College of Medicine, Department of Pathology (Neuropathology), New York, New York, USA

Background: Multiple sclerosis (MS), primary autoimmune demyelinating disease of the central nervous system (CNS), is been characterized by the presence of the demyelinating lesions (plaques) within the CNS tissue. Histopathologically MS lesions are divided into acute, chronic active and chronic inactive plaques.

Objectives: To understand the genes transcription status of the two most often MS lesions: chronic active and chronic inactive plaques we have performed a comparative cDNA microarray analysis of these two lesion types.

Methods: Differential gene expression (DGE) was performed by cDNA microarray analysis of CNS tissue from 4 multiple sclerosis (MS) subjects in which different regions of chronic active (n=2) and chronic silent lesions (n=2) were compared.

Results: DGE analysis shown a significant differences between the lesion margin and lesion center in both types of lesions. Nearly 10% of the genes were differentially expressed in the lesion margins and centers in the chronic active lesions whereas less then 2% in the chronic nonactive type of lesions. 14 genes were identified as overlapping in the analysis of DGE genes from different active type of lesions. These genes were mostly of inflammatory characteristic or associated with activation of cell death. To compare differences between chronic active and silent lesions, we performed DGE comparison of the pooled data from both types of lesions. The major DGE occurred at the lesion margin, 156 (26%; 5%), the greater number representing upregulated genes at the margin of active lesions (15%).

Conclusions: We have identified a set of genes to be differentially expressed within the MS chronic type lesions that may be related with the activity of the MS chronic lesions. Thus, using microarray analysis we were able to highlight a genes associated with lesion activity in MS, many of them not previously linked with the disease.

Disclosure: K Selmaj has nothing to disclose.

Funding: Supported by: National Institutes of Health (NS35058) National Institutes of Health (NS38667) National Institutes of Health (NS35058) National Institutes of Health (NS35058)

Multiple Sclerosis
16
MULTIPLE SCLEROSIS: EXPANDED CSF B CELLS ARE ALSO PRESENT IN THE BRAIN TISSUE

Goebels N1b, Weber H1, Hofbauer M1, Wekerle H1, Hohlfeld R1b
1Institute for Clinical Neuroimmunology, Klinikum Grosshadern, Muenchen, Bavaria, Germany; 2Department of Neuroimmunology, Max-Planck-Institute for Neurobiology, Martinsried, Bavaria, Germany

Background: Multiple sclerosis (MS) is a multifocal inflammatory disease of the central nervous system (CNS) characterized by demyelination and axonal damage. Although the responsible components of the immune system and the precise molecular targets are still unidentified, expanded lymphocyte populations have been implied in the pathogenesis. Previously we and others have demonstrated the presence of expanded B cell clones in brain tissue and in the cerebrospinal fluid (CSF) of MS patients.

Objectives: To study, whether brain tissue and CSF of MS patients contains distinct or identical B cell repertoires.

Methods: We employed a PCR - based method to identify and characterize clonally expanded B cells. The method (“CDR3 spectratyping”) relies on the natural length variation of the third hypervariable region (CDR3) of the rearranged immunoglobulin gene: whereas a polyclonal B cell population shows a random, Gauss-distributed length variation of the CDR3, a clonally expanded population has a uniform CDR3 length, which can be identified as a single band on a sequencing gel. The identity of these expanded B cell clones can often be determined by subsequent cycle sequencing.

Results: We analysed matched pairs of cDNA from CSF cells and brain tissue of two MS patients who underwent brain biopsy for diagnostic reasons in the initial phase of their disease. Histological examination of the brain tissue, CSF and clinical/MRI follow up supported the diagnosis of multiple sclerosis. CSF cells were obtained 6 months (patient A) and 5 years (patient B) after brain biopsy. Using CDR3-spectratyping, we repeatedly detected identical clonally expanded B cell clones both in the CSF and brain tissue compartments.

Conclusions: For the first time we have shown that CSF and brain tissue of MS patients contains partly identical repertoires of expanded B cell clones. This is especially remarkable since the time interval between brain biopsy and spinal tap was up to 5 years. Whereas some B cell clones maintained identical CDR3 region sequences, others showed signs of ongoing hypermutation. These findings strongly support that spinal fluid B cells at least partially represent disease relevant lymphocytes infiltrating MS brain tissue.

Disclosure: N Goebels has nothing to disclose.

Funding: Supported by Deutsche Forschungsgemeinschaft (SFB 571/A3), Giessen, Germany.

17
HIGH VULNERABILITY OF HUMAN NEURONS TO T CELL CYTOTOXICITY: A NEW MODEL TO EXPLAIN NEURODEGENERATION IN MULTIPLE SCLEROSIS

Giuliani F, Yong V
Clinical Neurosciences, University of Calgary, Calgary, Alberta, Canada

Background: MS is considered a T cell-mediated autoimmune disease of the CNS. MS lesions are characterized by infiltration of inflammatory cells, demyelination, axonal loss and neuronal degeneration. It has been reported by others that CD8+ T cells can induce the apoptosis of rodent neurons via an MHC-I mediated mechanism; MHC-I expression on neurons was induced by treatment with IFNγ and tetrodotoxin. Given that neurons are usually negative for MHC-I, these findings suggest that MHC-I expression on neurons may provide a novel approach to the treatment of MS.

Objectives: To determine whether activated human T cells can kill human neurons and to elucidate the mechanisms of this toxicity.

Methods: We used a co-culture system of human fetal neurons and T cells from allogeneic PBMC or syngeneic splenocytes. T cells were activated with an anti-CD3 antibody for 72h and then incubated with neurons. After, neurons were stained with mouse anti-MAP-2 antibody conjugated to Cy3. The number of surviving MAP-2 positive neurons was counted. In some experiments, neutralizing antibodies to defined antigens were introduced to the co-culture system to modify the killing.

Results: When activated T lymphocytes were added to neuronal cultures, they aggregated around neuronal elements and death to neurons occurred promptly. By 3h of co-culture, the number of MAP-2 positive neurons was reduced by 50% when compared to controls. Neuronal toxicity required the activation of T cells since unactivated T lymphocytes did not produce any death. Allogeneic or syngeneic activated T cells were equally deleterious to neurons. The mechanism of T cell mediated neuronal toxicity required cell-cell contact, and was attenuated by neutralizing antibodies to FasL, LFA-1 and CD40. Finally, no T cell cytotoxicity was evident on oligodendrocytes or astrocytes.

Conclusions: These data demonstrate that activated human T cells can kill human neurons in vitro without the apparent need for MHC-I. Toxicity is not a result of a graft-versus-host response as demonstrated using syngeneic co-culture. Furthermore, cytotoxicity is selective for neurons. We suggest that when T cells are activated, they enter the CNS to induce disruption of neural elements and cause neuronal death.

Disclosure: F Giuliani has nothing to disclose.

Funding: Supported by Alberta Heritage Foundation for Medical Research - Canadian Institutes of Health Research.
Abstract Body: There is good evidence that relapses in MS are the clinical counterpart of acute focal inflammation of the central nervous system whereas progression is that of chronic diffuse neurodegeneration. The classical view is to consider that MS is an organ-specific auto-immune disease, i.e. that inflammation is the cause of the neurodegeneration. The succession of relapses eventually leads to accumulation of disability and clinical progression could result from infraclinical relapses. A series of recent observations tend to challenge this classical concept. Interferons beta have well-known effects in MS. They lead to a 30% reduction in the relapse rate and to a more than 50% reduction in conventional MRI activity. Despite this strong effect on inflammation, the effect of interferons on disability is only marginal and possibly relapse-reduction driven. Administration of Campath-1H to MS patients results in a profound and prolonged lymphopenia, and the suppression of clinical and MRI activity. In spite of this, progression of clinical disability and cerebral atrophy still occurs. The relapse rate decreases dramatically during pregnancy, notably during the third trimester. By contrast, the three-month post-partum period is characterized by a 60% increase of the relapse rate. Despite these dramatic changes in the frequency of relapses, progression of disability goes on. Striking results have also come from the study of the natural history of MS in the Lyon MS Cohort. Progression of irreversible disability from the assignment of a score of 4 on the DSS Kurtzke scale to the assignment of a score of 6 or 7 is unaffected by the presence or the absence of a relapsing-remitting phase before the progressive phase of MS. The same observation is true regarding the presence or the absence of superimposed relapses during the progressive phase, either primary or secondary. All these observations give some credit to the fact that relapses do not essentially influence irreversible disability in the long term in MS. They are consistent with what has been shown at the individual level in the 70’s by performing serial quantitative neurological examinations over several years, and with what is currently emerging from early and serial structural brain MRI studies.

Disclosure: Christian Confaivreux has participated in meetings sponsored by pharmaceutical companies marketing treatments for multiple sclerosis and received grants from them. He has received fees and honoraria for his expertise and lectures from pharmaceutical companies marketing or developing programmes for the treatment of multiple sclerosis. The Department of Neurology A has received financial support for taking part in randomized, controlled trials from these pharmaceutical companies.

Funding: Biogen, Schering, Serono, Teva Pharma laboratories.

Session II Impact of Relapses on Disability: Natural History and Clinical Trials Data

19 CILIARY NEUROTROPHIC FACTOR ENHANCES MYELIN FORMATION: A NOVEL ROLE FOR CNTF AND CNTF-RELATED MOLECULES.

Bruno S1,2, Frederic N1, Marie Stephane A1, Bernard Z1, Catherine L1
1centre d’investigation clinique, hôpital de la Salpêtrière, Paris, France; 2Inserm U 495, Hôpital de la Salpêtrière, Paris, France

Background: In multiple sclerosis myelin repair is generally insufficient despite relative survival of oligodendrocytes within the plaques and recruitment of oligodendrocyte precursors. Promoting remyelination appears to be a crucial therapeutic challenge

Objectives: Using a newly developed enzymatic index of myelination, we screened different neurotrophic factors for their ability to enhance myelination.

Methods: Neurotrophic factors were added to the culture medium of myelinating cocultures between 11 and 25 days in vitro.

Results: Neurotrophins (NGF, NT-3, NT4/5, BDNF), GDNF related factors (GDNF, neurturin) and growth factors such as PDGF-AA, FGF-2, or insulin did not increase myelogenesis. In contrast, among factors belonging to the CNTF family, CNTF, LIF, cardiotrophin-1, and oncostatin M induced a strong pro-myelinating effect. We provide evidence that CNTF acts on oligodendrocytes by favoring their final maturation, and that this effect is mediated through the gp-130 receptor common to the CNTF family, and transduced through the janus kinase pathway.

Conclusions: Our results demonstrate a novel role for neurotrophic factors of the CNTF family, and raise the possibility that these factors might be of therapeutic interest to promote remyelination in multiple sclerosis.

Disclosure: S Bruno has nothing to disclose.

Funding: Supported by Inserm (institut national de la santé et de la recherche médicale), and ARSEP (association de recherche sur la sclérose en plaques).

20 THE ROLE OF EXACERBATIONS IN PERSISTENT IMPAIRMENT IN MS

Lublin F1, Cutter G2, Baier M3
1Corinne Goldsmith Dickinson Center for MS, Mount Sinai Medical Center, New York, New York, USA; 2Pythagoras, Inc., Reno, Nevada, USA; 3AMC Cancer Center; Denver, Colorado, USA

Abstract Body: Although it is intuitively obvious that repeated exacerbations may lead to step-wise worsening of neurological function in patients with MS, the actual role of exacerbations has not been quantified. For this study, we queried a database of placebo patients from several completed clinical trials of relapsing forms of MS to determine the effect of exacerbation on clinical course. In these trials, patients had periodic (usually q 3 month) assessments and also examinations at the time of exacerbation. Thus, we had a pre-exacerbation assessment of EDSS and Scripps score, intra-exacerbation scores and follow-up evaluations. We found that there was a mean worsening of 0.4 EDSS units. This finding persisted when patients were assessed more than 30 days after an exacerbation. Of those patients with exacerbations, 45% had residual impairment. The finding were similar for the Scripps score, with an average worsening of 2 points. The percent of patients with residual deficits increased with the length of time between the exacerbation and the follow-up visit, supporting the permanence of the effect. These results indicate that exacerbations in MS can leave residual deficit, in a step-wise fashion, and as such are an important therapeutic target. The results are consistent with the outcomes of clinical trials in RR MS.

Disclosure: F Lublin has nothing to disclose.

21 RELAPSES ARE NOT AN IMPORTANT CAUSE OF DISABILITY

Confaivreux C
1Neurology A, Hôpital Neurologique, Lyon, France; 2EDMUS Coordinating Center, Lyon, France

Abstract Body: There is good evidence that relapses in MS are the clinical counterpart of acute focal inflammation of the central nervous system whereas progression is that of chronic diffuse neurodegeneration. The classical view is to consider that MS is an organ-specific auto-immune disease, i.e. that inflammation is the cause of the neurodegeneration. The succession of relapses eventually leads to accumulation of disability and clinical progression could result from infraclinical relapses. A series of recent observations tends to challenge this classical concept. Interferons beta have well-known effects in MS. They lead to a 30% reduction in the relapse rate and to a more than 50% reduction in conventional MRI activity. Despite this strong effect on inflammation, the effect of interferons on disability is only marginal and possibly relapse-reduction driven. Administration of Campath-1H to MS patients results in a profound and prolonged lymphopenia, and the suppression of clinical and MRI activity. In spite of this, progression of clinical disability and cerebral atrophy still occurs. The relapse rate decreases dramatically during pregnancy, notably during the third trimester. By contrast, the three-month post-partum period is characterized by a 60% increase of the relapse rate. Despite these dramatic changes in the frequency of relapses, progression of disability goes on. Striking results have also come from the study of the natural history of MS in the Lyon MS Cohort. Progression of irreversible disability from the assignment of a score of 4 on the DSS Kurtzke scale to the assignment of a score of 6 or 7 is unaffected by the presence or the absence of a relapsing-remitting phase before the progressive phase of MS. The same observation is true regarding the presence or the absence of superimposed relapses during the progressive phase, either primary or secondary. All these observations give some credit to the fact that relapses do not essentially influence irreversible disability in the long term in MS. They are consistent with what has been shown at the individual level in the 70’s by performing serial quantitative neurological examinations over several years, and with what is currently emerging from early and serial structural brain MRI studies.

Disclosure: Christian Confaivreux has participated in meetings sponsored by pharmaceutical companies marketing treatments for multiple sclerosis and received grants from them. He has received fees and honoraria for his expertise and lectures from pharmaceutical companies marketing or developing programmes for the treatment of multiple sclerosis. The Department of Neurology A has received financial support for taking part in randomized, controlled trials from these pharmaceutical companies.

Funding: Biogen, Schering, Serono, Teva Pharma laboratories.

22 ONSET OF CLINICAL BENEFIT OF GLATIRAMER (COPAXONE®) ACETATE IN PATIENTS WITH RELAPSING REMITTING MULTIPLE SCLEROSIS (RRMS)

Johnson KP1, Brooks BR2, Ford CC1, Goodman A1, Guaraccia JB1, Lisak RP1, Myers LW1, Pantich HS2, Pratt AA1, Kachuck N1, Wolinsky JS1, and the Copolymer 1 MS Study Group
1University of Maryland, Baltimore, Maryland, USA; 2University of Wisconsin, Madison, Wisconsin, USA; 1University of New Mexico, Albuquerque, New Mexico, USA; 4University of Rochester, Rochester, New York, USA; 5Yale University, New Haven, Connecticut, USA; 6Wayne State University, Detroit, Michigan, USA; 7University of California, Los Angeles, California, USA; 8University of Vermont, Burlington, Vermont, USA; 9University of Pennsylvania, Philadelphia, Pennsylvania, USA; 10University of Southern California, Los Angeles, California, USA; 11University of Texas, Houston, Texas, USA

Background: RRMS is a chronic debilitating disease requiring early and sustained treatment. Various agents with distinct mechanisms of action and effects...
are currently available for the treatment of RRMS. The onset of action of these agents can be measured by their effect on several biologic, imaging, and clinical parameters.

Objectives: To evaluate the time course of early clinical effects of glatiramer acetate (GA) in decreasing the rate of relapse in patients with RRMS.

Methods: The effect of SC administration of GA 20 mg QD on decreasing the primary endpoint of relapse rate was evaluated in a randomized, placebo-controlled trial of 251 patients with RRMS. Regular quarterly and as needed clinic visits were performed to assess outcome and relapse events with a standardized criteria. Mean relapse rate was analyzed using ANCOVA, with tests for study-drug-by-center interaction and including the prior-defined covariates: sex, disease duration (years), prior 2 year relapse rate, and baseline EDSS to evaluate the time course of effect on relapse rate with GA treatment compared to placebo.

Results: The beneficial treatment effect of GA treatment compared to placebo on decreasing relapse rate at month 3 was 17%, p<0.001; month 6 was 27%, p<0.001; month 9 was 25%, p<0.047; month 12 was 27%, p<0.018; month 15 was 24%, p=0.034; month 18 was 27%, p=0.014; month 24 was 29%, p=0.007; and at approximately 30 months was 32%, p=0.002.

Conclusions: The clinical benefit of GA on decreasing relapse rate is seen relatively quickly with widening divergence at 6 months and statistically significant divergence seen at 9 months. Sustained and increasing benefit was observed throughout the 30 month follow-up.

Disclosure: KP Johnson is a consultant for Aventis Pharm., Amrad Corporation Ltd., Berlex Laboratories, Teva Pharm., Wyeth Ayerst. KP Johnson is on the Speakers Bureau for Berlex Laboratories and Teva Pharm. KP Johnson receives research support from Berlex Laboratories and Teva Pharm.

Funding: Supported by: Federal Food and Drug Administration Orphan Drug Program, National Multiple Sclerosis Society, and Teva Pharmaceutical Industries Ltd.

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EFFECT OF EARLY INTERFERON TREATMENT ON CONVERSION TO DEFINITE MULTIPLE SCLEROSIS: THE ETOMS STUDY 4-YEAR RESULTS.


- Clinica Neurologica, Ospedale S. Raffaele, Milan, Italy;
- Clinica Neurologica, Ospedale S. Raffaele, Milan, Italy;
- Clinica Neurologica, Ospedale S. Raffaele, Milan, Italy;
- Commissariat à l'Energie Atomique, CLRC, Saclay, France;
- Insitute for Neurology 2082, Rigshospitalet, Copenhagen, Denmark;
- Institute for Inflammation Research, Rigshospitalet, Copenhagen, Denmark

Background: The ETOMS Study showed a beneficial effect of IFN-beta-1a Rebif 22 mcg qw on conversion to clinically definite MS (CDMS) amongst patients with clinically isolated syndromes and MRI signs suggestive of MS.

Objectives: The study was extended to 4 years to assess longer term outcome.

Methods: 308 patients were randomized to double blind-treatment with IFN-beta-1a 22 mcg qw or placebo for 2 years, followed by a two-year extension with open-label treatment (IFN-beta-1a 22 mcg qw). Patients who reached the primary endpoint, conversion to CDMS, could stay in the study or withdraw and start best available therapy, at the physician's discretion.

Results: Fewer patients initially randomized to IFN converted to CDMS compared to those randomized to placebo, over the 4 years (67/154 (43.5%) vs 79/154 (51.3%). Although the difference at 2 years was statistically significant, it was not at 4 years (p=0.115). The time to conversion (40th percentile) was prolonged but not significantly (27.9 months IFN vs 16.3 placebo, p=0.114). Annualised relapse rate was not significantly different between treatment groups over four years. Of the 263 patients who entered year 3, 174 (66%) had not converted to CDMS before the end of year 2 (96 IFN, 78 placebo). Of these, 25 (14%) 15 (16%) randomised to IFN and 10 (13%) randomised to placebo converted to CDMS during years 3 or 4 (p=0.604). The global conversion rate dropped from 47% in the first two years to 14% in the second 2 years.

Conclusions: During the extension phase the conversion rate was lower in both groups, compared to the double blind phase. The benefit of low doses of IFN seem to be limited to the early phases of the disease.

Disclosure: Professor Comi has served as a Consultant to Serono. Funding: Sponsored by Serono International.

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NEUTRALIZING ANTIbODIES AGAINST INTERFERON (IFN)-BETA REDUCE THE CLINICAL EFFECT IN RELAPSING-REMITTING MULTIPLE SCLEROSIS


- Neurology 2082, Rigshospitalet, Copenhagen, Denmark;
- Danish Multiple Sclerosis Study Group, MS Registry, Copenhagen, Denmark;
- Institute for Inflammation Research, Rigshospitalet, Copenhagen, Denmark

Background: Therapy-induced neutralizing antibodies (NAB) against IFN-beta may interfere with treatment efficacy. Reported frequencies and clinical impact of anti-IFN-beta NAB vary depending on the IFN-beta preparation and administration.

Objectives: To evaluate the effect of different serum concentrations of NAB on the therapeutic efficacy in all IFN-beta treated patients in Denmark, using different sensitivities of the bioassay.

Methods: We measured NAB every 6 months for up to 48 months in 422 consecutive MS patients who from 1996 to 1998 started treatment with a commercial IFN-beta preparation. Measurements of NAB were performed in a blinded fashion, using anti-viral neutralization (A549/EMC) bioassays with high (3 LU/ml), medium (10 LU/ml), and low (100 LU/ml) sensitivity and employing different neutralizing capacities as cut-off value for definition of NAB-positive samples.

Results: NAB generally appeared within 12 months after start of treatment and faster with IFN-beta-1a than IFN-beta-1b. However, after 36 months of treatment we observed a significant reduction in the number of NAB-positive patients treated with IFN-beta-1b. The presence of NAB had a significant effect on the relapse rate. During NAB-positive periods, we found a significantly higher relapse rate with odds ratios from 1.42 to 1.55 (p<0.01) that were relatively independent of the sensitivity of the assay (medium or low) and cut-off values for neutralizing capacity between 50% and 50%. The time to first relapse in NAB-negative patients was significantly increased by 270 days in Kaplan-Meier analysis of the probability of remaining exacerbation-free (log rank test p=0.028). In this short study, we found a trend but no significant effect of the presence of NAB on disease progression measured on EDSS.

Conclusions: The results document that the occurrence of NAB reduces the clinical effect. The frequency of NAB against IFN-beta depends on the sensitivity of the neutralizing assay. In patients who are not doing well on IFN-beta therapy, the presence of NAB should raise the question about change of treatment.

Disclosure: PSS has received honoraria for lecturing and advisory councils, travel expenses for attending meetings, and financial support for his department from Biogen, Schering and Serono.

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THE SYLVIA LAWRY CENTRE FOR MULTIPLE SCLEROSIS RESEARCH (SLCMSR): BACKGROUND AND PROGRESS REPORT

Noseworthy JH and SLCMSR Staff, Scientific Oversight Committee and Working Groups

Neurology, Mayo Clinic, Rochester, Minnesota, USA

Background: The SLCMSR was founded in 2001 at the Technical University of Munich following an international competition. The centre is sponsored by the MS International Federation, multiple national MS societies and private
Session III
Inflammation, Demyelination and Axonal Loss: Insights From Imaging

26
RELATIONSHIP BETWEEN CONTRAST ENHANCING LESIONS AND AXONAL LOSS
Frank JA
LDRR/NHI, National Institutes of Health, Bethesda, Maryland, USA

Abstract Body: Contrast enhancing lesions (CEL) are a marker of acute inflammation and have provided an understanding of the pathophysiology of multiple sclerosis (MS). CEL have also been used as outcome measures to monitor MS activity, either natural or modified by experimental treatments and increases in CEL are correlated to clinical exacerbations. The relationship between CEL and axonal loss or permanent tissue damage is not completely elucidated, as it is apparent that there is continued tissue damage and atrophy that occurs independent of detected inflammation by MRI. Serial monthly MRI studies have shown a modest relationship to T1 “Black Holes” (i.e., demyelination, axonal loss, gliosis and possibly remyelination) and relatively weak correlation to the progression of cerebral atrophy over short term (i.e., < 2 years). However, long-term follow-up suggests that MS patients with increasing numbers of CEL have a greater degree of atrophy at >8 years. Therefore it is necessary to explore the natural history of CEL, the progression and relationship of these lesions to T1 hypointensities over long follow-up periods and determine if there is a relationship between CEL and exacerbations or clinical outcome with time. Lastly, the long term (i.e., >8 years) relationship CEL and cerebral atrophy (a cumulative measure of both microscopic and macroscopic disease activity) will be discussed.

Disclosure: J Frank has nothing to disclose.

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IN VIVO MONITORING OF AXONS AND MYELIN IN MULTIPLE SCLEROSIS
Caramanos Z, Arnold DL
McGill University, Montreal, Quebec, Canada

Abstract Body: Introduction Although conventional MR techniques allow us to image MS lesions with great sensitivity, they are not capable of fully characterizing and quantifying the extent of damage to specific cells and tissue types. Recently-developed MR techniques are, however, better suited for such a role. These techniques include: (i) magnetization transfer imaging (MTI), (ii) short T2 imaging, (iii) proton magnetic resonance spectroscopy (MRS), (iv) diffusion weighted imaging (DWI), (v) diffusion tensor imaging (DTI), and (vi) MR-based measures of brain atrophy. Imaging Myelin Integrity MTI provides contrast based on the exchange of magnetization between water bound to macromolecules, which is normally MR-invisible, and bulk water. In cerebral white matter, MTI is relatively specific for myelin because most of the exchange originates from myelin. Quantitative MTI is technically more demanding than standard MTI but has the advantage that it is not affected by edema. Short T2 imaging quantifies water trapped within the layers of the myelin sheath and appears to specifically measure the amount of myelin (both normal and abnormal) that is present. Increases in MRS-measured choline, a metabolite associated with membrane turnover, and MRS-measured mobile lipids are associated with active myelin breakdown. Imaging Axonal Integrity MRS measurement of the neuronal marker, N-acetylaspartate (NA), provides a specific surrogate of axonal integrity but at much lower spatial resolution than that available from water-based imaging. Imaging Structural Integrity DWI and DTI provide contrast based on the structural integrity of both myelin and axons. MR-based measures of brain atrophy show loss of tissue that includes myelin and axons; importantly, however, any such observed volume loss is attenuated by any co-occurring gliosis or expansion of extracellular spaces. Findings in MS When appropriately applied, the above methods show abnormalities, not only of MR-visible lesions, but also of normal-appearing white and grey matter. Furthermore, changes in MT, DWI, and MRS may precede the development of MR-visible lesions. The correlations between these different surrogates, as well as between these measures and disability, indicate that they measure aspects of MS pathology that are related but not necessarily the same.

Disclosure: D Arnold has nothing to disclose.

Funding: Supported by: CHF and MSSC

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CAN WE IMAGE REMYELINATION?
Barkhof F
VU Medical Centre, Amsterdam, Netherlands

Abstract Body: Little is known about the imaging appearance of remyelination. While positron emission tomography (PET) may have the required sensitivity to visualize remyelination, no tracer is available for in vivo imaging. Magnetic resonance (MR) imaging is very sensitive to alterations in brain composition, but lacks histopathological specificity. Remyelinated lesions have increased signal on T2-weighted images, and a mildly decreased signal on T1-weighted images. Serial images reveal a decrease in T1-signal intensity after cessation of enhancement, suggestive, but certainly not specific for, remyelination. For the magnetic transfer ratio (MTR), a similar temporal profile has been linked in animal models to the occurrence of remyelination.

Disclosure: F Barkhof has nothing to disclose.

Funding: Supported by: CHR and MSSC
WHAT IS NORMAL-APPEARING WHITE MATTER?

Grossman R
University of California, San Francisco, California, USA

Abstract Body: It is clear that the normal appearing white matter (NAWM) on conventional magnetic resonance (MR) imaging is not “normal” at all. The term NAWM is misleading. What is normal at 0.3 tesla is not normal at 1.5 tesla. What is normal at 1.5 tesla is clearly not normal at 7 or 8 tesla. Images at such high field strengths clearly reveal tiny MS lesions. What is normal by imaging is not normal by diffusion or spectroscopy. It is just as important to distinguish what the abnormalities represent as to detect them. As MR methodology becomes more powerful and resolving power improves there will be much less discussion of NAWM and much more characterization of what is abnormal. Biophysical measures including magnetization transfer and diffusion clearly reveal abnormalities that cannot be detected on conventional images. Higher field imaging (3 T or >) will enable better delineation of these abnormalities. Magnetic resonance spectroscopy can detect metabolic equivalents of structural abnormalities including low levels of N-acetyl aspartate and elevated levels of choline in what is now considered NAWM. Abnormalities in what is today considered NAWM appear to be diffuse in nature and contribute to the neurological deficit. They are potentially the most interesting aspect of the MS lesion for this may represent an early reversible component of the disease. How we quantitate and characterize such lesions will allow us to detect treatment effects and potentially provide prognostic information in rapid and accurate fashion. Our research goals must be to exploit all MR techniques that facilitate improving our sensitivity and specificity with respect to the so-called “NAWM”.

Disclosure: R Grossman has nothing to disclose.

Funding: NIH grants - R37-NS29029, R01-NS39135, 1 RO1-NS371739

BRAIN VOLUME CHANGES IN PATIENTS AT PRESENTATION WITH SUSPECTED MULTIPLE SCLEROSIS: RESULTS FROM THE ETOMS STUDY

1Clinical Trials Unit, Department of Neurology, Scientific Institute and University HSR, 2Neuroimaging Research Unit, Department of Neuroscience, Scientific Institute and University HSR, Milan, Italy; 3University of Siena, Siena, Italy; 4University of Oxford, Oxford, UK, United Kingdom; 5The ETOMS Study Group, Nijmegen, Netherlands

Background: Two phase III, double-blind, placebo-controlled trials of patients at presentation with suspected multiple sclerosis (SMS) have been demonstrated that interferon beta-1a is effective in delaying the onset of a second relapse leading to a diagnosis of clinically definite MS. They also showed positive treatment effect on MRI-derived metrics.

Objectives: This study reports the results of normalized brain volume (NBV) measurements from patients at presentation with SMS recruited in the ETOMS trial.

Methods: Of the original 309 patients, electronic MRI data for NBV assessment were available for 131 patients randomized to receive 22mg of interferon beta-1a (Rebif, Ares Serono) subcutaneously once a week for two years. The mean percentage changes of NBV over the 24 month study period was significantly higher for placebo (-1.6%) than for treated (-1.8%) patients (p<0.001). Over the entire study period, significant correlations were found between the NBV percentage change and the number of enhancing lesions (r=-0.18, p=0.006) and the number of new T2 lesions (r=-0.26, p=0.001). The number of enhancing (r=-0.21, p=0.003) and new T2 (r=0.29, p<0.001) lesions formed during the first year correlated significantly with brain volume changes during the second year of the study.

Conclusions: The rate of brain tissue loss in the SMS patients is close to that reported in patients with more advanced disease and an early treatment with interferon beta-1a is able to reduce the rate of brain tissue loss in these patients.

Disclosure: G Comi has nothing to disclose.

Funding: The ETOMS Study was supported by Serono Pharma.

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PREDICTIVE VALUE OF INFARCTENTIAL LESIONS IN PATIENTS WITH CLINICALLY ISOLATED SYNDROMES FOR LONG TERM DISABILITY

Minneboo A1, Barkhof F*, Polman CHP, Uitdehaag B*, Knol D*, Casteljns JA*
1Radiology, MR Center for MS Research, VU Medical Center, Amsterdam, Noord-Holland, Netherlands; 2neurology, Center for MS Research, VU Medical Center, Amsterdam, Noord-Holland, Netherlands; 3Clinical Epidemiology and Biostatistics, VU Medical Center, Amsterdam, Noord-Holland, Netherlands

Background: Abnormalities on baseline brain MRI in CIS are known to predict outcome in terms of conversion to clinically definite multiple sclerosis (CDMS) and disability. However no long-term follow-up data exist on the role of enhanced lesions and “black holes”.

Objectives: To assess the long-term predictive value of baseline MRI parameters including location of the lesions, gadolinium-enhancement and “black holes” in patients presenting with a clinically isolated syndrome (CIS) for mild disability as defined by EDSS≤3.

Methods: After a median follow-up period of 8.7 years, hospital charts of 42 patients presenting with CIS were reviewed and assessed for date of conversion to CDMS, date at which EDSS 3 or 6 was reached and EDSS at last follow-up. MRI parameters were dichotomized taking maximum accuracy and subsequently hazard ratios were calculated.

Results: Conversion rate to CDMS was observed in 26 patients (62%) of which 14 patients progressed to EDSS≤3. Infarctential lesions are the best predictors for long-term disability (hazard ratio 6.3). Predictive value of enhanced lesions and “black holes” seems limited.

Conclusions: Infarctational lesions predict long-term disability and may help to identify patients at high risk for progressive disease.

Disclosure: A Minneboo has nothing to disclose.

Funding: A Minneboo is supported by a grant (98-348 MS) of the Dutch Foundation for the Support of MS Research.

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A 48-MONTH LONGITUDINAL STUDY ON THE RELATIONSHIP BETWEEN THE DURATION OF THE ENHANCEMENT IN AN ACTIVE LESION AND THE DURATION OF A BLACK HOLE IN MULTIPLE SCLEROSIS

Bagnato F*, Jeffries NP, Ohayon P*, Stone R*, Frank JA*, McFarland HF*
1Neuroimmunology Branch NINDS, NIH, Bethesda, Maryland, USA; 2Bioscience Branch NINDS, NIH, Bethesda, Maryland, USA; 3Laboratory of Diagnostic Radiology Research, NIH, Bethesda, Maryland, USA

Background: A Black Hole (BH) is a possible evolution of a contrast-enhancing lesion (CEL) in Multiple Sclerosis (MS), and when they persist BHs are believed to represent a marker of axonal loss. However, why some CEL are more likely to form a persistent BH over time is not completely understood. The duration of the inflammatory process is a possible explanation for this observation.

Objectives: To evaluate the influence of duration in time of a CEL on the duration of a BH over the natural history of MS patients followed monthly for four years.

Methods: Eight MS patients (male/female: 7/1, mean±SD age 35±2.59, EDSS 2.4±1.4, MS duration 3±5.0) had 48 monthly MRIs. The number of CEL and of BH in the corresponding un-enhanced scan was evaluated. A BH was defined as any hypointense region visible on the T1-Weighted Images (WI)
with high signal intensity on the T2-WI and without enhancement in the corres-
pondent post-contrast scans. Only BHs with a previously identified CEL were
considered for the analysis. Cox Proportion Hazards models were used to eval-
uate the effect of enhancing time on BH duration.
Results: Out of 878 new CEL, 158 turned into a BH (17.9%). The mean dura-
tion in time of these BHs was 9.3±9.7 months. The results of Cox Regression
Analysis showed that among those CEL that formed a BH the increase in the
duration of the enhancement significantly influenced the duration of the corre-
sponding BHs (p=0.04), with each additional month of enhancement leading to
a 22% reduction in the rate at which BHs disappear.
Conclusions: The enhancement duration is an important factor in BH duration.
The longer the duration of the enhancement in a given active lesion, the higher
is the chance to persist as a BH over time, thus accumulating a permanent dam-
age and disability in MS.
Disclosure: F Bagnato has nothing to disclose.

Session IV
The Blood Brain Barrier as a Target for Treatment
33 ADHESION MOLECULES AND THEIR ROLE IN PATHOGENESIS
Antel JP, Biernacki K, Seguin R, Prat A
Neurology and Neurosurgery, McGill University, Montreal, Quebec, Canada

Abstract Body: Cellular immune trafficking across the blood brain barrier
(BBB) involves a series of molecular interactions between such cells and the
cells and extra cellular matrix (ECM) that comprise the BBB. These interac-
tions include the processes of cell-cell adhesion; chemotraction, and ECM
degradation by matrix metalloproteinases (MMPs). With specific regard to
adhesion molecules, expression of these molecules is up-regulated on lympho-
cytes and monocytes when such cells are activated, as is observed to occur in
corent with active phases of multiple sclerosis (MS). In situ studies of central
nervous system (CNS) microvesels derived from MS cases, demonstrate up-
regulation of the ligands for a number of adhesion molecules on endothelial
cells (ECs) when compared to ECs from non-inflammatory control cases. To
model the role of cell-cell adhesion in cell trafficking across the BBB, we have
examined the interaction of lymphocytes and monocytes derived from the
peripheral blood of MS patients and controls with human brain (HB)ECs
derived from non-inflammatory surgical tissue specimens. These HBECs con-
stitutively express moderate levels of ICAM-1 but only very low levels of
VCAM-1. Expression of both of these adhesion molecules is up-regulated when
the HBECs are exposed to supernatants from Th1 cytokine producing
CD4 T cell lines. Th2 cytokine producing cell lines neither up-regulate nor
inhibit adhesion molecule expression. Using a Boyden chamber assay system,
we can demonstrate that both lymphocyte and monocyte migration across a
barrier of HBECs grown on a fibronectin matrix can be inhibited by antibodies
directed at ICAM-1 but not VCAM-1. Antibodies directed at the VCAM-1 lig-
and VLA-4 do inhibit migration, implicating VLA-4 binding to an alternate
ligand (Connecting Segment (CS)-1 fragment of fibronectin) as the functional
event. Adhesion molecules remain targets for therapeutic intervention in MS
(eg anti-VLA-4 antibodies). Neither Copaxone nor interferon β (IFNβ)
directly modulate adhesion molecule expression on HBECs. However, IFNβ
induced lymphocytes induce VCAM on the HBECs with a subsequent release
of soluble VCAM-1 (sVCAM) ( Kalabrese et al 2001). sVCAM binding to its
ligand VLA-4 would provide a means to down-regulate trans-endothelial
migration.
Disclosure: J Antel has received honoraria from TEVA Marion, Schering,
Biogen and Serona
Funding: Supported by Multiple Sclerosis Society of Canada

CHEMOKINES AND CHEMOKINE RECEPTORS: WHAT’S THE ATTACHMENT
Ransohoff RM
*Neurology, Cleveland Clinic Foundation, Cleveland, Ohio, USA; †Departments of Neurosciences and Neurology, Cleveland Clinic
Foundation, Cleveland, Ohio, USA

Abstract Body: Chemokines are small peptides that govern leukocyte trafficking
and activation. There is a substantial and growing literature concerning their
biological functions in development, inflammation and degeneration of the
nervous system. The core hypothesis of our research is that chemokines and
their receptors are significantly involved in leukocyte invasion, differentiation,
activation, tissue destruction and repair in the nervous system. Furthermore,
resident neuroepithelial cells both make and respond to chemokines. To address
this hypothesis and identify the molecular targets for therapeutic endeavors, we
examine chemokine and chemokine receptor expression and function. These
studies comprise material from patients with neurological disorders, disease
models in mice, and tissue culture studies. We make extensive use of trans-
genic and knockout mice to clarify how chemokines exert their remarkably
specific effects in vivo in the face of apparent functional redundancy in vitro.
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hoff RM. Absence of monocyte chemoattractant protein 1 in mice leads to
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1 immune response in experimental autoimmune encephalomyelitis. J Exp
Kivisakk P, Trebst C, Eckstein DJ, Kerza-Kwiatecki AP, Ransohoff RM. Chemokine-based therapies for MS: how do we get there from here? Trends
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cytes accumulate in the central nervous system of patients with multiple sclero-
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N, Griffin JW, Toyka K, Ransohoff RM, Hartung H-P. Chemokines and chemokine
receptors in inflammatory demyelinating neuropathies: a central role for IP-10.
Brain 2002; 125(4): 823-834.
Disclosure: RM Ransohoff is a member of the Scientific Advisory Board of
Chemocentryx, San Carlos, CA and has received honoraria for scientific con-
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Millennium.
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Fogarty International Center (FIRCA TW00724) and the NCI (PO1 CA66220).

MATRIX METALLOPROTEINASES IN MS V. WEE YONG, DEPARTMENTS
OF ONCOLOGY & CLINICAL NEUROSCIENCES, UNIVERSITY OF
CALGARY
Yong V
Oncology & Clinical Neurosciences, University of Calgary, Calgary,
Alberta, Canada

Abstract Body: Matrix metalloproteinases (MMPs) are implicated in MS
This presentation will review the evidence that MMPs have a role in the pathol-
gy and the progression of disease in MS. It will also address which MMP fam-
ily members (of 26) may be important to consider as having important roles in
MS. Evidence will be presented that in experimental autoimmune encephalomyelitis (EAE), an animal model of MS, specific MMP members are
expressed at the initiation of clinical disease while others become prominent
during the evolution of the disease. The mechanisms by which MMPs may contribute to CNS pathology will be discussed. It is important to appreciate that some MMP members may be expressed following injury in an attempt to limit destruction or to attempt repair. In this regard, it is worthy of consideration that during myelogenesis, the extension of oligodendrocyte processes through a CNS matrix may require MMPs to remodel the extracellular environment. Evidence will be presented that MMP-9 plays a role in process formation by oligodendrocytes and that there is deficient remyelination in MMP-9 null mice following lysolecithin induced demyelination of the mouse spinal cord. In summary, MMPs have important roles in the pathology of MS. Inhibitors of MMP activity are appropriate therapeutic agents to consider in the disease. Nonetheless, caution is advised that specific MMPs, under particular circumstances, may have reparative roles.

Disclosure: V.W. Yong has received honoraria from Teva Neuroscience, Serono, and Berlex Laboratories. V.W. Yong has received research grants from Teva Neuroscience and Berlex Laboratories.

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CLINICAL TRIALS OF AGENTS TARGETING THE BLOOD BRAIN BARRIER: SUCCESSES AND FAILURES

Miller D
NMR Research Unit, Institute of Neurology, London, United Kingdom

Abstract Body: Several lines of evidence implicate blood brain barrier (BBB) abnormality as an important component in the development of multiple sclerosis lesions. New lesions exhibit BBB breakdown and perivascular lymphocytic infiltrates. The location of lesions around cerebral venules suggests that BBB breakdown may have a key role in lesion genesis. Serial MRI studies using gadolinium-chelate contrast agents have demonstrated BBB breakdown as a consistent feature of new lesions in relapse onset MS. However, new or enlarging lesions may develop without BBB breakdown in primary progressive MS and possibly in other forms of the disease. Diffuse abnormalities of the normal appearing white matter also occur, and the relationship of these to BBB breakdown is uncertain. It is however, likely that BBB breakdown is more extensive than the regions of gadolinium enhancement that are detectable to the eye. There is good evidence for low grade leakage in chronic lesions, and it may also exist in normal appearing white matter. Using gadolinium enhanced MIR, there is a relationship of BBB leakage with relapses, but not with progressive MS. Many immunosuppressive and immunomodulatory treatments have been shown to suppress new areas of focal BBB leakage. High dose intravenous steroids have a similar but transient effect on pre-existing and new enhancing lesions. Recently, the monoclonal anti-adhesion molecule antibody, anti-VLA4 (natalizumab), has shown dramatic effects in reducing by 90% the frequency of new areas of BBB leakage, but unlike intravenous steroids, does not effect the existing areas of leakage. Natalizumab treated patients also experienced a reduced relapse rate by 50% in the 6 month placebo controlled exploratory trial, and exhibited increased well being compared to those on placebo. Two year studies of this agent are now underway to evaluate its long term safety and efficacy, and in particular the effect on disability. Such long term studies are important, in view of the uncertain relationship between focal BBB changes and progressive disability.

Disclosure: D Miller has received grants from Elan and Biogen for MRI analysis in clinical trials of Natalizumab
Funding: MS Society of Great Britain and Northern Ireland.

Friday, September 20, 2002

Keynote Address

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NEURAL STEM CELLS TO REBUILD THE DISEASED BRAIN: HOW REALISTIC IS THIS APPROACH?

Snyder E
Harvard University, Boston, Massachusetts, USA

ABSTRACT NOT AVAILABLE FOR PUBLICATION

Session V

Neuroprotection

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MOLECULAR BASIS OF LIMITED REMYELINATION IN MULTIPLE SCLEROSIS

Raine CS, John G, Brosnan CF
Pathology (Neuropathology), Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, New York, USA

Abstract Body: Although CNS remyelination in MS is well-known, that its occurrence and extent is not more widespread has perplexed investigators for many years. Among frequently proposed causes of incomplete remyelination in MS, scarring astroglia, inflammation, macrophage activity and serum factors are the most studied, but to date, no satisfactory explanation has emerged. During an investigation of gene expression by astrocytes in vitro following activation by the inflammatory cytokine, TGFβ1 (John et al., submitted), induced expression was noted of Jagged1, a transmembrane protein shown to suppress the differentiation of oligodendrocytes, through activation of Notch1. Since TGFβ1 expression and reactive astrocytes are common features of active MS, we conducted a study on MS lesions displaying demyelination and remyelination. By immunocytochemistry, a broad zone of reactive, hypertrophic astrocytes surrounded acute and chronic active lesions lacking remyelination, and these cells displayed decreasing expression of Jagged1 away from the lesion. These Jagged1 positive astrocytes were invariably associated with a population of small rounded cells revealed by immunocytochemistry to be oligodendrocytes. Using double-label immunocytochemistry, we found that astrocytes and oligodendrocytes expressed PDGFRα, O4 and some NG2, indicative of an immature, precursor phenotype. These oligodendrocytes expressed Notch1 and Hes5, a downstream target of Notch signaling. Gene expression microarray studies of astrocytes and oligodendrocytes showed a marked reduction in a number of molecules involved in the Notch1/Jagged1 signaling pathway. These data provide evidence for involvement of this novel molecular pathway in the limited remyelination process in MS lesions. The role of this molecular pathway in other MS lesions, both acute and chronic, remains to be determined.

Disclosure: C Raine has nothing to disclose.
Funding: Supported by NINDS.
COMPLEMENT: DUAL ROLE IN INJURY AND PROTECTION

Shin ML, Rus H

*Pathology, University of Maryland School of Medicine, Baltimore, Maryland, USA; **Pathology, University of Maryland School of Medicine, Baltimore, Maryland, USA

Abstract Body: We showed that the membrane attack complex C5b-9 causes myelin vesiculation and demyelination of explant cultures. Myelin lacking complement inhibitory CD55 allows C5b-9 assembly 4.5-fold more than CD55-expressing membranes, like oligodendrocyte (OLG). Myelin damage, therefore, may be induced in vivo by C5b-9 without OLG death. Myelin also activates complement, which is amplified >50-fold by anti-myelin Ab. Thus, C5b-9-dependent demyelination can be more prevalent in vivo in the presence of Ab. This hypothesis is supported by recent works by Mead and by Tran, using EAE in C6-deficient rats. This detrimental role on myelin contrasts with the OLG survival actually enhanced by sublytic C5b-9. In defined medium, OLG progenitors differentiate and undergo apoptosis. We found that C5b-9 rescues OLG from serum deprivation- and TNF-induced death, by regulating apoptotic and pro-apoptotic genes revealed, with a few exception, that C5(-) mice clustered separately from C5(+) mice. Together, our data support the beneficial role of C5b-9 in demyelinating disorders by down-regulating apoptotic gene expression and promoting OLG survival and remyelination.

Disclosure: M Shin has nothing to disclose.
Funding: Supported by NIH grants; NINDS (NS15662, NS36231, NS42011 to M Shin). Funding: Supported by NIH grants; NINDS (NS15662, NS36231, NS42011 to M Shin).

Background: Not much is known about the genetic factors determining disease progression and outcome in multiple sclerosis (MS). Ciliary neurotrophic factor (CNTF) is a survival factor that also promotes differentiation of oligodendrocytes. 3% homozygous deletions in the CNTF gene exist in the European population.

Objectives: We compared clinical course and neuropathological features in CNTF-knockout and wild-type C57BL/6 mice using experimental autoimmune encephalomyelitis (EAE) as a model for MS.

Methods: EAE was induced by immunization with MOG peptide 35-55. Animals were clinically scored and weighed on a daily basis. At various time points spinal cord, brain and optic nerves were analyzed by histo- and immunomunocytochemistry. For quantification, coded sections were counted by blinded observers. To investigate the peripheral priming and induction of the immune response, lymph node proliferation assays were performed.

Results: CNTF deficient mice exhibited a significantly earlier onset of disease (p<0.005, 80 mice per group) and showed enhanced disability during the course of disease until day 100 p.i. (p=0.002). Histopathologic changes were mostly confined to the anterior and lateral columns of the spinal cord with prominent vacuolation and myelin destruction. In early and later stages of the disease, CNTF deficiency was associated with histopathological features indicative of oligodendrocyte cell death. Increased apoptosis of oligodendrocytes was seen on semithin sections and by TUNEL assay. In parallel proliferation of oligodendrocyte precursor cells was decreased by 60% in CNTF knockout mice. The severe vacuolar pathology in CNTF deficient mice could be prevented by treatment with an antisera against TNF-alpha. No significant differences in proliferative response to MOG in primed lymphocytes or in numbers of infiltrating T-cells and macrophages could be observed between CNTF-knockout and wild-type mice.

Conclusions: These results underscore the critical role of CNTF for oligodendrocyte integrity in the inflamed CNS. CNTF may serve as a modulator of lesion severity in MS. Back-to-back-submission with ID13400.

Disclosure: R Linker has nothing to disclose.
Funding: Supported by DFG SFB 581 TP A1.

IS NEUROPROTECTION A REALISTIC OPTION IN MS?

Hohlfeld R

Institute for Clinical Neuroimmunology, University of Munich, Bavaria, Germany

Abstract Body: Recent evidence suggests that inflammatory reactions in the CNS can have beneficial and even neuroprotective effects. Intriguingly, immune cells are capable of producing neuroprotective molecules of the neurotrophin family. The concept of “neuroprotective immunity” has profound consequences for the pathogenesis and treatment of neuroinflammatory diseases like multiple sclerosis (MS), and is also important for neurodegenerative disorders, in which inflammatory reactions often occur. In MS lesions immune cells produce brain-derived neurotrophic factor (BDNF), whereas neurons and astrocytes express the appropriate tyrosine kinase receptor TrkB. This observation together with functional evidence for the neuroprotective effects of immune cells support the concept of “neuroprotective immunity”. In addition a protective role of endogenous CNTF has recently been demonstrated in EAE by comparing the severity of EAE in wild-type and CNTF-knockout mice. Several neurotrophic and growth factors have been employed for the treatment of EAE. Pilot studies clearly demonstrated that growth factors like NGF, IGF-1, or GGF-2 can have beneficial effects in EAE. However, some of these effects seem to be primarily mediated by immunomodulation rather than primary CNS protection or repair. These observations have important implications for the treatment of multiple sclerosis.

Disclosure: R Hohlfeld has nothing to disclose.

INTERFERON-BETA GENE THERAPY FOR CENTRAL NERVOUS SYSTEM DISEASE USING BONE MARROW CELLS AS A DELIVERY SYSTEM

Dhib-Jalbut St, Makar TK, Wilt S, Dong Z, Fishman Pa, Mouradian Md

*Neurology, University of Maryland; **Neurology, Department of Veterans Affairs, Baltimore, Maryland, USA; ***Cell Biology, University of Texas, Houston, Texas, USA; ****Genetic Pharmacology Unit, NIH, Bethesda, Maryland, USA

Background: The peripheral delivery of Interferon-beta (IFNb) for the treatment of central nervous system (CNS) diseases is only partially effective.
because of the blood-brain barrier. This is true for multiple sclerosis where IFNb does not reach inflammation sites.

**Objectives:** To circumvent this problem, we evaluated the feasibility of genetically altering bone marrow cells ex-vivo, and using them as vehicles to transfer the IFNb cDNA into the mouse CNS.

**Methods:** An IFNb retroviral expression vector (pLXS-IFNb) was used to stably transfect PA317 cells (producer cells). The supernatant from these producer cells, which contain IFNb-expressing provirus, were used to infect mouse bone marrow cells. IFNb-transduced marrow cells were then transplanted into irradiated SJL mice via intravenous injection. IFNb expression was subsequently examined in the CNS by RT-PCR and immunocytochemistry.

**Results:** IFNb-engineered marrow cells accessed the CNS and expressed IFNb mRNA and protein. Marrow cells transduced with a control neomycin vector entered the brain and expressed the neomycin but not IFNb gene. In the CNS, IFNb delivered by marrow cells induced the mRNA expression of 2-5 oligoadenylate synthetase (2-5 OAS) indicating biological activity.

**Conclusions:** Our findings demonstrating that bone marrow cells can serve as a delivery system for IFNb cDNA into the CNS could have implications for the treatment of neurological disorders such as multiple sclerosis, viral encephalitis and brain tumors. Preliminary therapeutic results in experimental allergic encephalomyelitis will be presented.

**Disclosure:** S Dhib-Jalbut has nothing to disclose.

**Funding:** Supported by grants from the National Multiple Sclerosis Society and Department of Veterans’ Affairs.

### Session VI Hot Topics in Neuroimmunology

#### 44 CYTOKINE REGULATION IN MULTIPLE SCLEROSIS

**Weiner H, Khoury SJ**

**Harvard University, Boston, Massachusetts, USA**

**Abstract Body:** MS is an inflammatory disease of the CNS presumed to be Th1 type cell mediated autoimmune disease. There is increased IL-12 by intracytoplasmic staining and anti-CD3 stimulation and IL-12 is increased more in progressive than relapsing-remitting (RR) disease MS. Increased IL-12 is linked to increased IFN-g and the interaction between T cells and APCs via CD40-CD40L interactions. There are twice as many IL-12 secreting cells in the peripheral blood if there is Gadolinium enhancement on MRI. Cyclophosphamide decreases IL-12 in MS which is related to clinical response to therapy. Patients with elevated IL-12 may not respond as well to IFN-beta. IL-18 is increased in RR and progressive MS. In controls and RR MS neutralizing anti-IL-12 and anti-IL-18 alone equally suppressed IFN-g production whereas in progressive MS, maximum suppression was only observed when neutralizing anti-IL-12 and anti-IL-18 were given together, suggesting that in progressive MS, IL-12 and IL-18 function in a non-linked manner to induce IFN-g. Elevated IL-18 production was also dependent on CD40-CD40L interactions and IL-18 levels correlated with disease duration in progressive MS. We found a defect in regulation of both IL-12 and IFN-g by endogenous IL-10 in progressive MS which could contribute to the transition of MS from the relapsing to the progressive stage. dendritic cells also may contribute to the cytokine milieu in MS and the increased Th1 milieu in progressive MS as dendritic cells from progressive MS are polarized in a Th1 type pattern. INF-g levels are linked to MRI measures of disease activity and polarization of dendritic cells. Th1 type chemokine receptor expression (CXC3 and CCR5) is increased in MS and to a greater extent in progressive MS. Taken together, a predominant Th1 type cytokine milieu exists in MS that is linked to clinical and MRI measures and is more pronounced in progressive MS. Currently used immunomodulatory drugs appear to act in part by decreasing this Th1 polarization and further development of treatments that decrease Th1 and increase the Th2 and Th3 (TGF-b) cytokine milieu are likely to benefit MS. Increased immune dysfunction in progressive MS must be reconciled with the disease becoming more degenerative progressive MS and less responsive to immunomodulatory therapy.

**Disclosure:** H Weiner is a consultant to and a speaker for Teva, Biogen, Immune.  
**Funding:** Supported by the NIH, Multiple Sclerosis Society, Nancy Davis Foundation.

#### 45 ARE SPECIFIC IMMUNOTHERAPIES AN OPTION FOR MS?

**Martin R**

**Neuroimmunology Branch, NINDS, National Institutes of Health, Bethesda, Maryland, USA**

**Abstract Body:** Specific intervention with the T cell-mediated autoimmune process in multiple sclerosis (MS) and other autoimmune diseases has long been an attractive goal. Such therapies require a sound understanding of the
specificity/ies of pathogenic T cells and the development of effective therapeutic strategies that allow to selectively target these cell populations. Both in the animal model of MS, experimental autoimmune encephalomyelitis (EAE), there is good evidence that certain myelin proteins including myelin basic protein (MBP) have pathogenic potential in EAE and are immunogenic in MS patients in the context of disease-associated HLA-DR2- and other DR alleles. In EAE, the definition of individual encephalitogenic peptides served as a basis for establishing various specific immunotherapies. These included the induction of antigen-specific apoptosis, anergy induction, T cell receptor (TCR) peptide vaccination, T cell vaccination, oral tolerization, and the induction of bystander suppression via altered peptide ligands (APL), but also other approaches. A few of the above therapies are also in clinical testing in MS or have already been explored in the past. So far, the evidence for efficacy and/or safety of specific immunotherapies in MS is sparse. Oral tolerization was not effective in a large phase III trial, an APL peptide based on MBP (83-99) raised safety concerns, to give only two examples. The current state and future perspectives of specific immunomodulation in MS will be discussed.

Disclosure: Roland Martin has a consulting agreement with Teva Pharmaceuticals
Funding: Research efforts describing the testing of an altered peptide ligand in MS have been supported by a collaborative research and development agreement between NIH and Neocure and Novartis.

TCR PEPTIDE THERAPY IN AUTOIMMUNE DISEASE
Vandenbark AA
*Neuroimmunology, Portland VA Medical Center, Portland, Oregon, USA; 1Neurology and Microbiology and Immunology, Oregon Health and Science University, Portland, Oregon, USA

Abstract Body: Inflammatory Th1 cells reacting to myelin antigens likely contribute to the pathogenesis of diseases such as MS, rheumatoid arthritis (RA), and psoriasis. One regulatory mechanism that may be useful for treating autoimmune diseases involves an innate second set of Th2 cells specific for portions of the TCR of pathogenic Th1 cells. These Th2 cells recognize internally processed V region peptides from the TCR expressed on the Th1 cell surface in association with MHC molecules. As assessed by ELISPOT, TCR-reactive T cells constitute nearly 8% of the T cell repertoire in healthy individuals, but are significantly reduced in MS patients, potentially allowing unregulated clonal expansion of Th1 cells. We have used a variety of strategies to identify disease-associated V genes present on pathogenic Th1 cells, including BV525, BV656 & BV131 in MS, BV3, BV14 & BV17 in RA, and BV3 & BV131 in psoriasis. TCR peptides corresponding to the mid region of these BV genes were found to be consistently immunogenic in vivo when administered either i.d. in saline or i.m. in incomplete Freund’s adjuvant. In MS patients, injection of low doses of peptides (100-300µg) significantly boosted the number of TCR-reactive Th2 cells, and the strength of response to TCR vaccination was correlated with clinical benefit. Once the regulatory Th2 cells were specifically activated, they non-specifically inhibited activation of other CD4+ T cells via mechanisms involving cell-cell contact (as for CD4+CD25+ Treg cells) and inhibitory cytokines including IL-10. These findings indicate the potential of regulatory Th2 cells to inhibit not only the target Th1 cells, but also bystander Th1 cells expressing different V genes specific for other autoantigens. TCR peptide vaccines have been used in our studies to treat a total of >200 MS patients (7 trials), 484 RA patients (7 trials), and 177 psoriasis patients (2 trials). Based on treatment of almost 900 patients with autoimmune diseases, TCR peptide vaccination is safe and well tolerated, and can produce significant clinical improvement in a subset of patients that respond to immunization. TCR peptide vaccination represents a promising approach that is well-suited for treating complex autoimmune diseases.

Disclosure: A Vandenbark is consultant for and holds stock options in The Immune Response Corp.
Funding: Supported by NIMSS R0309-A-2, NIH NS23221.

LARGE SCALE TRANSCRIPTIONAL AND PROTEOMIC ANALYSIS OF MS TISSUE YIELDS NEW TARGETS FOR THERAPY
Steinman L
Stanford University, Stanford, California, USA

Abstract Body: I shall review results on large scale transcriptional and proteomic analysis of MS tissue. These analyses yield new targets for potential therapy.

Disclosure: I have nothing to disclose.
Funding: Supported by the NIH

RE-INDUCTION OF TOLERANCE IN ESTABLISHED AUTOIMMUNE DISEASE: A STRATEGY FOR THE TREATMENT OF MULTIPLE SCLEROSIS
NMR unit, Institute of Neurology, Queen Square, London, United Kingdom

Background: Leukocyte depletion and myelin-Ag delivery in MS have been attempted independently with limited success. In relapsing EAE, transient T cell deletion, using CD4-specific mAb, delays the return of disease. Similarly, myelin Ag i.v. induces a readily reversible unresponsiveness.

Objectives: To test whether the combination of transient T cell deletion and i.v. myelin Ag induces tolerance that prevents relapsing EAE. To test whether leukocyte depletion with a cytotoxic agent (mitoxantrone) can replace mAb-mediated depletion.

Methods: Depleting/blocking CD4-specific mAb and mitoxantrone was used in actively induced mouse EAE. Ag-specific tolerance was induced by a single injection i.v. of myelin Ag either alone or attached to fixed cell-carriers.

Results: In contrast to non-depleting agents, a combination of transient CD4 T cell deletion and i.v. myelin Ag, administered during the period when primed T cells are regenerating, induce tolerance that is resistant to further disease induction and inhibits relapsing EAE. This was achieved by depletion with either anti-CD4 mAb or mitoxantrone. Dependent on the immunizing Ag, single peptides were effective or ineffective. Recombinant myelin proteins were effective and require no knowledge of the pathogenic epitopes.

Conclusions: Translation of this strategy into MS may go some way towards halting the immunological disease process and provide a platform for additional neuroprotective and repair strategies. The observation that mitoxantrone, a drug already licensed for use in MS, can substitute for CD4+ T cell depletion has the potential advantage of targeting pathogenic B cell responses, which in part are responsible for some of the adverse reactions following myelin Ag administration.

Disclosure: G Giovannoni has nothing to disclose.
Funding: Supported by The MS Society of Great Britain and Northern Ireland.

KVL13 IS A UNIQUE FUNCTIONAL MARKER OF EFFECTOR MEMORY T CELLS IN MULTIPLE SCLEROSIS
Allie R, Yun S, Calabresi PA, Wulf H, Chandy K, Pennington M-
*Neurology, University of Maryland, Baltimore, Maryland, USA; 1Physiology, University of California Irvine, Irvine, California, USA; 2Bachem Bioscience, King of Prussia, Pennsylvania, USA

Background: Activated T cells maintain high levels of intracellular calcium by upregulating K+ channels that allow for a counter regulatory efflux of potassium. T cells acutely activated with mitogen upregulate the K+ channel IKCa, but not Kv1.3, whereas in chronically activated T cell lines (TCL) the
converse situation occurs with marked upregulation of Kv1.3. ShK is a high affinity peptide antagonist for Kv1.3 and has been shown to ameliorate EAE.

**Objectives:** To determine whether myelin reactive T cells from MS patients differed in their expression of Kv1.3 depending on their stage of differentiation, as well as compared to T cells from healthy controls. Further, we wished to study the functional effects of the peptide antagonist ShK on Kv1.3 hi T cells.

**Methods:** Myelin reactive T cells were generated from 15 MS patients and 10 healthy controls. Antigen specific T cells were phenotyped by FACS using monoclonal antibodies for CD4, CD45RA, CD45RO, and CCR7. IKca and Kv1.3 channel number was determined by patch clamp analysis and fluorescence microscopy. The functional effects of ShK were studied by titrated thymidine proliferation assays and cytokine analysis.

**Results:** In healthy controls IKca was upregulated on acutely activated (2-3 stimulations) myelin reactive T cells, but Kv1.3 was not upregulated until T cell had been restimulated greater than 7 times. The expression of Kv1.3 correlated strongly with conversion from central memory T cells (Tcm=CD45RA-CCR7+) to effector memory T cells (Tem=CD45RA-CCR7+). Myelin reactive T cells from MS patients expressed high channel numbers of Kv1.3 after only 3 stimulations and were phenotypically Tem. ShK selectively inhibited proliferation of Tem, but not Tcm in keeping with the observed K channel patterns on these cells.

**Conclusions:** We conclude that Kv1.3 is a functional marker of Tem, but is not expressed on acutely activated naïve T cells. Our preliminary data suggest that acutely stimulated myelin reactive T cells derived from MS patients have the Tem/Kv1.3 phenotype, which is not present in myelin reactive T cells from healthy controls. The Kv1.3 peptide antagonist, ShK, may specifically target Tem and should be considered as a potential therapy in MS.

Disclosure: KG Chandy has received ShK peptide from Michael Pennington at Bachem Bioscience
Funding: Supported by NIH (NS41435) and NMSS.

### Session VII

**Methodological Issues in Clinical Trials**

#### 50

**The New Diagnostic Criteria and Their Implications for Clinical Trials**

**Wolinsky JS**
University of Texas, Houston, Texas, USA

**Abstract Body:** The International Panel on the Diagnosis of MS (IP) recommended revised diagnostic criteria for the disease (McDonald et al., Ann Neurol 50:121, 2001). The focus of the new guidelines remains on the objective demonstration of dissemination of lesions in both time and space. Advances in MRI mandated its integration with clinical and other sensitive paraclinical diagnostic methods. The revised criteria clearly facilitate the diagnosis of MS in patients with a variety of presentations, including the clinically isolated syndromes (CIS) or “monosymptomatic” disease suggestive of MS, and disease with a typical relapsing remitting course. Previously used terms such as “clinically definite” and “probable MS” are no longer recommended, with the outcome of a diagnostic evaluation designated as MS, “possible MS” (for those at risk for MS, but for whom diagnostic evaluation remains equivocal), or “not MS.” Stringent guidelines for diagnosis of disease with insidious progression, without clear attacks or remissions were also formulated that require characteristic CSF abnormalities. The near term specificity and negative predictive value of the IP MRI criteria at first presentation of CIS are well supported by several studies (Tintore et al. MS 7:359, 2001), and the serial use of MRI to provide earlier evidence of dissemination over time than can clinical events is well-established in CIS subjects with two or more lesions on their presenting cerebral MRI (Beck et al., Ann Neurol 51:481, 2002). Nevertheless, several issues are raised by the IP criteria that may impact clinical trial designs that use MRI. MRI activity for several agents in MS demonstrated partial efficacy in reducing clinical (relapse-related measures) and MRI (new and enhancing lesions) evidence of inflammatory-demyelinating disease activity for several agents in MS. There remains less certainty that these or other agents influence mid- and long-term clinical (disability) and MRI (atrophy) measures of disease progression. Multiple recent RCTs have exploited short-term markers of inflammation (relapses, MRI activity) to assess treatment benefit in trials lasting less than three years. Positive findings suggest bio-

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**Application of McDonald Criteria to Clinically Isolated Syndromes Suggestive of Multiple Sclerosis**


*Clinical Neuroimmunology Unit, University Hospital Vall d’Hebron, Barcelona, Catalonia, Spain; +Magnetic Resonance Unit - IDI, University Hospital Vall d, Barcelona, Spain

**Background:** Patients presenting with a first demyelinating attack may be diagnosed of MS when MRI shows dissemination in space (DIS) and dissemination in time (DIT) according to new MS diagnostic criteria (McDonald criteria).

**Objectives:** To apply the new MS diagnostic criteria in patients with clinically isolated syndromes (CIS) suggestive of MS.

**Methods:** 139 patients with CIS, followed for a median of three years, underwent brain MRI within three months of the onset of first attack and after 12 months. Number and topography of lesions at baseline, alone or coupled with CSF analysis, provided evidence for DIS and new T2 lesions at follow up for DIT. Diagnosis of clinically definite MS (CDMS) based on Poser criteria was compared to diagnosis of MS incorporating MRI (McDonald criteria). Accuracy of new diagnostic criteria was evaluated.

**Results:** At 12 months, 11% had CDMS according to Poser compared to 37% who had MS according to McDonald MRI definitions for DIS and DIT. Eighty percent of patients fulfilling these MRI definitions and followed for at least three years, converted to CDMS. The McDonald MRI criteria (DIS and DIT) showed a sensitivity of 74%, specificity of 86% and accuracy of 80% in predicting conversion to CDMS after three years. Specificity for DIS decreased when adding CSF abnormalities to MRI definitions.

**Conclusions:** McDonald criteria more than triple the diagnosis of MS at one year. Specially when CSF analysis is not considered, evidence of DIS and DIT using MRI accurately predicts conversion to CDMS, demonstrating the usefulness of these criteria in anticipating MS diagnosis.

Disclosure: X Montalban has nothing to disclose.

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**Methodological Issues in Short-Term Clinical Trials JH Noseworthy, MD**

**Noseworthy JH**
Neurology, Mayo Clinic, Rochester, Minnesota, USA

**Abstract Body:** Randomized controlled clinical trials (RCTs) have demonstrated partial efficacy in reducing clinical (relapse-related measures) and MRI (new and enhancing lesions) evidence of inflammatory-demyelinating disease activity for several agents in MS. There remains less certainty that these or other agents influence mid- and long-term clinical (disability) and MRI (atrophy) measures of disease progression. Multiple recent RCTs have exploited short-term markers of inflammation (relapses, MRI activity) to assess treatment benefit in trials lasting less than three years. Positive findings suggest bio-
logical benefit but this remains unproven by confirmatory studies of adequate length and rigor. The desire to hasten the approval and subsequent availability of effective agents for patients must be balanced with the need to confirm that short-term measures of efficacy predict long-term benefit. Positive findings from short-term trials provide treatment options but disrupt equipoise making confirmatory, controlled, long-term RCTs difficult to conduct and complete. The major challenges to short-term trials in chronic diseases include: 1. Methodological issues: accelerated enrollment, large sample size, limited eligibility spectrum (generalizability), 2. Biological issues: time limitations both for change in major outcome measures in the control group and for therapeutic agents to influence measures of axonal degeneration and regeneration despite potential to measure benefits on short-term markers of inflammation. 3. Safety monitoring: limited time for important, rare or delayed toxicities to appear. Although prognostic markers in relapsing-remitting MS provide guidance for estimates of time to disability, studies to date have not shown that treatment-related reduction in these variables (including relapse frequency, severity, new and active MRI lesions) will change time to and degree of clinical disability (or MRI atrophy). Contemporary short-term trials have provided evidence of biological benefit upon markers of inflammation in MS resulting in drug approval. These studies, however, have not been followed by definitive, confirmatory, trials to measure the presence or magnitude of clinically relevant effects on disability.

Disclosure: J Noseworthy received a consulting fee from LFB-France in 2001 and is a site co-investigator in studies funded by Teva Pharmaceuticals, Pythagoras and Pfizer.

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THE ROLE OF MRI AS A SURROGATE MARKER IN MS
McFarland H
Neuroimmunology Branch, National Institutes of Health, Bethesda, Maryland, USA

Abstract Body: After the demonstration of the remarkable sensitivity of MRI to detect abnormalities in the brains of patients with MS nearly two decades ago, it was assumed that MRI would be a better measure of disease than clinical measures and that MRI would be a sensitive outcome measure in testing new therapies in MS. Unfortunately, the relationship between MRI and clinical disease has been found to be weaker than expected and the role of MRI as a surrogate marker in clinical trials more problematic than initially thought. The most important criterion for a potential surrogate is that the marker must predict future clinical disease. Early in the course of disease, the extent of change on MRI does have value in predicting the course of disease over a period of several years. However as patients who are further into the disease course are studied, the relationship between these conventional MRI measures and disability as measured by EDSS is poor in a cross-sectional analysis and seems to fail in predicting future course. Thus, conventional imaging falls far short of being a validated surrogate. Several techniques have shown to be more sensitive for assessing the changes ascribed to destructive MS lesions including spectroscopy, MTR, T1 hypointensities, and atrophy. Although the correlations between some of these measures and disability does improve as compared to measures of T2 lesion load and although the correlations between MRI measures and other measures of disability such as the composite score are better than with EDSS, the correlations remain less robust than hoped and long term data which would allow assessment of the predictive value of the techniques is not available. Thus, MRI is not, at present, an appropriate surrogate for clinical disability. Despite the failures, evidence is accumulating which indicates that disability is related to irreversible damage to myelin and axonal loss. Imaging techniques that can accurately identify these processes early in the disease course should eventually prove capable of predicting future disability. Long term follow-up of patients initially assessed with a MRI evaluation using validated and reproducible techniques is, in the end, necessary to determine the value of MRI as a surrogate in MS.

Disclosure: Dr. McFarland has served as a consultant for Berlex, Biogen, Teva, Schering, AG, Immunece, Wyeth

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A STANDARDIZED MRI SCAN IN THE DIAGNOSIS AND FOLLOW-UP OF MS PATIENTS
Paty D1, Li DK1, Traboulsee A1, Simon P, Frank P2
1Department of Medicine, University of British Columbia, Vancouver, British Columbia, Canada; 2Department of Radiology, University of British Columbia, Vancouver, British Columbia, Canada

Abstract Body: Purpose: MRI is used in the diagnosis of multiple sclerosis (MS) as it reveals neurologically asymptomatic lesions that may satisfy the criteria for dissemination of disease in space and time. MRI has potential in the follow-up of patients, yet there are no standards for MRI use in clinical practice. A consensus meeting was convened and considered guidelines for a standard brain and spinal cord MRI protocol. Methods and Materials: An international group of experienced MS neurologists and radiologists, along with representatives from the AAN, the ASNR, and the RSNA met in Vancouver, November 3-4, 2001. The working groups developed detailed guidelines, and the meeting culminated by a joint clinical-MRI consensus session. Results: Results were: (i) when available, a brain MRI based on a standardized protocol should be acquired for follow-up; (ii) when presenting symptoms are at the level of the spinal cord, a spinal cord and brain MRI are required; (iii) when brain MRI provides equivocal results, spinal cord imaging may be justified; (iv) in the absence of clinical indications, routine follow-up MRI scans are not recommended. Clinical indications for follow-up MRI include unexpected clinical worsening, reassessment of disease burden for initiation of treatment, suspicion of secondary diagnosis; (v) follow-up MRI by standardized protocol should be compared to previous studies; (vi) contrast enhanced MRI recommended for diagnosis, but optional otherwise. The full protocol will be available on the Consoritum web site (http://www.mscare.org) Conclusion: Uniform imaging standards and guidelines should improve the quality, yield and value of MS follow-up. These recommendations are a part of a continuing quality improvement process based on interactions of the neurology and radiology communities.

Disclosure: D Paty has nothing to disclose.
analysis has identified linked and associated regions on chromosomes 1p, 5q, 6p (MHC), 11p, 17q and 19q. Because cases were not typed individually, GAMES is concerned only with genes conferring disease susceptibility and not those influencing disease progression or the clinical course.

Disclosure: Prof. Compton no disclosure of interest for genetics of MS Funding: The Wellcome Trust.

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INSIGHTS INTO THE GENETICS OF MS FROM THE CANADIAN COLLABORATIVE PROJECT

Fbers GC*, Sadovnick D*, Risch N*
*Clinical Neurology, Radcliffe Infirmary, Oxford, United Kingdom; ^Clinical Neurology, University of Oxford, Oxford, United Kingdom; †University of British Columbia, Vancouver, British Columbia, Canada; ‡Stanford University, Palo Alto, California, USA

Abstract Body: The Canadian Collaborative Study in Genetic Susceptibility to Multiple Sclerosis involves 18 Canadian sites, each staffed by neurologists whose special interest is in MS. To date there are nearly 20,000 patients in the CCNSMS database. For many clinics, the patient population can be considered “population based” because contemporaneous prevalence studies have shown that the proportion of patients identified in the region is extremely high. These have allowed for a concentric approach to all studies. The ability to carry out population-based studies in large numbers of patients has been beneficial for answering a number of questions relating to the genetic epidemiology of MS. The frequency of familial occurrence gradually increases in clinics proportional to some degree to the background prevalence rate of patients and has approached 25% in several sites. More than 2700 families having more than one case of MS have been identified including three families with more than 11 affected individuals in a single pedigree. More than 40 families have been identified with four or more affected individuals. These families may hold special value in looking for susceptible genes in MS and their similarities and differences to sporadic cases will be outlined. In a single pedigree having more than 15 cases who are affected, the mode of inheritance appears to be autosomal dominant and may differ both from other familial cases and from those with sporadic MS. The female predominance is generally lower in such complex families, the contribution from the MHC may be larger (always transmitted from heterozygous parents), and the phenotype appears to be surprisingly broad. Studies of phenotype in concordant monozygotic twins imply the existence of genes influencing outcome as distinct from those determining susceptibility.

Disclosure: Member of the MS Forum which is an educational activity funded by Schering called MS Forum which supports research symposia twice yearly.

Funding: Supported by the UK Government. Supported by the MS Foundation of Canada.

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THE ROLE OF THE HLA REGION IN MULTIPLE SCLEROSIS

Hauser S*, Barcellos LF†, Pericak-Vance MA†, Haines JL†, Lincoln RR†, Schmidt S†, Swedin A†, Oskenberg JR†
†, University of California, San Francisco, California, USA; †Duke University Medical Center, Durham, North Carolina, USA; †Vanderbilt University, Nashville, Tennessee, USA

Abstract Body: The etiology of MS is complex, involving both genetic and environmental components. Linkage to the HLA-DR locus, and association with the DR2 haplotype (DRB1*1501-DQB1*0602) within the class 2 subregion of the MHC on ch.6p21 has been consistently demonstrated. In a North American MS dataset, intrafamilial concordance for early manifestations was present, but was not associated with HLA-DR2. A more detailed screen of 6p21, using 24 markers spanning approximately 20Mb, indicated that associations to other markers could be attributed to close proximity to the DR locus and that no evidence for a second MS susceptibility locus distinct from the class 2 region could be found. A dose effect of DR2 haplotypes on MS risk was also observed, indicating that individuals who inherit two copies of a DR2 haplotype are at greater MS risk than those who inherit a single copy only. Furthermore, DR2 homozygotes were significantly less frequent in patients with a benign disease course, demonstrating a disease modifying role for HLA-DR2. A recently completed follow-up whole genome screen demonstrated that evidence of linkage to the 6p21 region was restricted to DR2+ families and that, by controlling for HLA influences, additional candidate loci emerged. These findings shed new light on the complex molecular mechanisms that underlie HLA contributions to MS pathogenesis.

Disclosure: S Hauser has nothing to disclose.

Funding: NIH, National Multiple Sclerosis Society, The Nancy Davis Foundation.

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HORMONAL INFLUENCES IN MS

Voskuhl R
Neurology, UCLA, Los Angeles, California, USA

Abstract Body: Hormonal influences in multiple sclerosis include the two widely accepted clinical observations that (1) young men are less susceptible to disease than young women and (2) that disease activity in multiple sclerosis (MS) is decreased during late pregnancy. In vitro and in vivo data suggest that high levels of testosterone are immunomodulatory and therefore may be protective in young males. On the other hand, the protective effect of late pregnancy may be due to high levels of estrogens. Estriol is the predominant estrogen of pregnancy. It is normally not detectable, but during pregnancy it is made by the fetal placental unit and gradually increases to reach its peak during late pregnancy. Pre-clinical studies, when estriol was administered to mice with EAE, disease was ameliorated and a favorable shift in the immune response was observed which recapitulated, at least in part, that which occurs during pregnancy. Oral estriol was then given in a pilot trial of female multiple sclerosis patients in an attempt to recapitulate the beneficial effect of pregnancy. As compared to pretreatment baseline, relapsing remitting patients treated with oral estriol (8mg/day) demonstrated significant decreases in delayed type hypersensitivity (DTH) responses to tetanus. In addition, interferon-g levels in peripheral blood mononuclear cells (PBMCs) were decreased significantly. Finally, during estriol treatment, gadolinium enhancing lesion numbers and volumes on monthly cerebral MRIs were decreased as compared to pretreatment baseline. Further, when estriol treatment was stopped, enhancing lesions increased to pretreatment levels. When estriol treatment was re-instituted, enhancing lesions were again significantly decreased. Based on these results, a larger, placebo-controlled trial of oral estriol is warranted in women with relapsing remitting multiple sclerosis. In summary, clinical observations of alterations in disease activity which correlate with alterations in hormone levels should be viewed as invaluable clues to the identification of modulators of disease which can then be explored as novel therapies.

Disclosure: R Voskuhl has nothing to disclose.

Funding: National MS Society.

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OVARIAN HORMONES DIFFERENTIALLY EFFECT NEURON DEATH MEDIATED BY TNF-α VIA EXPRESSION OF ANTI-APOPTOTIC PROTEINS AND ACTIVATION OF JNK1 PRO-APOPTOTIC SIGNAL CASCADE.

Koski CL*, Hila S*, Popescue T*, Hoffman GP*
*Neurology, University of Maryland School of Medicine, Baltimore, Maryland, USA; †Anatomy & Neurobiology, University of Maryland School of Medicine, Baltimore, Maryland, USA

Background: Our previous data indicate estrogen (E) improves neuron survival in an animal model of multiple sclerosis, experimental allergic
encephalomyelitis. In striking contrast, progesterone (P) makes neurons more vulnerable to death during inflammation.  

Objectives: To investigate possible mechanisms for our in vitro data in an in vivo model of TNFα-mediated neuronal cell death.  

Methods: PC12 cells were differentiated with NGF. Cell death was determined with a TUNEL assay and FACS analysis. Protein expression was detected by binding of specific antibodies, FACS and Western blot.  

Results: TNFα (0-150 ng/ml) induced death of up to 80% of PC12 cells in a dose dependent manner. Cell death was prevented by a 72 hr preincubation of cells with physiologic levels of E (1 nM); in contrast, a similar preincubation with P (100 nM) increased cell death by two fold over cells treated with TNFα alone (50-100 ng/ml). 24 hr pre-treatment with E prior to TNFα exposure was required to achieve maximum neuroprotection, whereas P was able to enhance cell death within 30 min. E preincubation for 72 hrs increased ERα expression and BCL-xL expression greater than 2 and 3.6 fold respectively while reducing TNFR1 to 19% of control. P (72 hr) decreased ERα expression to 29% of control, did not effect BCL-xL levels, but increased TNFR1 greater than 2 fold. A peptide inhibitor of TNFR1 terminal kinase, INK1, a pivotal kinase in a pathway implicated in neuronal apoptosis, abrogated the ability of a 30 min P pretreatment to enhance TNFα induced injury of PC12 cells but did not effect death induced by TNFα alone.  

Conclusions: These results are consistent with the hypothesis that during inflammation, E may regulate neuron survival via transcriptional mechanisms while P increases neuronal cell death via a dual mechanism: activation of INK1 which augmented the TNF mediated death signal cascade and up-regulation of TNFR1 expression.  

Disclosure: C Koski has nothing to disclose.  
Funding: Supported by NMSS-PP0754.

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A NEW GENE OVEREXPRESSION IN MULTIPLE SCLEROSIS AND RHEUMATOID ARTHRITIS  
Greene C, Crasio R, Chen L, Rose C, Connelly D, Grelkova M, Richert JR  
Microbiology and Immunology, Georgetown University Medical Center, Washington, District of Columbia, USA  

Background: Multiple Sclerosis (MS) is an inflammatory, demyelinating disease of the central nervous system that is thought to be an autoimmune consequence of a microbial infection in a genetically susceptible host.  

Objectives: To evaluate abnormal gene expression in MS immune cells directly or indirectly related to environmental influences.  

Methods: Differential display was performed to screen peripheral blood mononuclear cells (PBMCs) from identical twins who are discordant for MS. Genes found to be differentially expressed in the twins were then evaluated in larger populations of MS patients and controls.  

Results: A gene with no significant homology with any known gene family was identified in the MS twin. Real-time RT-PCR studies showed it to be expressed 5.25-fold higher in the MS population compared to the healthy control group. Preliminary data also suggest that it is over-expressed in rheumatoid arthritis (RA) PBMCs, and under-expressed in other autoimmune diseases and in healthy subjects post-vaccination. Study of a panel of normal tissues revealed ubiquitous expression with levels ranging from low (breast) to moderate (lung, kidney, spleen) to high (pancreas, liver, prostate). Northern blot analysis in healthy PBMCs revealed five transcripts: approximately 4.9, 2.6, 1.8, 1.2, and 1.0 kb in size. 5' and 3' RACE studies have revealed the 2.6 and 1.3 kb transcript sequences to date.  

Conclusions: This gene that is overexpressed in MS and RA requires further evaluation regarding its function and control of its expression.  

Disclosure: J Richert has nothing to disclose.  
Funding: Supported by the Dominion Guild Fund. C Greene was supported by an NIH pre-doctoral fellowship.
Some recent studies also suggest that pro-inflammatory cytokines contribute to the sense of tiredness. Nonspecific treatments are available. Management strategies include medications, exercise, and behavioural therapy; in most cases a combined approach is appropriate.

Disclosure: G Comi has nothing to disclose.

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STEREOTACTIC SURGERY
Montgomery EB
Neurology and Neuroscience, Cleveland Clinic Foundation, Cleveland, Ohio, USA

Abstract Body: Stereotactic surgery for the relief of severe action tremor has been performed for many years. Enthusiasm for the ablative thalamotomy had been modest at best because of the then poor prognosis of multiple sclerosis, surgical risks, and high recurrence rates. However, recent advances on several fronts have rekindled interest and include treatments that may alter the natural history of multiple sclerosis, improvement in surgical techniques, and the development of deep brain stimulation (DBS). A number of clinical studies have been published attesting to the clinical efficacy of thalamic DBS. In our own published clinical study of 15 patients there were statistically significant reductions in tremor. Resting tremor was virtually abolished. Postural tremor was reduced to nearly 0 on Clinical Tremor Rating Scales (zero equals no tremor, four means severe to the point where the patient is unable to perform the task). Action tremor such as shaking the finger to the tip of the nose was reduced from an average value of 4 preoperatively to 1 postoperatively. Tremor with bringing a cup to the lip improved from an average of four preoperatively to 0.8 postoperatively. Patients often developed tolerance requiring frequent adjustment of the simulator that nearly always resulted in regained improvement. Long-term follow-up is not available. Our clinical impressions suggest that if patients can continue frequent simulator adjustments their tremors can remain improved. However, it is been our experience that as the disabilities associated with the multiple sclerosis continue to increase, their ability to make the frequent visits diminishes especially in view of the limited availability of clinics to adjust the DBS stimulators. When assessing a patient’s candidacy for DBS, it is very important to have a realistic set of expectations. Our goal is to improved tremor in one arm so that the patient has greater independence. Unfortunately, clinical trials utilizing large inventories of assessments will not detect significant improvements in the quality of life associated with tremor reduction in one arm. Our criteria for candidacy include tremor that is significantly disabling, reasonably intact speech and swallowing, good strength and sensation in the upper extremity, and no significant exacerbations in the previous six months.

Disclosure: E Montgomery Jr is a consultant to and has received research support from Medtronic, Inc.

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DISEASE-MODIFYING DRUGS FOR MULTIPLE SCLEROSIS. CAN TREATMENT FAILURES BE PREDICTED?
Johnson M, Ford H, Denton S
Dept of Neurology, Leeds Teaching Hospitals NHS Trust, Leeds, West Yorkshire, United Kingdom

Background: In most series, about 20% of patients started on disease modifying treatments for M.S. will, for various reasons, stop treatment. Patient disappointment and waste of scarce resources would be avoided if some of these treatment failures could be predicted.

Objectives: To investigate whether practical measures in a clinical setting could be used to predict treatment discontinuation.

Methods: All patients treated with interferon beta in the Leeds Multiple Sclerosis Treatment Programme had a comprehensive pre-treatment assessment. This included a self-report disease-specific measure of quality of life, the Leeds Multiple Sclerosis Quality of Life Scale (LMSQoL) and the Guy’s Neurological Disability Scale (GNDS). These measures were repeated at intervals on treatment. The Expanded Disability Status Scale (EDSS) was assessed annually. Patients eligible for treatment had clinically definite relapsing remitting (RR) or secondary progressive (SP) multiple sclerosis. Patients with RRMS had at least two disabling relapses in the previous two years and those with SPMS had active secondary progression with superimposed relapses or a 1 point deterioration in the EDSS in the previous year. Those patients who discontinued treatment were compared with those who stayed on treatment at two or more years.

Results: At the end of two years, 20 out of 73 (27%) patients had stopped treatment. 3 had stopped because of pregnancy, 4 had stopped or reduced the dose because of side-effects and are now back up to full doses. Of 57 patients treated for RRMS, 11 (19%) stopped because of treatment failure, (relapses or progression). Of 16 patients treated for secondary progressive disease, 7 (44%) stopped because of treatment failure. Patients in whom treatment failed were, as a group, no different on measures of quality of life or disability when they started treatment but they did not show any improvement. They did have generally higher EDSS scores on starting treatment.

Conclusions: Patients with relapsing remitting MS are more likely to stay on treatment with Interferon and less likely to be counted as treatment failures than secondary progressive patients. In relapsing remitting patients, a higher EDSS score does not confer a greater risk of treatment failure in the first two years.

Disclosure: Dr. Johnson serves on an advisory committee for Teva Pharmaceuticals for which he receives an honorarium. Dr. Johnson and Dr
GADOLINIUM ENHANCING LESIONS AS A SURROGATE MARKER OF INTERFERON RESPONSE


aMellen Center, Cleveland Clinic Foundation, Cleveland, OH, Ohio, USA; bUniversity of Nevada, Reno, Nevada, USA; cAMC Cancer Center, Denver, Colorado, USA; dThomas Jefferson University, Philadelphia, Pennsylvania, USA; eSUNY-Buffalo, Buffalo, New York, USA; fOHSU, Portland, Oregon, USA; gBiogen, Inc., Cambridge, Massachusetts, USA; hUCHSC, Denver, Colorado, USA

Background: Gadolinium lesion frequency is a primary outcome measure in phase II MS trials, and an important secondary outcome in definitive trials. This measure has not been validated as a surrogate marker for clinical benefit.

Objectives: 1. To classify patients from the IFNβ1a (AVONEX) RR-MS trial as “complete responders” or “non-responders” based on gadolinium lesions at baseline and 1 year. 2. To determine the proportion of responders and non-responders for the two treatment arms. 3. To correlate 8-year EDSS, MSFC, and BPF change with these categories.

Methods: 172 patients followed for 2 years in the phase III IFNβ1a trial were evaluated. 8.1 years after randomization. EDSS was calculated in 163 pts; MSFC in 137; and BPF in 134. Complete responder was defined as a patient with lesions at baseline but not at year 1; non-responder was defined as a patient with more enhancing lesions at year 1 compared to baseline. EDSS, MSFC, and BPF change over 8 yrs were compared in responders and non-responders.

Results: 42% of IFN and 46% of plc patients were complete responders or non-responders. There were more complete responders in the IFN group (27% vs 21%); and more non-responders in the plc group (25% vs 15%). Over the 8-year study period, complete responders did better than non-responders in the IFN group - mean EDSS worsening 1.1 vs 3.0; mean MSFC worsening -0.78 vs -2.5; and mean atrophy worsening -2.3% vs -3.2%. In contrast, complete responder and non-responder groups did not differ in the plc arm - mean EDSS worsening 2.6 vs 2.2; mean MSFC worsening -1.1 vs -1.1; mean BPF worsening -3.9% vs -3.7%.

Conclusions: 1. Responder or non-responder status as defined correlates with long-term outcome more strongly in the IFN than the plc arm, suggesting that gadolinium change is a marker of therapeutic response to IFN. The results support use of gadolinium as a surrogate marker in MS trials, and provide support for biological studies that classify IFN responders based on gadolinium activity.

Disclosure: Dr. Al Sandrock is an employee of Biogen, Inc.

Funding: Supported by The original IFNβ1a Study was supported by the NIH (NINDS), the National MS Society, and Biogen, Inc.; The follow-up study was Supported by Biogen, Inc.

MITOXANTRONE (NOVANTRONE) FOR TREATMENT OF RECURRENT NEUROMYELITIS OPTICA

Weinstock-Guttman B, Feichter J, Bakshi R, Browncheilde C, Linoff N Neurology, Buffalo General Hospital, Baird MS Center, Jacobs Neurological Institute, Buffalo, New York, USA

Background: Neuromyelitis optica (NMO) is a severe demyelinating disease involving the optic nerves and the spinal cord, usually sparing brain parenchyma. Patients with recurrent NMO (R-NMO) generally do not respond adequately to treatment trials with glucocorticosteroids, cyclophosphamide, interferon beta, or azathioprine. Following treatment with mitoxantrone (MITO, Novantrone) we observed improvement in disability (EDSS from 6.0 to 4.0) in one patient with R-NMO. This prompted a pilot trial of MITO in R-NMO.

Objectives: To determine the benefit of MITO therapy in patients with R-NMO.

Methods: We are evaluating the effect of 2-year MITO therapy in 5 patients with R-NMO. Entry criteria included recurrent longitudinally extensive myelitis with or without optic neuritis and normal brain MRI. The treatment protocol consists of IV MITO infusions 12mg/m2 (maximum 20 mg) with methylprednisolone 1,000 mg monthly for 3 months followed by every 3 month up to 2 years (maximum dose 140mg/m2). Neurological assessment (EDSS) is performed every 3 months and during relapses. Brain and spinal cord MRIs are performed at baseline, 3, 6, 12, 18, and 24 months. Visual evoked potentials and ophthalmologic evaluations are performed at baseline and annually. Treatment failure is defined as: one severe relapse (complete paraplegia or blindness); 2 relapses with change in EDSS; four documented relapses but without change in EDSS during a 12 month interval, or step-wise disability progression sustained for 6 months.

Results: Five patients are already enrolled: 3F; 2M; age range 22-51 years; disease duration: 4 months to 19 years; EDSS 2.5 to 6. Previous treatments: repeated cycles of steroids, azathioprine, and plasmapheresis. Number of MITO infusions per patient: 3 to 4, follow-up 4-5 months. One patient, 4 months after an initial substantial benefit became treatment failure. The remaining patients are stable or improved by clinical and MRI parameters. The therapy was well tolerated by all patients.

Conclusions: Our preliminary results suggest a beneficial effect of MITO for R-NMO.

Disclosure: B. Weinstock-Guttman has received honoraria from Immunex Corporation.

Funding: Supported by a grant from Immunex Corporation.

Session X Late Breaking News

ECTRIMS Lecture

QUO VADIS? AGENDA FOR EUROPEAN MS RESEARCH.

Hommes OR
European Charcot Foundation, Nijmegen, Netherlands

Abstract Body: Scientific progress is strongly dependent on political aspects. It is reflected in societies’ willingness to direct attention and funding to specific fields of research. For health care, poliomyelitis and HIV are good examples. In the European Union research developments in general are lagging behind Japan and USA. Neurological and specifically MS research remain at lower organisational and political scales. Since 1990, when the first Concerted Action on MS Research was funded by the Commission of the European Communities, developments of European co-operation in MS Research have been slow. Reasons can be found in the fact that overall national activities and interests prevail over the European dimensions. Two organisations, ECTRIMS and the European Charcot Foundation, are endeavours for MS Research on a European scale, each with different, but fully complementary aspects. MS research expertise in Europe is high, and a number of excellent MS Research Centers have been developed. These developments could be enhanced by a strong organisation of MS Research on a European level. Pre-requisites, structure and funding of such an organisation are discussed, with emphasis on clinically and politically relevant goals, fund-raising and governance. Such an organisation is required now as the European Parliament on May 15, 2002 accepted the 6th cadre-programme (2003-2006) doted with...
had been determined. After 24 months of treatment, groups of patients posi-
tively and negatively reacted to the therapy, were distributed on the basis of clinical criteria.

Results: clinical criteria. Results: 15 patients positively responded to treatment and 6 patients - negatively. Patients who didn’t react, had higher level of IL-12 in serum (p<0.05) before treatment and coefficient IL-12 in fluid / IL-12 in a serum significantly differed in both groups ( p<0.05 ). However we didn’t observe significant differences in IL-10 level both in serum and cerebrospinal fluid. Measured IL-10 / IL-12 coefficient was markedly lower in patients with negative response to treatment ( p<0.05 ).

Conclusions: Definition of the dependence between levels of IL-10 and IL-12 in serum might be helpful in a distinction of patients with potentially positive response to interferon treatment.

Disclosure: H Bartosik-Psujek has nothing to disclose.

Funding: Drug supply Supported by Schering Plough

Thursday, September 19, 2002

Posters

Surrogate Markers

P1

CEREBROSPINAL FLUID PROTEIN STATUS AND ITS CLINICAL USE.

Adam P, Sobek O, Taborsky L, Vesela B, Prucha M

Neuroimmunology, Homolka Hospital, Prague, Czech Republic

Background: Till now, the biological role of many CSF proteins is not known. In the group of 7849 patients with neurological disorders, concentrations of CSF and serum proteins were measured using laser nephelometry.

Objectives: Concentrations of CSF immunoglobulins (IgG, IgA, IgM), albumin, prealbumin, transferrin, haptoglobin, CRP, complement (C3c, C4), orosomucoid, apolipoproteins (apo A-I, apo B), fibrinogen, beta2-microglobulin and proteinase inhibitors (antitrypsin, antithrombin III) were evaluated to find out if there is some specific biological role of these proteins present in CSF.

Methods: As method of measurement, immunonephelometry was used to find out the CSF and serum concentrations of these proteins. Measurements were followed by statistical analysis of data.

Results: Majority of these proteins has specific biological meaning which enabled to define their subgroups as inflammatory, destructive and tumorous markers.

Conclusions: Complete CSF proteinogram comprising evaluation of the functional state of CSF-blood barrier and evaluation of intrathecal synthesis of immunoglobulins followed by analysis of specific CSF protein markers, i.e. CSF Protein Status, enables to improve the quality of CSF investigation and diagnostics of inflammatory and autoimmune diseases of CNS.

Disclosure: P Adam has nothing to disclose.

P2

INTERLEUKIN-10 / INTERLEUKIN-12 COEFFICIENT AS POTENTIAL INDICATOR OF POSITIVE RESPONSE TO INTERFERON β TREATMENT

Bartosik-Psujek H, Mitosek-Szewczyk K, Belniak E, Stelmsiak Z

Neurology, Medical School, Lublin, Poland

Background: Only part of multiple sclerosis (MS) patients treated with interferon β preparations, positively responded to applied treatment. Up to now, no parameters were found which could enable the determination before treatment if the patient would respond positively. Interleukin-12 (IL-12) is the inflammatory cytokine, necessary for lymphocyte differentiation and interleukin-10 (IL-10) is it’s main inhibitor. Dependence between IL-10 and IL-12 is of key importance in immunopathogenesis of MS and is also recognised to be very substantial in the mechanism of interferon β operation.

Objectives: The aim of the study was to investigate the dependence between IL-10 and IL-12 levels before treatment and the clinic condition during the 2 years period of the use of interferon β preparation.

Methods: The study included 21 patients (14 women, 7 men, aged 26.9 ± 8.5) with clinically convinced relapsing-remitting MS. All patients were in a remission phase and during at least 8 weeks they weren’t being received any steroid therapy because of disease aggravation. The patients were given interferon beta-1a (30μi im once a week). Before the commence of the treatment with ELISA method, levels of IL-10 and IL-12 in the serum and cerebrospinal fluid had been determined. After 24 months of treatment, groups of patients posi-
basic features in the pathogenesis of MS, but there are currently no established in-vivo markers for monitoring axonal pathology in MS patients. We sought to investigate the association between axonal pathology and serum antibodies to the light (L) and the heavy (H) subunit of neurofilament (NF) in patients with relapsing remitting (RR), primary and secondary progressive forms of MS.

**Objectives:** Serum samples of 149 MS patients were investigated for antibodies to NF-L and compared to patients with nondegenerative diseases, patients with Guillain-Barre syndrome (GBS), patients with other inflammatory neurological diseases (IND), patients with other neurological diseases (OND; including back pain, headache and stroke) and to healthy controls.

**Methods:** Recombinant NF-L and NF-H cDNA were amplified from human brain cDNA by PCR. All cDNAs were cloned into pTrcHis2-TOPO-vector and overexpressed in E. coli TOP 10 F. Proteins were purified using affinity and hydroxyapatite chromatography. Human NF-L and NF-H were prepared from human white matter by the axonal floating technique and purified by hydroxyapatite chromatography and consecutive electro-elution from bis-tris SDS polyacrylamide gels. Serum NF antibodies were measured with an established sandwich ELISA.

**Results:** Serum-autoantibodies to NF-L were found to be significantly increased in MS patients with a progressive disease course. In addition, serum-autoantibodies to NF-L were significantly increased in patients with GBS, IND and OND, whereas serum-autoantibodies to NF-H did not differ significantly among the investigated probands.

**Conclusions:** Our findings suggest that elevated levels of antibodies to NF-L are associated with the disease course in MS and are valuable biological markers for monitoring disease progression in MS, which may allow for the identification of at least a subgroup of MS patients. Though the diagnosis of SP-MS is yet still a retrospective one, anti NF-L antibodies might be used for prospective monitoring of disease progression in MS in the future.

**Disclosure:** T Berger has nothing to disclose.

**Funding:** This study was supported by a grant from the Austrian Federal Ministry of Science (GZ 70.059/2-Pr/4/99).

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**P5**

**RELATIONSHIP BETWEEN THE MS FUNCTIONAL COMPOSITE AND MRI IN IMPACT**

Cohen JA, Goodman AD, Heidenreich FR, Kooijmans MF, Sandrock AW, Simon JH, Tsao EC

*Meilen Center, Cleveland Clinic; Neurology, University of Rochester, Rochester, NY, USA; Biogen, Inc., Cambridge, MA, USA; Neurology, Hannover Medical School, Hannover, Germany; Biogen, Inc., Cambridge, Massachusetts, USA; Radiology, University of Colorado, Denver, Colorado, USA*

**Background:** IMPACT was a randomized, double-blind, placebo-controlled, Phase 3 trial of interferon beta-1a (IFNβ-1a, Avonex®) in secondary-progressive MS. Benefit of IFNβ-1a was shown on the primary endpoint, 2-year MS Functional Composite (MSFC) change. Benefit also was shown on relapse rate, MRI, and quality of life, but not on Expanded Disability Status Scale (EDSS).

**Objectives:** These analyses assessed the relationship between measures of neurologic impairment and MRI parameters.

**Methods:** 436 subjects with SP-MS and EDSS 3.5-6.5 were randomized to IFNβ-1a (60 mcg) or placebo IM weekly for 2 years. MSFC and EDSS were measured every 3 months. MRI was performed annually. T2-hypointense lesion volume (T2vol), T1-hypointense lesion volume (T1vol), and brain parenchymal fraction (BPF) were determined using computer-assisted technology. Significant Spearman rank correlations for the pooled treatment groups are reported.

**Results:** Baseline T2vol and MSFC correlated moderately (r=−0.48), while EDSS correlated weakly (r=0.20). Among MSFC components, T2vol correlated best with Paced Auditory Serial Addition Test (PASAT, r=−0.47), compared to 9-Hole Peg Test (9HPT, r=−0.35) and Timed 25-foot Walk (T25FW, r=−0.20). Baseline T1vol also correlated better with baseline MSFC (r=−0.41) than EDSS (r=0.20) with the same pattern among the MSFC components. Baseline BPF showed a similar pattern of correlations with clinical measures: MSFC (r=−0.41), PASAT (r=−0.45), 9HPT (r=0.26), T25FW (r=0.18), EDSS (r=−0.20). The strengths and patterns of cross-sectional MRI-clinical correlations at Years 1 and 2 were similar to baseline. 2-year HPT change was the best correlate of T2vol change (r=−0.26) and T1vol change (r=−0.17). 2-year BPF change correlated only with PASAT change (r=0.13).

**Conclusions:** In this SP-MS cohort, T2 lesion volume, volume of T1 holes, and whole brain atrophy correlated substantially better with MSFC than EDSS. Among MSFC components, measures of cognition and arm function correlated with MRI better than did ambulation. These findings provide additional support for the validity of the MSFC as a clinical outcome measure.

**Disclosure:** J. Cohen, A. Goodman, F. Heidenreich, and J. Simon have served as speakers and consultants, and have received honoraria from Biogen, Inc. M. Kooijmans, A. Sandrock, and E. Tsao are employees of Biogen, Inc.

**Funding:** Supported by Biogen, Inc.

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**P6**

**A FOUR-MONTH LONGITUDINAL STUDY ON THE RELATIONSHIP BETWEEN CLINICAL ACTIVITY AND SERA CONCENTRATION OF S100 PROTEIN AND NEURON SPECIFIC ENOLASE IN MULTIPLE SCLEROSIS.**


*Neurological Sciences, University “La Sapienza”, Rome, Italy; Clinical Medicine and Neurology, University of Trieste, Trieste, Italy; Pathology and Experimental Medicine, University “La Sapienza”, Rome, Italy; European Biomedical Foundation, Rome, Italy*

**Background:** The calcium binding S100 protein (S100p) constitutes the major component of the citosol in glial cells. Neuron Specific Enolase (NSE) is a soluble cytoplasmatic protein localized mainly in neurons. Both these proteins have been reported to increase in blood concentration in patients with Multiple Sclerosis (MS) and other neurological disorders. T1 Black Holes (BH) and brain atrophy on Magnetic Resonance Images (MRI) are considered as radiological signs of axonal loss in MS.

**Objectives:** To investigate the role of serum levels of S100p and NSE as biological marker of tissue destruction in MS.

**Methods:** Thirty MS patients (male/female: 10/20, mean±SD age 34±8.2, EDSS 1.4±0.6, MS duration 5.4±5.1) were included. Patients were clinically assessed and imaged for four consecutive months. Samples’ sera were collected and enzymatically evaluated for the evaluation of S100p and NSE, by the means of an enzyme-linked immunosorbant assay (ELISA).

**Results:** A mean monthly value of 0.05±0.004 ng/ml of S100p and of 8.9±0.9 ng/ml of NSE were found in the sera of these MS patients. Such values were both within the range already reported in healthy subjects. A mean monthly value of 2±0.1 cm3 in T1BH lesion load and a mean monthly value of 0.769±0.76 for brain parenchymal fraction was found in the MRI of these MS patients.

**Conclusions:** S100p and NSE do not increase in patients with MS even in the presence of radiological signs of axonal loss. Thus such proteins cannot be considered a sensitive biological marker of tissue destruction for patients with MS.

**Disclosure:** P. Finamore has nothing to disclose.

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**P7**

**WHOLE BRAIN N-ACETYLASPARTATE ASSESSMENT IN RELAPSING-REMITTING MULTIPLE SCLEROSIS - EVIDENCE FOR DIFFERENT CLINICAL COHORTS**

Gonen CP, Moriatry DM, Li BS, Babb JS, Markowitz CM, Grossman RI

*Radiology, New York University School of Medicine, New York, New York, USA; Neurology, University of Pennsylvania Medical Center, Philadelphia, Pennsylvania, USA*

**Background:** Evidence is mounting that axonal damage, followed by neuronal cell death by Wallerian degeneration is the cause of permanent neurologic deficits in MS. This can be assessed non-invasively by proton MRS.
magnetic resonance spectroscopy (1H-MRS) quantification of the amino-acid derivative N-acetylaspartate (NAA), found almost exclusively in neurons and axons. Since MS pathology is diffuse, NAA assessment of the entire parenchyma is crucial to evaluate the full extent of the disease. Indeed, a new 1H-MRS method to quantify the whole-brain NAA (WBNAA) has recently shown that the latter can be more than 20% lower in RR MS patients than in their healthy contemporaries and declines x10 faster with age.

**Objectives:** To quantify the rate of concentration decline of the neuronal marker NAA in the entire brain of relapsing-remitting (RR) MS patients in relation to healthy, age-matched controls.

**Methods:** WBNAA was quantified in 49 RR MS patients, using MRI and 1H-MRS. It was statistically analyzed using Spearman rank correlation coefficients to test the within-group relationship between WBNAA and Expanded Disability Status Scale (EDSS) score and Mann Whitney analyses to test for differences between the groups’ EDSS scores versus WBNAA values of healthy subjects, disease duration and age.

**Results:** The analyses indicated three subgroups of WBNAA dynamics: Ten patients were “Stable,” exhibiting an insignificant change of ~0% per year of disease duration (p=0.54); 27 showed a “Moderate” decline, ~2.8%/yr (p=0.01); and 12 experienced “Rapid,” ~27.9%/yr (p=0.01) loss. The average EDSS in each of the subgroups, however, was the same ~2.0. No correlation was found between WBNAA deficit, EDSS score, or age.

**Conclusions:** MS metabolic progression is highly variable even in a cohort of patients of similar, mild, clinical impairment (average EDSS of 2). Consequently, ascertaining an individual’s NAA concentration dynamics might: (a) provide an early forecast of disease course; (b) reflect disease severity, hence, influence treatment decisions; and (c) improve clinical trial efficiency by selecting candidates based on individual WBNAA dynamics in addition to their clinical status.

**Disclosure:** O Gomen has nothing to disclose.

**Funding:** NIH Grants NS29029, NS33585 and NS37739

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**P8**

**INTERFERON BETA-1A IN COMBINATION WITH AZATHIOPRINE AND LOW DOSE STEROIDS FOR RELAPSING-REMITTING MULTIPLE SCLEROSIS: PILOT MODEL FOR PREDICTIVE MULTIVARIATE TYPOLOGY OF RELAPSES BASED ON CLINICAL AND MRI DATA**

Havrdova E, Horakova D, Krasensky P, Dusek L, Vanekova M, Seidl Z, Ticha V, Novakova P, Tyblova M

**Objectives:** To stratify relapses or progression according to MRI measures, a new - quantified - view into the course of disease in individual patients.

**Methods:** 183 pts planned for the study. 90 pts were already enrolled (27 males, 63 females, age 29+/−4,5yrs, Kurtzke EDSS 1.8+/−1), signed informed consent and were randomized into 3 groups receiving either i.m. IFNB + AZA 50mg/d + prednisone 10mg every other day, or IFNB + AZA 50mg/d + placebo S, or IFNB + placebo A + placebo S. MRI done every 5 weeks (T1, T2 and PD weighted images, 1-2mm slices, automatic measurement of lesion load and atrophy) enables to follow the development of lesions and atrophy. The study is planned for 2 years and data on MRI used for multivariate analyses were routinely monitored every 2 months during 1st year of the follow up. Survival analysis: time-to-event data were primarily based on EDSS score and censored at the point of sustained change.

**Results:** MRI parameters (volume of lesions) were found to be highly significantly correlated with changed EDSS status of each individual (p < 0.010) and on average level it clearly distinguished pts with repeated relapses or progression (group R) from cases without relapses (group W). Volume of lesions increased by nearly 65% in the group R (12 months follow-up with statistical significance of time-related trend: p < 0.01), while it remained within 100-111% of initial level in group W and reached statistically negligible trend changes.

**Conclusions:** Although MRI measures correlated positively with increasing EDSS score (with the best cut off at EDSS ≥ 2.5), the MRI parameters provide more detailed and intrinsically multivariate information related to time-related changes. Using pilot multivariate analysis, the MRI-based score was used as effective covariate for survival analysis modelling time to the first relapse or progression.

**Disclosure:** E Havrdova has nothing to disclose.

**Funding:** Supported by Research project MSM CR 111100001, and Schering-Plough, Praha, CR (MRI part of the study).

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**P9**

**PLASMA LEVELS OF BRAIN-SPECIFIC OXYSTEROL MAY REFLECT PROGRESSION OF MS**

Masterman T, Leoni V, Diczfalussy U, Melzi d’Eni G, Hillert J, Björkhjem P

**Objectives:** To compare plasma levels of a brain-specific oysterol, 24S-hydroxycholesterol (24S-OH-chol), in MS patients and in plasma alone from 183 age-matched controls, with a view to comparing levels in first, patients and controls subgrouped by age, and, second, patients subgrouped according to the results of brain MRI.

**Methods:** Blood and CSF were taken after an overnight fast. MRI scans were considered positive if judged by a neuroradiologist to be consistent with MS. Only scans performed within two months before or two months after sampling were assessed with regard to gadolinium (Gd) enhancement.

**Results:** In the oldest patient subgroups, plasma 24S-OH-chol levels were significantly lower than in age-matched controls. Spikes (levels more than 2 SD higher than the mean in the age-matched control subgroup) were found exclusively in patients with positive brain MRI scans, and CSF levels in patients with Gd-enhancing lesions were significantly higher than in patients without such lesions.

**Conclusions:** Compared to levels in controls, plasma 24S-OH-chol is increased in younger patients with MS and decreased in older patients, perhaps corresponding to the occurrence of inflammatory relapses in the former and of degenerative progression in the latter. Measurement of plasma 24S-OH-chol may thus represent a convenient method for evaluating different phases of MS.

**Disclosure:** T Masterman has nothing to disclose.

**Funding:** Supported by The Swedish Medical Research Council, The Swedish Heart-Lung Foundation, The Swedish Association of Neurologically Disabled.
P10
MATRIX METALLOPROTEINASE-9 IN MULTIPLE SCLEROSIS CLINICAL FORMS
aClinical Neuroimmunology Unit, University Hospital Vall d’Hebron, Barcelona, Catalonia, Spain; bMagnetic Resonance Unit, University Hospital Vall d’Hebron, Barcelona, Catalonia, Spain

Background: Inflammatory mechanisms are less prominent in primary and transitional progressive (PP/TP) multiple sclerosis (MS). Matrix metalloproteinase-9 (MMP-9) is an enzyme that degrades the extracellular matrix, and has been involved as part of the inflammatory cascade of MS. The role of MMP-9 in PP/TPMS has not been assessed.

Objectives: 1. To determine pro and active MMP-9 levels in PP/TPMS patients, relapsing-remitting (RR) MS patients, and healthy controls (HC). 2. To correlate the values of pro and active MMP-9 in PP/TPMS patients with clinical and radiological variables.

Methods: Seventy-three PP/TPMS, 50 RRMS patients (non-treated and stable for at least 3 months), and 46 HC were included. MMP-9 determinations (pro and active forms but not TIMP-complexed) were performed on serum samples using a commercial ELISA kit (MMP-9 assay system; RPN 2634, Bio-tak, Amersham-Pharmacia-Biotech, Little Chalfont, UK). EDSS scores were recorded at the time of blood sampling for PP/TPMS cases. T2- and T2-weighted MR scans for the PP/TPMS group were obtained at the time of blood sampling and one year later; increase in T1 and T2 lesion load and new or enlarging lesions at 12 months were determined.

Results: Mean MMP-9 levels were 6.32 ng/ml (SD: 3.47) for PP/TPMS, 7.5 ng/ml (SD: 3.48) for RRMS, and 8.57 ng/ml (SD: 4.32) for HC. Mean MMP-9 levels in PP/TPMS MS were significantly lower (Mann-Whitney’s U) when compared to HC (p<0.01) and RRMS (p<0.026). No statistically significant correlations between MMP-9 and EDSS scores or radiological parameters were found in the PP/TPMS group, although a trend for a correlation (p=0.06; r=0.312) between baseline T1 lesion load and MMP-9 levels was observed.

Conclusions: MMP-9 activity is different in PP/TPMS and RRMS. MMP-9 seems to be unrelated to clinical or MR variables in PP/TPMS, except for a possible role in brain tissue loss.

Disclosure: X Montalban has nothing to disclose.

P11
DYNAMICS OF AXONAL PATHOLOGY IN MULTIPLE SCLEROSIS
aResearch & Innovation, Padova, PD, Italy; bDept. of Neurological and Psychiatric Sciences, University of Padova, First Neurology Clinic, MS center, Padova, Italy; cEuganea Medica, Diagnostic Centre, Padova, PD, Italy

Background: Neurofilaments (NF) are principal components of the axonkeleton released during axonal injury.

Objectives: To identify Multiple Sclerosis (MS) patients with high cerebrospinal fluid (CSF) levels of NF and relate them to disability and progression in their disease.

Methods: Twenty-eight MS patients were included into this 3-year follow-up study along with 9 control patients. CSF levels of the phosphorylated NF (NfH-SMI35) and extensively phosphorylated NF (NfH-SMI34) heavy chains were quantified at baseline and 3-year follow-up using ELISA technique. Patients were stratified into relapsing remitting ‘RR’ or progressive ‘SP/PP’ disease. Patients ≥1 point increase in the EDSS over 3-years were classified as clinically advancing. Levels of NF phosphoforms, degree of phosphorylation (ratio) and changes between baseline and followup (delta) were related to phenotype, EDSS, ambulation index (AI) and 9 hole peg test (9HPT).

Results: 74% of patients with SP/PP disease experienced an increase in delta NfH-PP compared to 20% maintaining RR disease (p<0.05, Fishers exact test). SP/PP patients at baseline (80.5 pg/mL) and followup (131 pg/mL) had higher NfH-SMI35 levels than stable RRMS patients (below assay sensitivity, p<0.001). NfH-SMI35 correlated with the EDSS (r=0.54, p=0.01), the AI (r=0.42, p<0.05) and the 9HPT (r=0.59). No correlations were found for NfH-SMI34.

Conclusions: This study demonstrated that NfH-phosphoforms might predict the development of disability in RRMS, supporting the concept that early axonal injury is a poor prognostic sign. During the progressive phase of the disease an increase of NfH was observed, suggesting cumulative axonal damage. The correlation of NfH-SMI35 with all 3 clinical scales finally suggests that axonal pathology in MS is a dynamic process.

Disclosure: A Petzold has nothing to disclose.

Funding: Multiple Sclerosis Society of Great Britain and Northern Ireland

P12
INCREASING SERUM NITRIC OXIDE METABOLITES ARE RELATED TO DISEASE ACTIVITY IN PATIENTS WITH MULTIPLE SCLEROSIS.
aNeuroimmunology, Institute of Neurology, London, United Kingdom; bNeurology, Medical University, Lublin, Poland

Background: Nitric oxide (NO) is a reactive compound involved in acute inflammatory processes. Increased levels of the NO metabolites, nitrate and nitrite (NOx), are found in MS patients. The role of NO in the pathogenesis of MS is complex and remains unclear.

Objectives: To elucidate the relationship of serum NOx levels to disease activity measured using Gd-enhanced MRI and disease progression.

Methods: Twenty-five patients with early relapsing-remitting MS (RRMS) were enrolled in a longitudinal study. Patients were on average followed up for 12 months and underwent a series of clinical (EDSS) and magnetic resonance (MRI) assessments. Serum samples were obtained at each visit for measurement of total serum NOx using a vanadium-based assay.

Results: Mean serum NOx was raised in patients who had enhancing MRI lesions compared to those without or with very low enhancement (<260mm3) during the study period as well as to healthy controls (45.7±11.5uM vs. 35.0±8.1uM and 32.7±13.3uM; p=0.02, p=0.004; respectively). Similarly, patients who progressed on EDSS assessment during the study had higher serum NOx levels compared to stable patients (46.6±12.5uM vs. 35.3±10.0uM; p=0.02). There was no correlation between serum NOx levels and other MRI parameters of disease progression.

Conclusions: Serum NOx levels appear to be increased in the early relapsing-remitting phase of MS and are associated with MRI apparent inflammatory disease activity.

Disclosure: K Rejdak has nothing to disclose.

Funding: K. Rejdak is a Marie Curie Fellow.

P13
IMMUNOLOGICAL MARKERS OF DISEASE ACTIVITY IN NEWLY-DIAGNOSED MULTIPLE SCLEROSIS: A ONE YEAR FOLLOW-UP STUDY IN MRI MONITORED PATIENTS
aResearch & Innovation, Padova, PD, Italy; bDept. of Neurological and Psychiatric Sciences, University of Padova, First Neurology Clinic, MS center, Padova, PD, Italy; cEuganea Medica, Diagnostic Centre, Padova, PD, Italy

Background: Autoimmune aggression to myelin antigens is currently thought to be responsible for MS immunity-pathology and MS still today remains orphan of immunological markers of diagnostic/prognostic relevance.

Objectives: We designed a clinical protocol for the study of circulating parameters in MS, in attempt to correlate possible ongoing immunological changes with disease activity assessed by EDSS score and MRI burden.

Methods: The protocol entails a one year follow-up of 20 newly diagnosed untreated MS patients, that, every 45 days, undergo clinical examination and
cerebral MRI. At the same time points, immunophenotyping (FACS) of a broad-spectrum of lymphocyte subsets is performed: NKT, T regulatory, central/effector T memory, Th1/Th2, Tc1/ Tc2, NK and T CD8+ expressing KIRs, and CD1d+ APC.

**Results:** To date 18 patients have been recruited and initial results show that each MS patient is characterised by an individual pattern of lymphocyte subsets, the general features of which are maintained throughout the follow-up. Nevertheless subgroups of patients are distinguishable on the basis of the percentage of circulating lymphocytes (e.g., NK, γδ T) or the preferential expression of KIRs on NK and CD8+ T cells. In addition, preliminary evidence suggests an association between increased MRI lesion load, increasing EDSS and fluctuations in the frequency of NKT cells, CD4+CD25+ T cells, CXC8+CC5+CD4+ and CXCR3+CCR5+CD8+ T cells, and NK and CD8+ T cells expressing one or more KIRs.

**Conclusions:** Although the study is still in progress, results currently available show the occurrence in MS of a complex immunological interplay, which may underlie the observed MRI changes. This favours the possibility that well-defined immunophenotypic panels may be used in monitoring the immunopathological events of MS.

**Disclosure:** L Rinaldi has nothing to disclose.

**Funding:** Supported by Italian Ministry of Education, University and Research (MIUR)/project n° 3933.

**P14**

**COGNITIVE FATIGUE DURING A TEST REQUIRING SUSTAINED ATTENTION**

Schwid SR, Weinstein A, Goodman AD, Scheid EA, Tyler CM, McDermott MP

University of Rochester, New York, USA

**Background:** Fatigue is common in MS, but difficulty quantifying fatigue severity has impeded studies of its characteristics, mechanisms, and therapeutics. Motor fatigue can be objectively measured as the decline in strength occurring during sustained contractions. Analogous declines in cognitive performance occur during tasks requiring sustained attention.

**Objectives:** To objectively measure cognitive fatigue as a decline in performance during administration of the PASAT (3rd version), which requires sustained attention for 3 minutes.

**Methods:** Patients with clinically stable MS (n=20) and healthy controls (n=21) matched for age, gender, and education completed the PASAT at 2 identical test sessions separated by 4-10 days, within a month after 2 practice sessions. A cognitive fatigue index (CFI) was calculated from the number of correct responses in each block of 10 PASAT items. Reliability of the CFI was determined from the intraclass correlation coefficient (ICC), and validity was evaluated from correlations with self-reported fatigue (Fatigue Severity Scale [FSS], Modified Fatigue Impact Scale [MFIS], Rochester Fatigue Diary), cognition (PASAT total score, MMSE), and overall neurological impairment (EDSS, MS Functional Composite).

**Results:** MS patients had a mean of 9.3 items correct on the first 10 items of the PASAT and 8.5 on the last 10 items (CFI of 12%, p=0.01 rejecting the hypothesis CFI=0). Mean CFIs for MS patients were similar at both sessions, but within-patient reproducibility was relatively low (ICC=0.49). The CFI correlated with the FSS (r=0.58, P=0.008), but not with other measures of self-reported fatigue, including the cognitive subscale of the MFIS. Cognitive fatigue scores correlated with total PASAT (r=-0.68, p=0.001) and MMSE scores (r=-0.47, p=0.04) in MS patients but not in controls. Cognitive fatigue scores were not associated with overall neurological impairment, and there were no significant differences between patients with relapsing and progressive MS. Control patients did not demonstrate significant declines in performance.

**Conclusions:** MS patients, but not controls, have measurable cognitive fatigue during administration of the PASAT. Comparison of measures with self-reported fatigue, cognition, and impairment further establish the construct validity of this entity.

**Disclosure:** S Schwid has nothing to disclose.

**Funding:** Supported: A grant from the The National MS Society.

**P15**

**RELEVANT SENSITIVITY OF TIME TO WALK 25 FEET FOR PROGRESSION OF DISABILITY**

Schwid SR, Goodman AD, Scheid EA, McDermott MP

University of Rochester, New York, USA

**Background:** Time to walk 25 feet (T25FW) was selected as the test of LE function for the MS Functional Composite (MSFC) based on a dataset with limited information about 2 continuous and 3 ordinal tests.

**Objectives:** To determine the relative sensitivity of T25FW and other measures of LE function for progression of disability in patients with MS.

**Methods:** Thirty patients with MS, worsening LE symptoms in the past year, and measurable LE weakness were tested on five consecutive days during a period of clinical stability to establish baseline variability, and after 3, 6, 9, 12, 18, and 24 months to determine change over time. Relative sensitivity for progression was determined by t-tests (or Wilcoxon signed rank tests for ordinal measures) of the mean changes from baseline for measures of ambulation (T25FW, maximum distance walked [Dmax, up to 500m], velocity on 500m walk [V500]), AI, strength (manual motor testing in 18 muscles [MMT]), and coordination (foot taps in 5 seconds [FT], timed balance test [TBT]). Composite measures (EDSS, MSFC, Scripps Neurological Rating Scale [SNRS]) were also assessed. The 95% confidence interval for the within-patient variability on each outcome measure during the baseline period was used to determine the number of patients worsening.

**Results:** Participants had mean age 51.9 (sd=8.6) years, relapsing (n=11), primary (n=2) or secondary progressive (n=17) courses, mean MS duration 13.8 (10.6) years, and baseline EDSS 2.5-6.5. They were taking a variety of course-modifying and symptomatic therapies, and 70% were women. Mean T25FW scores were significantly worse than baseline at the 9-month visit (p = 0.01) and thereafter, with 8-12 patients demonstrating worsening beyond their baseline variability at each visit. None of the other measures demonstrated worsening as early, although V500 and AI were more sensitive to worsening (p = 0.002 and 0.009) and had a higher number of worsened patients (n = 14 and 9) by 24 months. Of the 19 patients who had worsened T25FW at least once, 4 (21%) were worse than baseline at only one visit. Eight (42%) remained worse than baseline at all subsequent visits.

**Conclusions:** Change from baseline evolves more rapidly for T25FW than for other measures of LE impairment, but it may not be the most responsive to change at later time points.

**Disclosure:** S Schwid has nothing to disclose.

**Funding:** Spororted: by National MS Society.

**P16**

**CYTOCHROME P46, A SUITABLE MARKER FOR NEURODEGENERATION IN EAE-MODELS AND MS?**

Teunissen CE+, Dijkstra CD+

*a Mol. Cell Biology, VU Medical Centre Amsterdam, Netherlands; b Mol. Cell Biology, VU Medical Centre Amsterdam, Noord Holland, Netherlands

**Background:** Cytochrome P46 (CYP46) is a brain-specific CYP450 enzyme catalyzing the formation of 24S-hydroxy-cholesterol. Oxidation of cholesterol into 24S-hydroxy-cholesterol likely is an important route for cholesterol transportation out of the brain, and this oxysterol is almost specifically formed in the brain. A recent report showed a decrease of CYP46 in neuronal cell bodies, axonal structures and oligodendrocytes in the cortex of patients with Alzheimer’s disease compared to controls. In astrocytes, in contrast, increased presence of CYP46 was observed. Since in MS axonal loss, demyelination as well as astrogliosis occur, it may be expected that the presence of CYP46 in neuronal axons or oligodendrocytes may be decreased and the presence of CYP46 in astroglia may be increased in this disease.
Objectives: The objective of our experiments was to investigate if CYP46 could be a suitable marker for neurodegeneration in MS.

Methods: The expression of CYP46 will be studied in MS lesions. Experimental autoimmune encephalomyelitis (EAE) stands as an animal model for MS and we will use this model to investigate if the axonal presence of CYP46 is altered in EAE. Both acute monophasic as well as chronic EAE will be studied for CYP46 expression in the brain and spinal cord. The aim of the present experiment was to study the immunocytochemical CYP46 staining in control brain tissue sections of humans, rats and mice.

Results: The results showed the presence of the CYP46 staining in large neurones in the human grey matter. CYP46 staining was also present in endothelial cells, Dendrocytes and microglia. In mice and rat brain, CYP46 staining was observed in neuronal structures in the hippocampus, cortex, striatum and thalamus.

Conclusions: The results obtained in control brain tissue of humans and rodents show that CYP46 staining is present in different neuronal subtypes known to be affected in MS lesions.

Disclosure: C Teunissen has nothing to disclose.

Clinical Aspects of MS (Part 1)

P17
SCREENING FOR DEPRESSION IN MULTIPLE SCLEROSIS: YALE SINGLE QUESTION VS. BECK DEPRESSION INVENTORY SCALE
Avasarala JR, Cross A, Trinkaus K
Neurology, Washington U School of Medicine, St Louis, Missouri, USA

Background: Screening for depression in patients with MS currently includes Beck Depression Inventory (BDI), a 21 item self-report rating inventory that measures characteristic attitudes and symptoms of depression. However, BDI might not be the most appropriate instrument if simpler techniques can be used to screen for depression. We compared the accuracy of a Yale single question screen (YSQ) response against BDI to screen for depression in MS patients.

Objectives: To examine if depression in MS can be accurately recognized using YSQ as compared to BDI, a 21-item self-report rating scale for depression.

Methods: One-hundred twenty consecutive MS patients seen in our MS Clinic were screened for depression by asking patients the YSQ - “Do you frequently feel sad or depressed?” followed by BDI administration. Depression was defined as a score > 13 on the BDI.

Results: Of the 120 patients studied, a total of 49/120 were clinically depressed as defined by a BDI cut-off of > 13; 71/120 were not. The sensitivity of YSQ was 32/49 or 65.3% with a 95% confidence interval (0.50, 0.78), specificity was 62/71 or 87.3% (0.77, 0.94), positive predictive value was 32/41 or 78.0% (0.62, 0.89) and negative predictive value was 62/79 or 78.5% (0.68, 0.87). Of the 49 patients depressed by BDI criteria, 17 patients responded ‘no’ to YSQ, yielding a false-negative rate of 34.7% (0.22, 0.50).

Conclusions: Our results show that YSQ cannot replace BDI as an instrument for screening for depression in MS. YSQ could not identify 34.7% of patients who were depressed by BDI criteria.

Disclosure: J Avasarala has nothing to disclose.

Funding: J. Avasarala is a postdoctoral fellow of the National MS Society.

P18
MULTIPLE SCLEROSIS AND HASHIMOTO’S THYROIDITIS: TWO CASES
Balcý BR, Yayla V, Özer F
Neurology, Haseki Hospital, Istanbul, Turkey

Background: Multiple sclerosis (MS) occurs with immune-mediated mechanisms and its pathogenesis is not known accurately. Coexistence with other autoimmune diseases reported. Although the real prevalence of association is unknown, there are some case reports of MS associated with autoimmune thyroid diseases.

Objectives: We present the clinical data of two patients who fulfilled Hashimoto’s thyroiditis diagnostic criteria.

Methods: Among 106 consecutive patients who fulfilled the diagnostic criteria of Poser for definite MS, two patients who had the diagnosis of Hashimoto’s thyroiditis by the determination of T3, TSH and antithyroidal antibodies were presented.

Results: We had two MS patients associated with Hashimoto’s thyroiditis (1.89%). The patients were 35 and 38 years old women and had relapsing remitting (RR) MS of benign course and secondary progressive form respectively. None of them were under interferon treatment. In both of them Hashimoto’s thyroiditis diagnosis was previous to MS diagnosis. Clinical manifestation of hypothyroidism was mild in both of the patients.

Conclusions: Hashimoto’s thyroiditis is relatively common in MS patients, especially in women (4.3%). This high association was not related to the interferon in our series. Thyroid autoimmune diseases should be carefully determined in patients suffering MS.

Disclosure: B Balcý has nothing to disclose.

Posters

P19
EFFECT OF INTERFERON-BETA-1B ON COGNITIVE FUNCTIONS IN MULTIPLE SCLEROSIS
BARAK Y, ACHIRON A
Psychogeriatrics, ABARBANEL Hospital, Bat-Yam, NA, Israel

Background: Multiple sclerosis (MS) is recognised as a central nervous system disease also affecting cognition. The rate of cognitive dysfunction in MS is in the range of 45-65% and adversely affect the quality of life.

Objectives: To evaluate the effect of 1 year of treatment with interferon-beta-1b (IFN -1b) on cognitive functions in patients suffering from relapsing-remitting MS.

Methods: A battery of cognitive tests was used to assess verbal learning, delayed recall, visual learning and recall, complex attention, concentration and verbal fluency at baseline and after 1 year of treatment with IFN -1b. A group of 23 relapsing-remitting MS patients matched for neurological disability served as controls.

Results: Eighteen of 23 patients treated with IFN -1b (74%) completed the study. In the IFN -1b-treated group, complex attention, concentration as well as visual learning and recall improved significantly (p = 0.024, p = 0.006 and p = 0.005, respectively), while no deterioration was observed in the other dimensions. In the control group, complex attention, verbal fluency, as well as visual learning and recall deteriorated significantly (p = 0.02, p = 0.004 and p = 0.01, respectively), while no deterioration was observed in the other dimensions.

Conclusions: Immunomodulating drugs that reduce the relapse rate and slow the disease progression also inhibit cognitive deterioration in patients with MS.

Disclosure: Y BARAK has nothing to disclose.

Funding: The study was Supported by an educational grant from Schering, Germany.

P20
COMPUTERIZED ISOMETRIC MUSCLE STRENGTH - NATURAL HISTORY IN MULTIPLE SCLEROSIS
Brooks BR*, Sanjak M*, Belden D*, Dogan S*, Konopaki R*, Roelke K*, Peper Sa,c, Parnell Ja,c
aMotor Performance Lab, Dept Neurology, University of Wisconsin-Madison Med Sch, Madison, Wisconsin, USA; bNeurol Svc, Wm S Middleton Memorial VA Med Ctr, Madison, Wisconsin, USA; cMS Clinical Research Ctr, Dept Neurology, University of Wisconsin Hospital & Clinics, Madison, Wisconsin, USA

Background: Clinical changes occur within Expanded Disability Status Scale steps that may be crucial to patient function such as walking or transfers.
Prospective longitudinal measurement of maximum voluntary isometric contraction [MVIC] in 10 arm and 10 leg muscles, as well as 2 neck muscles has been ongoing at the University of Wisconsin Hospital & Clinics and VA Medical Center MS Clinics [http://www.neurology.wisc.edu/MPL/index.htm] in 279 [227F/70M] MS patients.


Methods: CIMS measurement of participating MS patients occurred at each clinic visit. MVIC was transformed to muscle-specific percent predicted muscle strength according to equations developed by the National Isometric Muscle Strength (NIMS) Database Consortium for use of normal isometric strength data for weakness assessment [Archives of Physical Medicine & Rehabilitation. 77(12): 1251-5, 1996]. MS patients were studied cross-sectionally by age, gender(sex), site of disease onset, disease duration, EDSS, AI. MS patients were studied longitudinally by these parameters, as well as development of exacerbation or progression,treatment and response to treatment.

Results: MVIC decreases significantly in motor exacerbations involving arm and leg and, like recovery seen in polioleymatitis, seldom achieves recovery to normal MVIC. Leg MVIC correlates better with EDSS and AI than arm MVIC. In MS patients who require wheelchairs arm MVIC decreases from 87.04±24.21 [SD] percent predicted to 73.45±21.83 percent predicted [p=0.00010] while leg MVIC decreases from 86.54±26.34 percent predicted to 57.74±28.58 percent predicted [p=0.00001].

Conclusions: Longitudinal changes in MVIC may be studied in MS as a function of treatment with anti-inflammatory agents, immunosuppressive agents, immunomodulatory agents, anti-fatigue agents, anti-spasticity agents, anti-tremor agents.

Disclosure: BR Brooks has served as a consultant to Teva Neurosciences, tremor agents.

The MSFC is a sensitive scale to measure disability in MS, because it integrates function [p<0.0001] while leg MVIC decreases from 86.54±26.34 percent predicted to 73.45±21.83 percent predicted [p=0.0001], we found, also, a correlation to the mean evolution time was of -0.710 (Spermann rank correlation). The correlation to the normal MVIC was of -0.534 and to the EDSS was of -0.710 (Spermann rank correlation). The correlation between our series and the normalized population was 0.997 in relation to the population was reached by the NMSS task force, and as in the pivotal study from Cutten, a significant correlation between the EDSS and the mean evolution time for the three compounds and the total Z-score of the MSFC was demonstrated

Disclosure: B Casanova has nothing to disclose.

P22

EFFECT OF TRAINING ON THE MULTIPLE SCLEROSIS FUNCTIONAL COMPOSITE SCALE IN EARLY RELAPSING AND REMITTING MS. A LONGITUDINAL ONE YEAR FOLLOW-UP STUDY

Casanova B, Coret F, García E, Bernt A, Valero C, De Vera A, Pascual A

*Neurology, Hospital La Fe, Valencia, Valencian Country, Spain; **Neurology, Hospital Clinic, Valencia, Valencian Country, Spain

Background: The MSFC has been proposed as a more sensitive form to measure the clinical state from MS patients, but the first exams may be biased by practice, as has been demonstrated by Cohen who noted continuous improvement in the MSFC in the first three exams.

Objectives: To study the effect of training on the MSFC for one year in an early population of MS patients

Methods: We have studied 30 consecutive MS patients with clinical definitive MS (22) or probable MS (8) according to the Poser criteria, mean age 26 years old, mean evolution time 18 months and 1.4 of EDSS to PMS and 1.8 of EDSS to CDMS, with no statistical differences between groups. The Z-score from the FCMS has been calculated at basal time and each sixs months, in two ways; one in relation to the Task Force Database to allow comparison between studies, and another to longitudinal studies with the follow formula: ((Mean (1/9 NPHT) - Basal mean (1/9 NPHT)) / (Mean 8m - Basal medio (1/9 NPHT)) / (Mean 8m - basal mean 8m) / Basal DS 8m + (PASAT3 -Basal mean PASAT3 / Basal DS PASAT3) / 3, according to the guidelines published by the NMSS. The Z-scores values have been compared between the two different populations of MS patients, at basal time and after one year of evolution. We have used the Wilcoxon test, t-test for independent variables analysis of means and the Spermann rank for correlations.

Results: Correlations between our series and the normalized population was 0.892 (p=0.0001) at first examination, and 0.923 (p=0.000001) one year after (after undergoing two previous exams). At the baseline a significant difference for Z-score was demonstrated between PMS and CDMS patients (p=0.01), with no correlation with the EDSS (Spermann rank =-0.188, p=0.5). In the follow up the Z-score improved slightly from 0.004 to 0.11, with no differences between patients that had suffered from bouts or remained as PMS. The differences between the basal Z-score and the Z-score measure after one year wasn’t statistically significant (p=0.7)

Conclusions: The MSFC is a sensitive measure of the activity in MS patients, even in the beginning of the disease, but a training of almost two exams are necessary to compensate for practice effects and improve the sensitivity.

Disclosure: B Casanova has nothing to disclose.

P23

PROBABLE CUTANEOUS SARCOIDOSIS ASSOCIATED WITH INTERFERON-BETA 1B TREATMENT IN MULTIPLE SCLEROSIS.

Clere C, Chevalier Y, Bataillard M, Rumbach L, Richard P

*Service de neurologie, C.H. Bouloche, Montbeliard, France; **Service de neurologie, CHU Minjoz, Besancon, France

Background: Adverse cutaneous effects described after interferon-beta 1b injections are numerous and appear mostly near to the injection site. However sarcoid-like subcutaneous nodules are rarely reported.

Objectives: Description of an unusual adverse cutaneous effect during interferon-beta 1b therapy.

Methods: Case report

Results: A 49 years old woman was diagnosed with multiple sclerosis (MS) 20 years ago. In January and November 1999, she presented two attacks. Interferon-beta 1b therapy was prescribed, she did not inform her treating physician for symptoms described in the information sheet. In March 2000, she developed subcutaneous nodules. These nodules were without symptoms except for itching. They were less than 1 cm in diameter. Despite interferon-beta 1b therapy being stopped, the nodules persisted. After 3 months, these nodules disappeared.

Disclosure: B Casanova has nothing to disclose.
beta-1b (8 MUI self-administered by subcutaneous injections every other day) was started in March 2000. Ten months later, she developed subcutaneous nodules on her forearms. Biopsy showed features compatible with sarcoidosis and the serum angiotensin-converting enzyme level was elevated. After interferon beta-1b therapy disruption, cutaneous lesions disappeared.

Conclusions: Interferon alpha has already been implicated in the development or exacerbation of auto-immune diseases including sarcoidosis. According to this case report, the possible role of interferon beta-1b in the pathogenesis of sarcoidosis is discussed.

Disclosure: C Cocco has nothing to disclose.

P24

BENIGN MULTIPLE SCLEROSIS IN SARDINIA: A RETROSPECTIVE STUDY

Cocco EE, Lai MM, Marchi PP, Floris GG, Fadda LL, Sanna SS, Marroso MM

Background: Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system, which affects predominantly individuals in their most productive period of life. MS course and its clinical outcome could be extremely heterogeneous ranging from death in few weeks from onset to disease discovered casually at autopsy. Benign MS is defined by the presence of minimal or no disability in the long term, but there is lack of agreement in quantitative terms.

Objectives: To evaluate the frequency and characteristics of “benign MS” in MS Sardinian patients with long disease history.

Methods: Among all (1402) clinically defined MS Sardinian patients followed at the Multiple Sclerosis Centre of Cagliari, we selected patients with a long duration of disease (20 years or longer). We considered as “benign” patients presenting a disability measured by Kurtke EDSS scale <3 after 20 years of disease.

Results: Among 1402 Sardinian MS patients 276 (19.7%) had a disease duration longer than 20 years and 55 (3.9% of total patients and 19.9% of patients having >20 years of disease duration) of them presented disability <3, thus fulfilling definition of benign MS. Thirty three were female an 22 were male, the median age was 47.2 years (DS +/-7), the age at onset was 22.4 years (DS +/- 6.6) the median duration of the disease was 24.8 (DS +/-4.5). There no statistical difference between benign MS and the other MS patients for sex, age at onset and age at observation.

Conclusions: Our results sustain the existence of “benign MS” in Sardinian patients having more than 20 years of disease duration. However the percentage of benign patients is relatively small, thus suggesting than only in few cases MS in long term does not cause a consistent disability. The availability of disease modifying treatments for MS and the increasing evidence of the utility of early treatment sustains the need of a good definition of “benign MS” and of early predictive markers for its detection.

Disclosure: E Cocco has nothing to disclose.

P25

ACUTE PARTIAL TRANSVERSE MYELOPATHY: A 7-YEAR PROSPECTIVE STUDY


*neurology, university, Lille, lille, France; **neuroradiology, university, Lille, lille, France

Background: Clinical and radiological characteristics of myelopathy in multiple sclerosis (MS) are relatively well known. Nevertheless, it remains difficult for the clinician to ascertain an evolution into MS after a first episode of acute partial transverse myelitis (APTM). Furthermore, there is a need for predictive factors of bad evolution in order to decide which patients should be treated by immunomodulatory drugs early in the course of the disease.

Objectives: The aims of this study were to define predictive criteria of conversion into clinically definite MS after an APTM and to define predictive factors of severe disability progression.

Methods: Between 1994 and 2001, we prospectively included 55 APTM (28.85% men, 71.15% women, mean age of 35 years). Three patients were lost during the follow-up. We evaluated clinical, spinal cord and brain MRI, cerebrospinal fluid (CSF) and visual evoked potentials (VEP) at admission. Mean duration of follow-up was 35 months (range 12-86). At endpoint we evaluated diagnosis and among the MS subgroup, severity of the disease.

Results: Among the 52 APTM who completed the study, 30 became definite MS (23.33% men, 76.67% women, mean age 33 years, mean follow-up 37 months) and 22 remained of unknown aetiology (36.36% men, 63.64% women, mean age of 37.8 years, mean follow-up 31.5 months). All the MS patients remained in the relapsing remitting form of MS. The discriminating factors for the evolution in MS were: initial sensorial symptoms (p=0.009), lateral-posterior spinal cord lesion (p=0.01), abnormal brain MRI (p=0.002), oligoclonal bands in CSF (p=0.003), but not VEP. In the clinically definite MS subgroup, we did not find any predictive factors of severe disability progression.

Conclusions: Our study demonstrates that clinical symptoms, CSF, spinal cord and brain MRI are highly predictive of an evolution of APTM into clinically definite MS. These patients should be selected for an early treatment by immunomodulatory drugs.

Disclosure: C Cordonnier has nothing to disclose.

P26

MS GENDER ISSUES: CLINICAL PRACTICES OF WOMEN NEUROLOGISTS


Women Neurologist’s MS Initiative, WNMSI, New York, USA

Background: Lack of data on gender issues impacts female MS patients. The Women Neurologist’s MS Initiative (WNMSI) is a group of women neurologists interested in gender-based MS issues.

Objectives: To assess clinical practices of women neurologists regarding gender-related MS issues.

Methods: A 4-pg 16 question survey, mailed to 419 US Board Certified/eligible women neurologists (from academic, private practice, or MS centers). Non-respondents received 1 reminder after 6 wks.

Results: A total of 147 surveys were returned (35%). The majority were from private practitioners (68%), treating 50-100 MS patients/yr (23%); >100 patients/yr (39%). 91% treated ≥10 patients of child-bearing age/yr, with 57% treating ≥2 MS pregnancies/yr. Respondents ranked FDA Pregnancy Category B, company insert, and published data as 3 key factors guiding disease modifying therapy (DMT) use in pregnancy. 52% insist patients cease DMT immediately if pregnant, while 36% discourage use. In patients planning pregnancy, 81% recommend ceasing interferon therapy prior to pregnancy, 14% recommend continuing to conception. With glatiramer therapy, 70% recommend ceasing therapy before pregnancy, 22% recommend continuing to conception. Of DMTs, glatiramer acetate was described as safest in pregnancy by 52%, 48% felt there was no difference. When DMTs were not used during pregnancy, respondents restart therapy immediately after delivery or breastfeeding (53%) or within 1-3 menstrual cycles (43%). With regard to breastfeeding, nearly half (49%) make no specific recommendation, 38% recommend it, and 13% discourage it. DMTs were usually not recommended during breastfeeding (88%). With regard to contraception 2/3 of respondents do not recommend specific methods, but refer patients to an OB/GYN (100%) or a gynecologist (13%). Most MS patients use oral contraceptive, tubal ligation, or barrier methods. Menstrual irregularities were reported in 25% of MS patients. In the survey 36% recommend HRT very frequently or frequently, 25% occasionally or rarely, and 42% never recommend it; 92% refer patients to an OB/GYN.

Conclusions: Surveyed neurologists show a high interest in MS gender issues. Over 90% of respondents would participate in a prospective study. However, results indicate no real consensus on current clinical practices. Future
goals involve development of a consortium, database, and provide for future studies.

Disclosure: P Coyle has nothing to disclose.

P27

IS DEVIC NEUROMYELITIS OPTICA A SEPARATE DISEASE? A COMPARATIVE STUDY WITH MULTIPLE SCLEROSIS

de seze 1, Lebrun C, Stojkovic T, Ferraby D, Chatel M, Vermersch P
1Neurology, CHRU de Lille, Lille, Nord, France; 2Immunology, CHRU de Nice, Nice, Cote d’Azur, France

Background: Devic neuromyelitis optica (NMO) associates optic neuritis and myelitis without other neurological signs. Many patients with NMO may be diagnosed as having multiple sclerosis (MS), optic neuritis and myelitis being the inaugural symptom in 20% and 5% of MS cases, respectively. However, there have been no previous studies comparing these two pathologies and it is still unclear if NMO is a separate entity or a syndrome including MS, systemic or infectious diseases.

Objectives: To compare NMO patients with MS patients revealing by optic neuritis or myelitis, in order to determine the place of NMO in the spectrum of MS.

Methods: We retrospectively studied 30 patients diagnosed with NMO. We compared these patients with 50 consecutive MS cases revealed by optic neuritis or myelitis, in order to determine the place of NMO in the spectrum of MS.

Results: NMO patients were older and more frequently female than MS patients but the differences were not significant. CSF and MRI data were different: oligoclonal bands were found in 23% of NMO cases and 88% of MS cases (p<0.001 for both EDSS and IP). If we include MRI data only two of the 10 patients with PPMS and SS criteria (60%). The frequency of anti-alpha-fodrin autoantibodies was statistically higher in this last group compared with MS patients without SS criteria both when we considered all MS patients (p<0.01) and the 20 PPMS without the SS criteria (p<0.05).

Conclusions: Our study confirmed that aAb against alpha-fodrin are reliable markers for SS and may differentiate MS and SS, especially in the progressive form of MS. These results are consistent with the fact that SS and PPMS are different diseases rather than PPMS could be associated with SS.

Disclosure: J de seze has nothing to disclose.

P28

DIFFERENTIAL DIAGNOSIS BETWEEN MULTIPLE SCLEROSIS AND SJOGREN SYNDROME: INTEREST OF ALPHA-FODRIN ANTIBODIES

de seze 1*, Dubaequoit S, Matthias T, Lefranc D, Dussart P, Vermersch P, Witte T
1Neurology, CHRU de Lille, Lille, Nord, France; 2Immunology, CHRU de Lille, Lille, Nord, France; 3Immunology, University of Hanover, Germany

Background: Sjogren syndrome (SS) with neurological manifestations raised a major problem for the diagnosis because anti-Ro (SSA) and anti-La (SSB) antibodies are found in less than 50% of cases. Furthermore, SS with neurological manifestations may mimic multiple sclerosis (MS) and especially in the primary progressive forms of MS (PPMS). We previously observed that SS could be misdiagnosed with PPMS in around 15% of cases. A recent study underlined the interest of a new biological marker for SS, showing IgA or IgG autoantibodies (aAb) against alpha-fodrin in almost 70% of patients with primary SS and in less than 5% of blood donors.

Objectives: To investigate the interest of aAb against alpha-fodrin in order to discriminate SS and MS patients especially PPMS.

Methods: We tested alpha-fodrin aAb, with an ELISA method, in 30 SS patients with neurological manifestations, 60 MS patients without SS (20 patients with relapsing-remitting MS, 20 patients with PPMS and 20 patients with secondary progressive MS) and 10 patients who responded both to PPMS and SS criteria.

Results: IgA and/or IgG levels against alpha-fodrin were observed in 19 of the 30 SS patients (63%) with neurological disease, in 8 of the 60 MS patients without SS (13.3%) unrelated with the clinical form of MS, and in 6 of the 10 patients with PPMS and SS criteria (60%). The frequency of anti-alpha-fodrin autoantibodies was statistically higher in this last group compared with MS patients without SS criteria both when we considered all MS patients (p<0.01) and the 20 PPMS without the SS criteria (p<0.05).

Conclusions: Our study confirmed that aAb against alpha-fodrin are reliable markers for SS and may differentiate MS and SS, especially in the progressive form of MS. These results are consistent with the fact that SS and PPMS are different diseases rather than PPMS could be associated with SS.

Disclosure: J de seze has nothing to disclose.
P30
CLINICAL, CEREBROSPINAL FLUID AND NEUROIMAGING FINDINGS IN OPTICOSPINAL INVOLVEMENT IN THE SPECTRUM OF INFLAMMATORY DEMYELINATING DISEASES
Eraksoy M, Turan N, Kurtunec M, Akman-Demir G, Yapici Z, Deniz E, Ozcak H
Neurology, Istanbul University, Faculty of Medicine, Istanbul, Turkey

Background: The selective involvement of optic nerve(s) and spinal cord may be encountered in the spectrum of inflammatory demyelinating diseases. There has been some debate on the classification and the naming of these group of diseases.

Objectives: The objective of this study was to determine the clinical, neuroimaging, cerebrospinal fluid and other laboratory findings of 26 patients with inflammatory demyelinating disease presented with opticospinal involvement.

Methods: The case records of patients seen in our department between June, 1987 and April 2002 and diagnosed as Devic’s syndrome, Devic’s neuromyelitis optica, Devic’s disease, opticneuropathy/transverse myelopathy and opticospinal MS were reviewed. We classified patients who met the criteria set by O’Riordan at al. The findings of these patients were compared with the clinically definite multiple sclerosis (CDMS) patients presented with uni- or bilateral optic neuropathy (ON/BON).

Results: There were 21 women and 5 men with a mean age 26 range (4-52) years. This study revealed that most of the patients in this group developed multiphasic Devic’s neuromyelitis optica (DNO), small group of patients had monophasic Devic’s disease, opticneuropathy/transverse myelopathy and opticospinal MS were reviewed. We classified patients who met the criteria set by O’Riordan at al. The findings of these patients were compared with the clinically definite multiple sclerosis (CDMS) patients presented with uni- or bilateral optic neuropathy (ON/BON).

Conclusions: This study revealed that the occurrence of opticospinal involvement in inflammatory demyelinating diseases seemed a result of different etiopathogenetic factors. Some of the clinical and laboratory clues showed that there has been a close relationship between inflammatory demyelinating diseases, vasculitis and DNO showing an opticospinal presentation. The genetic and environmental factors which modify the acuteness and tempo of the process might explain the differences of the syndrome presented with opticospinal involvement.

Disclosure: M Eraksoy has nothing to disclose.

P31
RECURRENT SYNCOPE AS THE PRESENTING SYMPTOM IN MULTIPLE SCLEROSIS
Fazio MC, Musolino R, Buccafusca M, Dattola V, Scalﬁari A, Girlanda P, Messina C
Neurosciences, Psychiatry and Anaesthesiology, University of Messina, Messina, Italy

Background: Syncopal episodes are relatively underestimated presenting symptom of Multiple Sclerosis (MS). The cause and the pathophysiology of these episodes and their incidence in MS are unknown. In literature only two isolated cases are reported (Sakakihara et al. 1997, Funakawa et al. 1998).

Objectives: We describe two patients, who presented with recurrent episodes of loss of consciousness. Case report: a male and a female, 51 and 31 years old respectively. The first patient had been presenting syncopal episodes for five years and the second one for twelve years before our observation. They both had not consulted a neurologist and had received the diagnosis of “sincopal episodes”. Due to an increase of episodes, the patient were addressed to neurological evaluation.

Methods: Neurological examination, brain and spinal cord MRI, visual and somatosensory evoked potential and cerebrospinal fluid examination were performed in both patients.

Results: The neurological examination revealed in the male patient mild tendon hyperreflexia and a left Babinski sign; the female patient had tendon hyperreflexia, decreased deep sensation and mild ataxia. The brain and spinal cord MRI showed in both the presence of typical lesions of a demyelinating disease. In both patients one lesion in the paramedian tegmentum and another one in the high cervical spinal cord (C1-C2) with enhanced gadolinium were found. Visual and somatosensory evoked potential were also abnormal and cerebrospinal fluid examination revealed oligoclonal bands. The patients were treated with high dose IV methylprednisolone and syncopal episodes did not recur after more than one year.

Conclusions: We underline that syncopal episodes can be presenting symptom of MS. A possible relationship with localization of demyelinating lesions in brainstem and high cervical spinal cord has been hypotized.

Disclosure: M Fazio has nothing to disclose.

P32
THE ITALIAN DEVIC’S STUDY GROUP (IDESG) - PART 1: CLINICAL CHARACTERISTICS OF DEVIC’S NEUROMYELITIS OPTICA AT ONSET
1 Centro Studi SM, Gallarate, (VA), Italy; 2 Clinica Neurologica, Pavia, (PV), Italy; 3 Clinica Neurologica, Milano, (MI), Italy; 4 Clinica Neurologica, Ferrara, (FE), Italy; 5 Clinica Neurologica, Bari, (BA), Italy

Background: It is not clear whether Devic’s Neuromyelitis Optica (DNO) is a form of MS or a separate syndrome. Apart from one study (Wingerchuk et al. 1999) data are not available on the clinical and laboratory characteristics of DNO.

Objectives: Our study was undertaken to evaluate these aspects: in this study clinical findings at onset will be presented.

Methods: several Italian neurologics departments involved in MS research were invited to collect patients with DNO, diagnosed on a clinical ground, according to these characteristics: clinical attack suggesting involvement of optic nerve/spinal cord, no other site of CNS involvement. Brain MRI was required to be normal in initial stages of the disease (or with < 3 lesions). Cases with intrathecal IgG synthesis and brain MRI lesions were considered as affected by MS and were not included. Fifteen neurological departments participated to the study.

Results: 45 subjects were collected. The mean age was 40.2 years (+/- 15.1) with a peak at age 30-39 ys and a second peak at age > 59ys. Nine were males (20%). Symptoms suggesting spinal cord involvement were found in 17 subjects, suggesting optic nerve involvement in 26, both spinal cord and optic nerve in 2. EDSS at onset was 3.9 +/- 1.5 during the acute phase, the residual score was 2.1 +/- 1.9. Concomitant diseases: fever or viral infections in 5 subjects, ANA positivity in 4, thyroid dysfunction in 3, HCV positivity in 3, epilepsy in 2, myasthenia gravis in 1.

Conclusions: Our results show a strong preponderance of females affected (80%), a bimodal distribution of age at onset, with a first peak at age 30-39 ys and a second peak at age > 59 ys., a frequent association with infectious/autoimmune/thyroid dysfunction

Disclosure: A Ghezzi has nothing to disclose.

P33
THE ITALIAN DEVIC’S STUDY GROUP (IDESG) - PART 2: COURSE AND PROGNOSIS OF DEVIC’S NEUROMYELITIS OPTICA
1 Centro Studi SM, Gallarate, (VA), Italy; 2 Clinica Neurologica, Pavia, (PV), Italy; 3 Clinica Neurologica, Milano, (MI), Italy; 4 Clinica Neurologica, Ferrara, (FE), Italy; 5 Clinica Neurologica, Bari, (BA), Italy; 6 Centro Studi SM, Gallarate, (VA), Italy

Background: It is not clear whether Devic’s neuromyelitis optica (DNO) is a form of MS or a separate syndrome. Apart from a few studies, data are not available on the long term evolution of this disease.

Objectives: Our study was undertaken to evaluate the course and prognosis of DNO.

Methods: 45 patients affected by DNO were collected by 15 Italian neurological departments. Patients were included if presenting optic nerve/spinal cord involvement in a mono/multiphasic pattern (for details see the presentation: Part 1)

Results: The duration of follow up was 8.8 +/- 7.0 years (range 1-26 ys). The number of attacks was 6.3 +/- 3.5 (range 2-16). The number of attacks/year was 1.3 +/- 1.2 (range 0.4-5.5). The inter-attack interval between the first 6 attacks...
was 16-17 months for each interval. Kurtzke’s EDSS during the acute phase was 3.9, 4.4, 4.9, 5.4, 6.3 in the first 6 attacks, the residual score was respectively: 2.1, 3.0, 3.9, 4.3, 4.6, 5.4. The % of reduction was respectively: 46%, 32%, 20%, 20%, 18%, 14%. Data concerning 103 brain MRI scans and 91 spinal cord MRI scans will be presented.

Conclusions: The clinical course was variable but, in general, disability increased progressively with time, with a tendency to a poor recovery. The prognostic value of different variables (age, gender, inter-attack interval, initial EDSS score) will be presented.

Disclosure: A Ghezzi has nothing to disclose.

P34

THERAPY RELATED ACUTE MYELOGENOUS LEUKEMIA IN A PATIENT WITH MULTIPLE SCLEROSIS TREATED WITH MITOXANTRONE

Hessen C*, Bruegmann M*, Koch E, Gold SM*, Moench A*, Gbadamosi P
*Neurology, University Hospital Eppendorf, Hamburg, Germany; †Hematology and Oncology, University Hospital Eppendorf, Hamburg, Germany

Background: In 2000, mitoxantrone (mitox) was approved for treatment of progressive relapsing-remitting and secondary progressive multiple sclerosis (MS) in the US. Therapy related leukemia (t-AML) is a rare but serious adverse event of mitoxantrone therapy described in about 1% of mitoxantrone treated cancer patients. We report the case of a patient out of 59 treated at our center who developed acute myelogenous leukemia (AML) after 108 mg mitoxantrone.

Objectives: To describe a case of therapy-related leukemia (tAL) under mitoxantrone treatment.

Methods: Casereport.

Results: A now 34-year old patient developed relapsing-remitting MS 16 years ago. After disability ratings worsened considerably in 1998 (EDSS 5.0 to 6.0), mitoxantrone therapy was initiated at a 12mg/m2 dose every third month. Medication was well tolerated. Mitoxantrone was administered 6 times until November 2000. In April 2001, the patient presented with fever, fatigue and marked leukocytosis. Bone marrow biopsy and immunophenotyping led to the diagnosis of AML, French-American-British Group classification type (FAB) M4Eo. Cytogenetic analysis showed an inversion of chromosome 16. Chemotherapy and autologous stem cell support chemotherapy were performed. Up to now, no recurrence of leukemic cells could be detected. At her last presentation in April 2002, she reported increased fatigue but mitoxantrone was stable at 6.0, no deterioration of hand function or cognitive dysfunction were seen.

Conclusions: This is the third reported case of t-AML in MS patients treated with mitox. We conclude from these studies that MS patients should not only be educated about the risk of cardiomyopathy but also about the risk of t-AML, which might be in the range between 0.05-1%. However, since only three cases have been described so far, the empirical evidence does not allow for definite conclusions to be drawn. Cytogenetic studies may help to clarify a causal relation to therapy and identify prognostic factors.

Disclosure: S Gold has nothing to disclose.

P35

RETROSPETIVELY ANALYZING MONOSYMPTOMATIC DEMYELINATING DISEASES, ACCORDING TO NEW RECOMMENDED DIAGNOSTIC CRITERIA FOR MULTIPLE SCLEROSIS

Guerrero A, Bueno V, Hernández M, Martín-Serradilla J, Díez S, Calvo A
Neurology Unit, Hospital Rio Carrion, Palencia, Spain

Background: There have been recently proposed revised criteria for the diagnosis of multiple sclerosis (MS). According to these criteria, a monosymptomatic demyelinating disease can be diagnosed as MS, when it fulfills criteria of dissemination in time and space.

Objectives: We retrospectively analyzed our patients with a isolated demyelinating disease, in order to apply in the usual clinical parameters, these new criteria.

Methods: In January 2002 we analyzed 12 cases seen in our unit among April 1997 and January 2001 due to a clinically isolated demyelinating disease. None of them suffered a new clinical episode during the follow-up. Accordin to the new recommended criteria we analyzed the RMN performed during clinical event, and a new one performed later in all the cases.

Results: Among our 12 cases, 3 clinically corresponded to spinal cord lesions, 5 to infratentorial ones, and 4 were optic neuritis. Only 2 of our patients presented space and time dissemination, so fulfilling MS new diagnostic criteria. Five more presented dissemination only in space and one dissemination in time.

Conclusions: The analysis of the patients seen in our unit due to monosymptomatic demyelinating disease, leads us, according to the new recommended criteria, to diagnose two new cases of MS, as well as to identify 6 more in which dissemination either in space or in time will lead us to observe more carefully the evolution of these patients.

Disclosure: A Guerrero has nothing to disclose.
P37

A PROSPECTIVE STUDY OF MULTIPLE SCLEROSIS PRESENTING WITH ACUTE MYELOPATHY.

Jean P., Bertrand A., Jean Philippe R., Laurent S., Alban D., Sylvaine C., Patrick C., Andre A.

*Neurology, CHU Timone, Marseille, France; †Laboratoire de Neurophysiologie et de Neuropsychologie, Faculté de Médecine

Background: The risk of progression to multiple sclerosis (MS) after an episode of acute non compressive episode involving the spinal cord remain uncertain.

Objectives: To determine the risk of progression to MS in a population of 50 patients presenting with clinically isolated lesions of the spinal cord, and to evaluate the clinical course of these patients in a long term follow-up study.

Methods: The population consisted of 50 patients (30 women, 20 males; median age : 26 / 9.2 ys) presenting with a first clinically isolated acute syndrome of the spinal cord suggestive of MS. At baseline, spinal cord and brain MRI (T2 and T1 with and without gadolinium injection weighted sequences), cerebrospinal fluid (CSF) analysis, evoked potentials (VEPs, BAEPs, SEPs) were performed. During the follow-up, brain MRI was performed in all cases.

Results: Spinal cord MRI demonstrated cervical lesions in 78% of the cases. Initial brain MRI was strongly suggestive of MS in 25 cases (Barkhof criteria) and normal in 7 cases. 40% of the patients presented abnormalities on evoked potentials (VEPs and/or BAEPs and/or SEPs) while CSF analysis showed oligoclonal bands (OB) in 80%. During the follow-up period (7/3 ys ), 76% of the patients presented one or more exacerbations and time to the first recurrence was 23/12 months. Among these patients, 90% had CSF OB and initial brain MRI was strongly suggestive of MS 65%. During this follow-up period, brain MRI showed emergence of lesions in 5 of the 7 cases with normal initial examination.

Conclusions: This study confirm the predictive value of brain MRI and CSF OB for the diagnosis of MS in patients presenting with clinically isolated acute syndrome of the spinal cord.

Disclosure: P Jean has nothing to disclose.

P38

CLINICAL PROFILE AND APPLICATION OF DIAGNOSTIC CRITERIA IN PRIMARY PROGRESSIVE MULTIPLE SCLEROSIS

Khan O., Caon C, Ching W, Sonenwirth E, Tselis A, Zwartvand-Hind M

Neurology, Wayne State University, Detroit, Michigan, USA

Background: PPMS represents 15% of all MS patients. New diagnostic criteria for PPMS were proposed by Thompson et al. We examined the usefulness of these criteria in a clinical setting outside the context of clinical trials.

Objectives: To apply the diagnostic criteria proposed by Thompson et al. and study the clinical profile of PPMS patients.

Methods: PPMS patients followed at a large urban hospital MS Clinic were evaluated. Detailed history was obtained. Investigations including brain and cervical spine MRI, CSF, and evoked potentials (EP) were evaluated. Kurtzke’s EDSS were recorded. Clinical course was analyzed retrospectively. Diagnostic criteria proposed by Thompson et al. were applied. At the time of neurological assessment, no patient had received therapy with disease modifying potential. Our clinic has a large African-American (AA) MS patient population who were also analyzed as a subgroup.

Results: 67 patients with PPMS were identified. 37 (55.2%) of 67 patients were women with a female to male ratio of 1.23 : 1.0. Mean age was 51.6 ys, mean age of onset was 40.4 years, and mean EDSS was 5.4. 44 (65.6%) of 67 fulfilled the Thompson criteria for definite PPMS. However, with the addition of somatosensory (tibial) EP to the criteria, 53 (79%) of 67 fulfilled the criteria for definite PPMS. 62 of 67 patients had undergone lumbar puncture for CSF studies. In one case, results could not be available. Review of CSF abnormalities i.e. presence of OCB (greater than 2) and/or raised IgG index (n=46 or 75.4%) revealed no effect on the clinical course compared to the CSF negative (n=15 or 24.6%) PPMS patients. Additional analyses of brain and cervical spine MRI abnormalities will also be presented. 16 (23.8%) of 67 were AA. Details of this sub-cohort will also be presented.

Conclusions: Diagnostic criteria for PPMS by Thompson et al. are applicable in a clinic setting. However, the addition of SSEP may increase the diagnostic yield for definite PPMS. Based on the current understanding of disease mechanisms in PPMS, it is not clear if CSF abnormalities should be a mandatory requirement for definite PPMS. PPMS appears to be more aggressive in AA.

Disclosure: O Khan has nothing to disclose.

P39

RELIABILITY OF EDSS AND FS-SCORE RATING CAN BE IMPROVED BY STANDARDISED TRAINING

Lienert C., Lechner-Scott I., Müller U., Kappos L

Neurology, University Hospitals, Basel, Switzerland

Background: The EDSS of Kurtzke is the most widely used outcome measure in clinical trials

Objectives: To improve reliability of Kurtzke’ EDSS and FS assessment in clinical trials by standardised training

Methods: 721 neurologists and neurologists in training participating in four ongoing multicenter trials, underwent either EDSS-training sessions at 11 different investigator meetings (ETS, n= 494), including detailed verbal instructions of EDSS and FS rating and demonstration of video vignettes, or used a training CD-rom for self-training and assessment (ECD, n=227). Level of knowledge about EDSS and FS scoring was assessed with a questionnaire, containing 25 questions. Participants were asked to fill in questionnaires before and after the ETS, ECD trained participants after training. A score of 2 was allocated for the correct answer, a score of 1 for the second best, thus resulting in a maximum score of 50. A minimum score of 31 was required for passing the test.

Results: Post-training results were significantly better than pre-training results in the ETS group. Mean score pre-training: 33.98; mean score post-training: 38.36; p<0.001, Wilcoxon-test. This overall result was confirmed in the evaluation of each separate meeting, but in different magnitude. Performance at post-training tests was not statistically different in ETS and ECD.

Conclusions: Standardised training improves level of knowledge for EDSS and FS scoring. Post-ETS results did not differ significantly from post ETS results, indicating that self-training with CD-rom may be as effective as participation in training sessions, although the different environment at completion of the questionnaires must be taken into account.

Disclosure: C Lienert has nothing to disclose.

P40

This abstract was also presented at the platform.

ISOLATED SPINAL DEMYELINATING EVENTS WITH NORMAL BRAIN MRI: PROGRESSION TO MS, CLINICAL AND MRI FOLLOW UP

Milo I., Katz T., Corat-Simon P.

*Neurology, Barziliat Medical Center, Ashkelon, Israel; †Assaf Harofeh Medical Center, Zerifin, Israel

Background: Most clinically definite MS patients show multiple hyperintense lesions on T2W MRI images in the white matter of the brain. However, up to 10% of patients with spinal cord lesions may have no, or few, brain lesions. The number of MRI lesions at presentation is predictive of the development of MS and the long-term outcome.

Objectives: To characterize MS patients with initial spinal demyelinating events and normal brain MRI.

Methods: Among 150 consecutive patients with definite or probable MS, 48 (32%) presented initially with demyelinating lesions in the spinal cord. These
were further divided according to brain MRI to those with positive scans compatible with MS, and those with negative (normal) scans, which were followed up for clinical and MRI findings.

Results: Sixteen patients (F-10, M-6) with an initial clinical spinal demyelinating episode and a corresponding MRI lesion but normal brain MRI were detected. Their mean age was 31.8 (19-47), mean EDSS at presentation 2.9 (1.5-6.5) and mean EDSS during first remission 1.1 (0-2.5). Location was cervical in 12, thoracic in 3 and conus cauda in 1. During the follow-up period of 7-130 (average 35.3) months, 12 (75%) converted to clinically definite MS, while 4 did not have further relapses. All patients showed the relapsing-remitting form of MS. Although most patients continued to have spinal relapses, the optic nerve, brainstem or cerebellum were involved in 50%. In only 4 patients, a second brain MRI became positive within a year; in the other 12 patients, additional 1-4 scans performed 3-84 months after the first one were still negative. Disease progression was usually slow, with EDSS = 1.5 (0-6.5) after 7-130 months. Oligoclonal bands in the CSF were detected in 1/15 (7%) only.

Conclusions: A considerable number of patients with an initial spinal demyelinating event have normal brain MRI scans at presentation. Albeit high conversion rate to clinically definite MS, most of them show normal brain scans repeatedly. This group of patients is characterized by a relapsing-remitting and relatively benign course of the disease (despite repeated spinal relapses), and by the absence of oligoclonal bands in the CSF. We speculate that these characteristics may be associated with a relatively minor activity of the disease in the brain.

Disclosure: R Milo has nothing to disclose.

P41

ASSESSMENT OF SIGNS AND SYMPTOMS IN MULTIPLE SCLEROSIS (MS) AND EVALUATION OF DISABILITY CONDITION IN PATIENTS REFERRED TO MS RESEARCH CLINICS FROM 1997-2001 (2412 CASES)

Pakdaman R1, Pakdaman R2

1Neurology, Beheste University, Tehran, Iran; 2Neurology, Behesti University

Background: Multiple Sclerosis is one of the most common neurologic disorders in young adult. It is noted because of making disability and disfunction in youth. Epidemiologic studies show that there are differences in prevalence, manifestation and disability condition between various races and geographical locations.

Objectives: From four years ago, three clinics of MS established in Tehran (which has high prevalence of MS) in order to syudy on sign and symptoms and disabilities of patients which some of the results discussed.

Methods: This is a prospective cross-sectional study, which is carried out during 1997-2001. The patients with MS announced to these three MS clinics. After taking history and complete examination, computerized data analyzed with statistical method EPI6.

Results: In this study 2412 patients were diagnosed as having MS as based on Poser criteria. Mean age of patients was 32/2. 71% of patients were female (F/M = 2.4). 64% of patients were educated, 26% were single and 73% were married. Family history was positive in 7-7%. Mean duration of disease was 5.7 years. Initial symptoms were weakness (50%), paresthesia (30%), sensory deficit (23%), diplopia (21%), ataxia (14%), vertigo (11%), bladder dysfunction (11%). The course of disease is relapsing-remission in 56% and progressive in 31% of cases. Mean EDSS - Kurtzke number was 4.2.

Conclusions: According to results obtained in this study, it seems that clinical manifestation and symptoms in Iranian patients with MS are similar to those in developed countries. More incidences of MS in high-educated persons are considerable. In spite of mean duration of disease (5.7 years) Kurtzke number in our patients is 4.2 which is exactly equal with the number in other countries. So, it does not seem to be significant difference in MS prognosis in Iran and other countries.

Disclosure: H Pakdaman has nothing to disclose.

P42

ONE CASE OF VERY LATE ONSET OF MULTIPLE SCLEROSIS WITH ACUTE NEUROPSYCHOLOGICAL IMPAIRMENT

Radu T1, Marc D1, Rene A1, Luc T1, Herve V2

1Neurology, Nancy Central Hospital, Nancy, 81, France; 2Neuroradiology, Nancy Central Hospital, Nancy, 81, France

Background: Objectives: We report the case of a 70-year-old woman with cognitive impairment and Magnetic Resonance Imaging (MRI) and Cerebrospinal Fluid (CSF) criteria for Multiple Sclerosis (MS).

Methods: The patient had a family history of MS (father) and was treated for hypercholesterolemia. In March 1998, she presented with alexia during 30 minutes. MRI showed multiple and disseminated periventricular hypointens on T2 weighted images and a CSF oligoclonal profile was found. In April 1999, a new MRI performed because of alexia showed right frontal and left parietal enhancing lesions. The neuropsychological examination found a pattern of frontal subcortical cognitive dysfunction. In April 2001 aphasias, central vestibular syndrom and VI paralysis led to realize a new MRI. This exam showed new lesions located at subcortical frontal-parietal, right temporal, left frontal and central with contrast enhancement. Spinal cord MRI revealed enhancing lesions at cervical and thoracic levels. The vascular investigations (cardiac and cervical vascular ultrasonography) and immunological profile (inflammatory markers, autoantibodies including ANCAp, ANCAc, AntidsDNA, AntissDNA, Anti Ro/SS-A, Anti La/SS-B, Anti-ribosomal P, antiangiop- allines and antiphospholipids antibodies, VDRL etc.) were negative, except a minimal monoclonal gammapathy and non significant values of ANA (1/160).

Results: Conclusions: The interest of this case is given by the presence of typical MacDonald criterias for MS in an old patient with cognitive impairment, without any other immunological or inflammatory definite disorder.

Disclosure: T Radu has nothing to disclose.

P43

APHASIA IN MULTIPLE SCLEROSIS

BERGER E, CHOPARD G, VIDRY E, REVENCO E, MOULIN T, RUMBACH L

Neurology, CHU Minjoz, Besancon, France

Background: A large proportion of patients with MS are cognitively impaired memory deficits are the most frequently reported. MS can impair speech production but aphasia is considered to be uncommon.

Objectives: To describe 4 patients with MS whose main cognitive complaints were aphasia that appeared at onset (1 case) or during (3 cases) the disease.

Methods: The clinical and imaging pictures of 4 patients with aphasia are described.

Results: Case 1. A 28-year-old man presented with the acute onset of right arm decreased sensation, an inability with speaking; he also reported problems in comprehending spoken language. Exam showed right Babinski sign. MRI demonstrated several white matter lesions and CSF showed oligoclonal bands. Case 2. A 45-year-old man was diagnosed as having MS for 10 years. He experienced several relapses; he has been treated by Interferon for 5 years. In July 2000, his main complaint was speaking difficulties; he also had a right hemiparesis. MRI showed several lesions and a new large frontal one. He did not recover. Case 3. A 28-year-old man had a typical relapsing-remitting MS history for 4 years. In one of his relapse he presented word-finding problems, speech abnormalities with normal comprehension. Case 4. A 40-year-old man had 5 previous episodes of relapses. Five years after the disease onset, a relapse was characterized by difficulties in reading and comprehending spoken language.

Conclusions: Although memory impairments have been the most reported, these cases stress the involvement of other cognitive domains.

Disclosure: L RUMBACH has nothing to disclose.
P44
FACTORS INFLUENCING PATIENTS' CHOICE OF IMMUNE MODULATING THERAPY
Stoian CA, Metz LM
University of Calgary, Calgary, Alberta, Canada

Background: Patients with MS often play a role in determining which immune modulating therapy they will use. However, little is known about which factors influence their decisions.

Objectives: To determine which factors influence patients’ treatment choices.

Methods: This was an observational cohort study of 104 adults with RRMS from 3 Canadian provinces. Consenting subjects completed self-administered questionnaires after patient education and prior to initiation of one of four MS therapies (Copaxone, Rebif, Betaseron, Avonex). Patients were asked to rank the three most important factors that influenced their choice of therapy.

Results: Sample characteristics: median time since diagnosis was 0.7 years, mean age was 39 years, and 82% were women. Physician recommendation was the most common factor influencing choice of therapy (71.2%) with no statistical differences between the four drugs (p = 0.378). The two most frequently reported factors to influence choice of therapy, by treatment, were: (1) physician recommendation (76.7%) and tolerability (75.0%) for Copaxone (n = 60, 58%); (2) physician recommendation (64%) and pre-filled syringes (52%) for Rebif (n = 25, 24%); (3) physician recommendation (80%) and tolerability (60%) for Betaseron (n = 5, 5%); and (4) injection frequency (85.7%) and physician recommendation (57.1%) for Avonex (n = 14, 13%). When available, the following devices were also rated as important: auto-injectors - Copaxone (50%), Rebif (44%) and pre-filled syringes - Rebif (52%). Relapse data, disability data, MRI data, type of injection, cost, availability of more than one dose, and ‘other’ were infrequently identified.

Conclusions: While the sample size of this study limits the precision of the reported frequencies, physician recommendation is fairly consistently the most frequently reported factor that influenced patients’ choice of MS immune modulating therapy. Tolerability, injection frequency, and the availability of auto-injectors and pre-filled syringes are, however, also important to patients.

Disclosure: This study was designed and Supported by TEVA Neuroscience. L. Metz and her research team (C. Stoian) coordinated data management, analysis and interpretation.

Funding: Supported by TEVA Neuroscience.

P45
FACTORS ASSOCIATED WITH DIFFICULTY INITIATING AND MAINTAINING IMMUNE MODULATING THERAPY
Stoian CA, Metz LM
University of Calgary, Calgary, Alberta, Canada

Background: Adherence to immune modulating therapy is often challenging.

Objectives: To identify factors associated with difficulty initiating and maintaining therapy over the first month of treatment.

Methods: This was an observational cohort study of 104 people with RRMS. Consenting subjects completed self-administered questionnaires after patient education and prior to initiation of therapy. Ease of therapy initiation was defined as reporting “somewhat” to “very easy” to maintain therapy, tolerance of full dose, and feeling normal or better over the first month of treatment. Failure to meet these criteria defined difficulty of therapy initiation. Ease of therapy maintenance was defined as reporting “some” to “very easy” to maintain therapy, and feeling normal or better from 3 months to 6 years. Failure to meet these criteria defined difficulty of therapy maintenance.

Results: Sample characteristics: median time since diagnosis was 0.7 years, mean age was 39 years, and 82% were women. Therapy initiation was difficult for 38.5%. Impaired vision and having any significant concerns about therapy initiation were associated with difficulty initiating therapy. Patients with impaired vision (difficulty reading newsprint) were more likely to find therapy initiation difficult than those without (OR=3.7, 95% CI: 1.2-10.9, p = 0.019). The odds ratios of reporting difficulty initiating therapy were higher for people “very concerned” about any of 18 listed potential treatment concerns compared to those with no “very concerned” ratings and increased with the increase in the number of “very concerned” ratings. The odds ratio of experiencing difficulty was 7.6 (95% CI: 2.1-27.8, p=0.002) for those with 1 to 5 “very concerned” ratings and 18 (95% CI: 3.6-89.6, p<0.001) for those with 6 to 10 ratings compared to those with no “very concerned” ratings.

Conclusions: Patients with ‘impaired vision’ or ‘significant concerns’ may benefit from additional support during therapy initiation and early treatment.

Disclosure: This study was designed and Supported by TEVA Neuroscience. L. Metz and her research team (C. Stoian) coordinated data management, analysis and interpretation.

Funding: Supported by TEVA Neuroscience.

P46
NON-ADHERENCE TO THE BETA-INTERFERONS FOR MULTIPLE SCLEROSIS IN CLINICAL PRACTICE
Tremlett H, Oger J, Special Therapies Group M
Medicine (Neurology), University of British Columbia, Vancouver, British Columbia, Canada

Background: The beta-interferons (IFNB) are long-term treatments for MS. Drug adherence is essential to maximize cost-effectiveness. Identification of patients that are likely to stop treatment and reasons for non-adherence may help in the prescribing, patient-counseling and education process.

Objectives: 1) To identify why MS patients stopped an IFNB 2) To ascertain if particular demographic factors (age, gender, disease duration, EDSS) were predictors of non-adherence

Methods: In December 2001 we reviewed the charts of MS patients who had started on one of the three IFNBs between July 1st 1995 and May 31st 2001. IFNB trial patients were excluded.

Results: 590 patients were identified whom had been prescribed an IFNB ‘first-line’ and 585 charts were reviewed. Mean treatment length was 1.8 years (range 7 days to 6.3 years). In all, 190 patients were non-adherent (ie had stopped treatment for at least one month). Of these, 60/190 (32%) were non-adherent by 6 months, and 103 (54%) by one year. The most frequently cited reason for drug cessation was perceived lack of efficacy (63/190 33%) followed by injection site reactions (62, 14%), ‘flu like symptoms (16; 8%), depression (16; 8%), headache (14; 7%) and abnormal liver enzymes (13; 7%). No reason was documented for 5 patients. Compared to the adherent group, the non-adherent group had a significantly higher median baseline EDSS (2.5 versus 3.5; Mann-Whitney p=0.001) and longer disease duration (mean 12.1 versus 14.5 years; Fishers exact test p=0.001). There were no differences between the groups for age or gender (p<0.05). Of the non-adherent patients, 10/190 (10%) were re-started on the same IFNB, 73 (38%) on a different IFNB and 29 (15%) on glatiramer acetate. Overall, 69/585 (12%) totally discontinued immunomodulatory drug therapy, averaging 6% of patients per year.

Conclusions: Lack of efficacy was the single most frequently cited reason for non-adherence, followed by injection site reactions. EDSS score and disease duration were both significant factors for non-adherence to the IFNBs indicating that those more disabled at the start of treatment were more likely to cease therapy. The low discontinuation rate found was attributed to the compulsory education session and frequent follow-up with trained nurses for all patients.

Disclosure: JO has received consulting fees and honoraria for speaking from Berlex, Biogen and Serono, although none in support of this abstract. HT has a fellowship from the MS Society, Canada.

Funding: HT has a fellowship from the MS Society, Canada.
Multiple Sclerosis

P47
VISUAL FUNCTION IN MULTIPLE SCLEROSIS PATIENTS: 20 YEARS LATER
Vorobeychik GP, Anderson D3, Lindley JP, Paty DW. UBC MS Clinic
1Neurology, UBC, Vancouver, British Columbia, Canada; 2Ophthalmology, UBC, Vancouver, British Columbia, Canada

Background: Optic neuritis (ON) and diplopia are common presenting symptoms in patients with multiple sclerosis (MS). There is little published experience on long-term assessment of visual function for these patients.

Objectives: To compare visual function in clinically definite MS (CDMS) patients 20 years after onset, who presented with ON or diplopia

Methods: We included patients from the UBC MS Clinic computerized database (MS-COSTAR) who met the following criteria: (i) had CDMS, (ii) initial manifestation was ON or diplopia, (iii) visual function was assessed (by neurologist) 20 or more years after onset. The groups were compared for course of MS, age of onset, and sex ratio.

Results: ON was a presenting symptom in 13.2% (479/3617) and diplopia in 6.5% (234/3617) cases with CDMS in the UBC MS Clinic (September 1980-May 2000). The mean age of onset, duration of observation, and sex ratio was similar in the ON group (104 patients) and the diplopia (45 patients) groups with follow up for 20 or more years after onset. The proportion of patients with primary progressive course of MS (PPMS) was lower in the ON group (3/104 patients, 2.8%) than in the diplopia group (6/45, 13%, p=0.01). The percentage of patients that switched to secondary progressive (SP) MS during observation period was similar in both groups (61% in ON and 67% in diplopia group). Visual acuity remained 20/40 or better in majority of patients in both groups (69.2% in ON and 84.4% in diplopia group). Severe decrease of visual acuity to 20/200 was rare in both groups (13/104, 12.5% in ON group and 3/45, 6.7% in diplopia group). Progression of optic neuritis to multiple sclerosis (MS) was rare in both groups (15/104, 14.4% in ON group and 2/45, 4.4% in diplopia group). Severe decrease of visual acuity to 20/200 was rare in both groups (13/104, 12.5% in ON group and 3/45, 6.7% in diplopia group). Visual acuity remained 20/40 or better in majority of patients in both groups (69.2% in ON and 84.4% in diplopia group). Severe decrease of visual acuity to 20/200 was rare in both groups (13/104, 12.5% in ON group and 3/45, 6.7% in diplopia group). Visual acuity remained 20/40 or better in majority of patients in both groups (69.2% in ON and 84.4% in diplopia group). Severe decrease of visual acuity to 20/200 was rare in both groups (13/104, 12.5% in ON group and 3/45, 6.7% in diplopia group).

Conclusions: CDMS patients who presented with ON have less frequently PPMS than those who presented with diplopia. 2/3 of both groups switched to SPMS during observation period. Majority of the patients in both groups have good visual acuity after 20 years of disease. Only very small proportion of patients become legally blind after 20 years of disease.

Disclosure: G Vorobeychik has nothing to disclose.

Epidemiology

P48
COMPARISON OF DIAGNOSTIC CRITERIA FOR MULTIPLE SCLEROSIS
Balciyp, Yaya V, Ozer F
Neurology, Haseki Hospital, Istanbul, Turkey

Background: The diagnosis of MS is relied upon the accumulation of information, neurological examination and laboratory findings. Until date, Poser criteria were used most for MS classification, recommended in 1983. By 2001, after Recommended Diagnostic Criteria for Multiple Sclerosis: Guidelines from the International Panel on the Diagnosis of Multiple Sclerosis published by McDonald et. al, these criteria are taken into consideration for diagnostic approach.

Objectives: The aim of this study is to determine the status of the patients followed up in Haseki Educational and Research Center - Neurology Department as definite MS according to Poser criteria by McDonald criteria.

Methods: Total 106 patients (62 women, 44 men) age ranged 19 - 67 (mean: 35) diagnosed as definite MS according to Poser criteria were reassessed by the new criteria, taking disease duration into consideration.

Results: By McDonald criteria 88 (83%) of 106 patients who previously diagnosed as definite MS by Poser criteria (92 CDMS, 14 LSDMS) were diagnosed as MS. 81% of the patients diagnosed as MS by McDonald criteria were CDMS and 2% wer LSDMS. The mean duration of the disease was 6.17 years for 106 patients with definite MS according to Poser and 7.06 years for 88 patients diagnosed MS according to McDonald.

Conclusions: Difficulties of diagnostic approach at the onset period of the disease continue by McDonald criteria. By McDonald criteria the diagnosis gets easier with longer disease duration.

Disclosure: B Balci has nothing to disclose.

P49
LOOKING FOR THE DEFINITION OF BENIGN MULTIPLE SCLEROSIS
Coustans M, Le Page E, Le Duff F, Leray E, Sartori E, Edan G
Neurology, CHU Pontchaillou, Rennes, France

Background: Benign multiple sclerosis (BMS) has been variably defined in quantitative terms and no consensus had been reached to define this entity. BMS commonly defined as EDSS <= 2 or 3 after 10 years of disease duration is questionable.

Objectives: To determine the best definition of BMS taking either EDSS <= 2 or 3 at 10, 20, 30 y of disease duration.

Methods: In our EDMUS (European database for multiple sclerosis) database, among 1019 clinically definite relapsing remitting MS (RR MS), 695 patients had a disease duration more than 10 y. We analysed evolution of BMS with two definitions : RR MS population with an EDSS <=2 and EDSS <=3 after 10 y of disease duration and we examined these two groups of patients at 20 y and 30 y disease duration.

Results: Among the 695 RR MS patients followed more than 10 y, 280 were followed more than 20 y and 100 more than 30 y. Proportion of BMS defined as EDSS <=3 was respectively 62.3%, 36.4% and 25%. Proportion of BMS defined as EDSS <=2 was respectively 48%, 26% and 16%. Moreover among the 433 patients with a definition of BMS as EDSS <=2 after 10 y of disease duration, 217 had a disease duration more than 20 y, only 47% (102) of them were still BMS. Among these 102 still BMS patients at 20 y, 49 had 30 y follow-up and only 51% (25) of them were still BMS. For BMS with an EDSS <=2 at 10 y (334), 184 had a disease duration more than 20 y, 73 (39.7%) were still BMS. Among these 73 BMS patients, 41 had 30 y follow-up, only 16 (39%) were still BMS.

Conclusions: In the population of BMS defined as either EDSS <=2 or EDSS <=3 at 10 y, a majority of these patients had a worsening of their disability when they are followed at 20 and 30 y and so did not deserve the term benign MS. Benign MS is a questionable entity.

Disclosure: M Coustans has nothing to disclose.

P50
INCIDENCE AND PREVALENCE OF MULTIPLE SCLEROSIS IN AN HMO IN ARGENTINA
Cristiano E, Patrucco LB, Soriano ER, Videla GC, Figar SP, Hares DP, Bauso Toselli PL
1Neurology, Hospital Italiano de Buenos Aires, Buenos Aires, Capital Federal, Argentina; 2Epidemiology, Hospital Italiano de Buenos Aires, Buenos Aires, Capital Federal, Argentina

Background: There is a lack of incidence and prevalence studies in Multiple Sclerosis (MS) in Latin-america.

Objectives: To estimate the incidence and prevalence of MS in an HMO in Buenos Aires, Argentina. (Lat 34 38’S; long 58 28’W)

Methods: Population: the population at risk was all members of a Hospital based HMO, who were affiliated at least since December 1992 or at least twelve months before the date of diagnosis of each case included up to December 2001. Each person was followed until voluntary disenrollment, death or finalization of the study (final dates), contributing time at risk since December 1992 or enrollment date (whichever occurred later) to that final date. Case ascertainment: multiple methods for case finding were used
to ensure complete ascertainment: a) patients included in neurologists databases, b) patients with the ICP/C (International Classification for Primary Care) code N86 in the HMO Computer-based Patient Record System, patients with ICD 9 codes 340-341 on admission to Hospital, and d) patients receiving MS specific drugs. Medical records of all patients found were reviewed and only cases with MS definite diagnosis (Poser) were included. For incident cases the date of diagnosis was considered. To be included, cases must have been affiliated to the HMO at least 12 months before diagnosis. Statistical analysis: incidence density was calculated with 95% confidence intervals. Prevalence was estimated at December 2001, and the denominator population was the number of active members at December 31, 2001.

Results: In the study period 120,442 patients were followed for a total of 446,842 persons-year, of whom 10 developed MS. The incidence density rate (IR) was 2.24/100,000 (95% CI: 0.85-3.62/100,000). On December 2001, 21 prevalent cases (13 females) were identified, among 81,855 members. Prevalence: 25.6/100,000 (95% CI: 17-38/100,000); 27.2 for females and 23.5 for males/100,000. Mean age at diagnosis of these patients was 35.6 years (20-56 years).

Conclusions: Incidence rate estimated in this selected population was similar to countries with median risk for MS. Prevalence detected was higher than reported in previous studies in our country.

Disclosure: E Cristiano has nothing to disclose.

P51

DEVIC’S NEUROMYELITIS OPTICA (NMO) AND MULTIPLE SCLEROSIS (MS): CLINICAL AND EPIDEMIOLOGIC FINDINGS IN AN MS CENTER IN ARGENTINA.

Patrucco LB, Cristiano E, Videla GC, Basso Toselli PL
Neurology, Hospital Italiano de Buenos Aires, Buenos Aires, Capital Federal, Argentina

Background: Argentina is a country of medium risk for developing MS (prevalence: 18-25/100,000). In Latin-america there is a lack of epidemiological data regarding NMO.

Objectives: To determine the proportion of NMO regarding a population of MS patients followed in our MS center and to describe some of its clinical and epidemiological features.

Methods: We reviewed the clinical charts of 569 patients followed up at our MS clinic. Patients who developed definite MS (Poser) and NMO (Wingerchuk) between January 1996 and March 2002 were included. We compared initial symptoms, sex prevalence and age at onset between both groups. In the NMO group we also analyzed clinical evolution and paraclinical findings.

Results: Of 134 patients who fulfilled the inclusion criteria, 124 (77 females) were definite MS (92.5%) and 10 (7 females) NMO (7.46%). The MS: NMO ratio was 12.4:1. The mean age of onset was 29.6 for MS and 40.6 for NMO. 30 (24.1%) MS patients presented spinal symptoms at the beginning of the disease (18 males and 12 females), and 14 (11.3%) optic neuritis (2 males and 12 females). In the NMO group, 3 (2 males) out of 10 patients presented spinal symptoms and 7 (6 females) optic neuritis at onset. Clinical presentation in NMO was monophasic (less than 3 months between initial symptoms) in 3 patients and polyphasic in 7. During the follow up period (0.4-6.2 years), 5 out of 10 NMO patients suffered one or more relapses (5 myelitis and 5 ON) with partial recovery of spinal symptoms and minimal recovery of visual function. In the acute period, pleocytosis with increased neutrophil count in CSF, absence of oligoclonal bands in all patients, extensive spinal cord lesions and normal brain MRI, were the main paraclinical findings in NMO patients.

Conclusions: In this study the proportion of NMO compared with MS patients was higher than expected according to published data. In NMO the age of onset was greater, ON was the most frequent onset symptom, polyphasic clinical presentation showed a clear female predominance and 50% of the patients suffered relapses during this follow up period. Further studies are needed to clarify the prevalence and relative incidence of NMO compared with MS in different areas and its clinical features.

Disclosure: E Cristiano has nothing to disclose.
forms, duration and degree of disability. These findings were compared in two groups of patients - with initial ON and without ON (non-ON) in onset of MS.

**Results:** ON as an isolated initial symptom was in 29 cases (18.2%). Average age of patients in the time of occurrence of ON was 27.9 years of age, what is in comparison with non-ON group 2.8 years less. In ON-group there were 73% of patients with RRMS and their EDSS was about 2 degrees better than in non-ON group, 27% with SPMS and PPMS was not observed. Average duration of the disease up to December 2000 is in ON-group 9.4 years, in non-ON group 11.7 years.

**Conclusions:** First retrospective analysis of ON as early manifestation of MS in East Slovakia. Our results do not exclude the upper opinion in background.

Disclosure: K Eleonora has nothing to disclose.

**P54**

MULTIPLE SCLEROSIS PREVALENCE AND HLA CLASS II ALLELE DISTRIBUTION IN GYPSIES FROM MALAGA, SOUTHERN SPAIN


Neurology, Hospital Regional Universitario Carlos Haya, Málaga, Spain; Research Unit, Hospital Regional Universitario Carlos Haya, Málaga, Spain; 2Neurology, Hospital Virgen de las Nieves, Granada, Spain; 3Neurology, Hospital Ciudad de Jaén, Jaén, Spain

**Background:** There are occasional reports about the low prevalence of multiple sclerosis (MS) among Gypsies. Few HLA studies have been carried out in the MS gypsy population.

**Objectives:** To study MS prevalence and HLA class II distribution among MS gypsies in Malaga.

**Methods:** MS gypsy patients in our geographical area were actively searched with a yield of 12 cases. The HLA class II DRB1 and DQB1 were investigated by PCR/SSO and PCR/SSP. HLA distribution of a healthy control gypsy group from the same geographical area was taken from published data.

**Results:** Estimated MS prevalence for gypsy population in Malaga is 5/100,000 inhabitants. DRB1*1404, DQB1*0503 was a frequent allele combination in our gypsy patients (25%). Just one positive association was detected between MS gypsy patients and the allele DQB1*0602 (41.7% vs. 2.5%, corrected p=0.0009)

**Conclusions:** These results indicate that in Malaga population, MS prevalence in gypsies is about the same as in Caucasians (53/100,000 in 1991). Gypsies from Malaga have the same anthropological origin as other European Gypsy groups. We have detected a positive association of HLA class II allele DQB1*0602 with MS gypsy patients.

Disclosure: V Fernández has nothing to disclose.

**P55**

COURSE, DISABILITY AND IMMUNOMODULATORY TREATMENT OF PATIENTS WITH MULTIPLE SCLEROSIS BASED ON A POPULATION BASED REGISTRY

Frederiksen J

Neurological department, Glostrup university hospital, Glostrup, Denmark

**Background:** We underwent a population based epidemiological study of patients with multiple sclerosis (MS) from the County of Copenhagen with about 610,000 inhabitants and two departments of neurology.

**Objectives:** The aim was to create a clinical database for research purposes with demographic data, date of onset og MS, date and result of various paraclinical examinations, and informations about course and degree of disability of MS. The previous and actual immunomodulatory and immunosuppressive treatment is registered. Patients were consecutively referred, either as in- or outpatients, to Gentofte or Glostrup University Hospital during a three years period from March 1, 1999 to Feb. 28, 2002.

**Methods:** Patients were identified based on the diagnose of MS, G 35.9 based on the ICD-10 code system. We also went through files of patients diagnosed with opticus neuritis (H 46.9) to see if they fulfilled the Poser criteria of definite MS.

**Results:** At the time of the ECTRIMS congress we expect to have classified the patients according to course (relapsing-remitting, primary progressive and secondary progressive, benign) of MS and to have registered the disability on the Kurtzke EDSS scale. We will calculate the frequency of patients with relapsing-remitting MS and secondary progressive MS, who are treated with betainterferon or copaxone. Likewise, we will judge how many would have been eligible for such treatment but did not want to receive it, and the characteristics of these patients. In addition, we will analyse how many patients who stopped the above mentioned treatment and the reason why.

**Conclusions:** Based on the prevalence of MS in Denmark we expect to register about 700 patients. As the study population is population based from a well defined geographical area with free admittance to the local neurological departments and to immunomodulatory treatment, the results may be representative for a population of patients with MS.

Disclosure: J Frederiksen has nothing to disclose.

**P56**

INCIDENCE OF MULTIPLE SCLEROSIS IN A NINE-YEAR PERIOD IN THE PROVINCE OF SEVILLE (SOUTH-WEST SPAIN).

Izquierdo G, Navarro G, Garcia Moreno J, Durán E, Gamero M, Ruiz-Peña J, Dinca L, Páramo D

Neurology, Hospital Virgen Macarena, Sevilla, Spain

**Background:** The prevalence of Multiple Sclerosis (MS) is well known in south Europe, but the actual data for MS frequency depend on incidence and not prevalence data. The incidence data are missing in our region.

**Objectives:** We studied incidence and prevalence rate in a well-delimited area of our region.

**Methods:** Patients were included as MS when fulfill the Poser et al. 1983, criteria. The incidence data were studied in a prospective study in a nine-year period (from January the first 1993 to December 31th, 2001) in the province of Seville (Spain) in a well-delimited rural area depending of our hospital. The total population of the area was the 142,776 inhabitants in 16 villages.

**Results:** Seventy patients were detected in the area, 62 of them were diagnosed in the follow-up period. The global prevalence of the area was 49.02 per 100,000. The annual incidence rate changes from 1.5 to 8.3 per 100,000 inhabitants. The mean annual incidence rate of the 9-year period was 5.2 per 100,000 inhabitants.

**Conclusions:** The incidence and prevalence rates are not concordant. Missing cases of pre-study period explain these differences. The frequencies of MS in our region are higher than that has been previously reported.

Disclosure: G Izquierdo has nothing to disclose.

**P57**

THE PREVALENCE OF MULTIPLE SCLEROSIS IN BELO HORIZONTE, BRAZIL.

Lana-Peixoto MA, Frota E, Campos GB, Botelho CM, Araújo AL

CIEM INAS UFMG Center for Investigation of Multiple Sclerosis, Brazilian Committee for Treatment and Research in Multiple Sclerosis, Belo Horizonte, MG, Brazil

**Background:** The epidemiology of MS in South America is largely unknown. In a recently published study the prevalence of MS in Sao Paulo city was found as 15.0/100 000 inhabitants.

**Objectives:** To describe the prevalence of MS in Belo Horizonte, Brazil.

**Methods:** The city of Belo Horizonte is the capital of the state of Minas Gerais in Southeastern Brazil. The city covers an area of 330.9 Km2 at a mean altitude of 858 m and is situated at a latitude of 19 55S and a longitude of 43 56E. The climate is subtropical. The estimate population in the prevalence day (July 1, 2001) was 2258,857 inhabitants. Case ascertainment was done through most of neurologists, most of the hospitals in the city area, two local societies of patients, the Brazilian Association of Multiple Sclerosis and the State Health
Department. Only patients living in the city area and with the diagnosis of CDMS were included.

**Results:** This search provided a list of 453 patients, 409 of whom qualified for inclusion. The MS prevalence was 18.1/100,000 population. There were 95 men and 314 women; 243 were white, 63 mulatto and 11 black. The age at onset was below 20 years in 13.5%, between 20 and 39 in 65.5% and 40 years or above in 20.9% (median 36 years). The most frequent symptoms and signs at presentation were motor (28.1%), sensory (27.7%), optic neuritis (20.6%) and brainstem/cerebellar (20.0%). There were 216 patients with RRMS, 84 with SPMS and 39 with PPMS. The EDSS was 3.5 or below in 49.0%; 4.0 to 6.5 in 36.6%; 7.0 and 7.5 in 6.5% and 8 or above in 7.9%.

**Conclusions:** This study adds strength to previous investigations on the prevalence of MS in Southeastern Brazil.

Disclosure: M Lana-Peixoto has nothing to disclose.

**Funding:** Supported by Serono Brasil.

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**P58**

**STUDIES ON MULTIPLE SCLEROSIS: A GEOGRAPHICAL DISTRIBUTION.**

Lana-Peixoto MA, Araujo CR, Macedo R, Haase VG

**CIEM MINAS UFMG Center for Investigation of Multiple Sclerosis, Brazilian Committee for Treatment and Research in Multiple Sclerosis, Belo Horizonte, MG, Brazil**

**Background:** The understanding of MS has had a striking progress in the last decade as a result of an increasing number of investigations dealing with different basic and clinical features of the disease.

**Objectives:** To analyze the growth of the scientific production on MS in different parts of the world.

**Methods:** The search was conducted through the MEDLINE from 1991 to 2000. All publications containing the key word Multiple Sclerosis as a major topic were considered. The search was conducted for each country separately in the Americas, Europe, Asia, Africa, Australia and New Zealand. In the search strategy the term affiliation was used to indicate the country of origin of the institution the first author was affiliated to.

**Results:** The MEDLINE contains 6816 citations of papers on MS published during the last decade. In 4919 papers the system provided information about the country and institution the first author was affiliated to. The origin of the papers are as follows: 2089 came from countries in the American continent, 2511 from Europe, and 310 from Asia, Australia and New Zealand. There is no paper from African countries. There has been a regular growth in the number of papers from 1991 (495) to 2000 (1018). This trend is observed in all continents and most countries. As individual countries are concerned the largest number of papers came from the USA (1735 in the decade; 96 in 1991 and 259 in 2000), Canada (298), Brazil (28), Mexico (14), Cuba (10) and Chile (5) followed the USA in the American continent. The most productive countries in Europe have been Italy (561), UK (478), Germany (254) and France (228). They were followed by The Netherlands, Sweden, Denmark and Spain. Russia (4), Portugal (3) and Luxemburg (1) were the least productive countries. Most papers from Asia came from Japan (143) and Israel (87). Investigators from Australia published 49 papers and from New Zealand 10.

**Conclusions:** Interest in the study of MS has markedly increased during the last decade. Other factors in addition to differences in prevalence may play a role encouraging investigators in different parts of the world to conduct studies on MS.

Disclosure: M Lana-Peixoto has nothing to disclose.

**Funding:** Supported by Serono Brasil.

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**P59**

**THE NATURAL HISTORY OF MULTIPLE SCLEROSIS IN BRAZIL. I. CLINICAL DATA AND DISABILITY.**

Lana-Peixoto MA, Callegaro D, Moreira MA, Gama PD, Maciel D, Sá PN

**CIEM MINAS UFMG Center for Investigation of Multiple Sclerosis, Brazilian Committee for Treatment and Research in Multiple Sclerosis, Belo Horizonte, MG, Brazil**

**Background:** There has been an increasing interest in the study of MS in Brazil. Its prevalence has been described in Sao Paulo and Belo Horizonte, two of the largest cities in Southeastern Brazil which share common features regarding immigration, genetic background and economical development.

**Objectives:** To describe the demographic, clinical and disability features in a sample of MS patients in Southeastern and Southern Brazil.

**Methods:** Cases were randomly ascertained from Belo Horizonte, Sao Paulo, Sorocaba, Florianopolis and Londrina. All patients had CDMS, and the disability was assessed according to the EDSS.

**Results:** The study comprised 1035 cases, 156 of them were discarded. Analysis of the remaining 859 cases showed that 633 were women and 226 men; median age at onset was 29 years; Whites comprised 82.4%, Mulattoes 13.8%, Blacks 2.4% and Orientals 1.3%. The mean duration of the disease was 10.9 years. MS presentation was monosymptomatic in 87.8% of the cases with motor symptoms and signs in 31%, sensory in 26.1%, brainstem and cerebellar dysfunction in 19.7% and optic neuritis in 13.5%. The clinical course was relapsing-remitting in 74%, secondary progressive in 14% and primary progressive in 12%. The mean frequency of relapses was 1.6 in the first year, 2.6 in the first three years and 3.6 in the first five years after onset. Cross-section analysis of disability showed that 305 patients were full ambulatory, 388 had minimal to severe ambulation disability, 94 were bound to wheelchair, and 46 were bedridden.

**Conclusions:** The present study fulfills the methodological requirements for reliability as it derives from an extensive database, a geographically defined population and cases were selected at random. Further analysis of this database will provide more detailed information about the natural history of MS in Brazil.

Disclosure: M Lana-Peixoto has nothing to disclose.

**Funding:** Supported by Serono Brasil.

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**P60**

**CLINICAL ASPECT, COURSE AND PROGNOSIS OF MULTIPLE SCLEROSIS IN SOUTH OF FRANCE: STUDY FROM 500 CONSECUTIVE MS PATIENTS USING EDMS.**

Soriani M, Lebrun C, Bourg V, Chatel M

**Neurology, CHU Pasteur, Nice, Am, France**

**Backround:** The course of multiple sclerosis (MS) was assessed in a group of 500 consecutive patients observed in our neurological department. The data were collected using EDMS between May 2000 and April 2002.

**Objectives:** To evaluate the clinical characteristics and the evolution of our MS patients and to compare among them prognosis of 2 subgroups classified according to the number of relapses during the first year of evolution.

**Methods:** According to McDonald criteria, 462 patients (92.4%) presented a definite MS. Among them, 166 (36%, M/F ratio=1/2.4) had an active MS defined by an interval from MS onset to second event <1 year (group A). 249 patients (54%, M/F ratio=1/3.1) had at least 2 relapses with an interval >1 year (group B) and 47 (10%) had primary progressive course. Student test was performed for statistical analysis.

**Results:** The mean age at onset was 32.2+/−10.1 years (median 30.7; group A) and 30.2+/−9.6 years (median 29.5 ; group B) (p<0.05). Record of sequelae after the first relapse was not determinant between the 2 groups (28.3% group A/26.5% group B). Time to progression in RR with sequellae and in secondary progressive (SP) forms was significantly shorter in group A (2.4+/−3.4 years) than in group B (8.3+/−6.2 years ; p<0.001). SP ; group A (8.3+/−7.2 years), group B (12.6+/−6.9 years ; p>0.05). The most common symptom at onset was lower limbs involvement (motor or sensitive): 51.5% (group A)/47.8% (group B ;p=ns), sensory 41.9% (group A)/39.2% (group B ;p=ns), upper limbs involvement (motor or sensitive) : 34.1% (group A)/29.4% (group B ; p<0.05), and optic neuritis: 19.8% (groupA)/36.5% (group B ;p<0.05). After a 5 and 10 years follow-up, more patients with active or progressive form had reached EDSS 3 : group A=13.2% and 24.1%, group B = 8.8% and 2.8%, group C = 29.8% and 51.1%. This difference was still observed for time to reached EDSS 7 at 10 years: group A=24.7%, group B=4.4%, group C=10.6%.

**Conclusions:** Our study confirm that patients presented an active MS had a different clinical profile and a more severe prognosis than those who have a remittent course with a two-first relapses interval >1 year.

Disclosure: e lebrun has nothing to disclose.
P61
SEASON OF BIRTH IN MULTIPLE SCLEROSIS
Luetic GG
Multiple Sclerosis, Sanatorio Britanico, Rosario, Santa Fe, Argentina

Background: MS is a complex disease. It has been strongly suggested that influence of environmental factors early in life may play a key role in the latter development of the disease. There is a great variability in environment features between different seasons (sunlight hours, weather, viral agents, diet). There are few studies regarding the association between birth’s season and MS, and the results are controversial.

Objectives: To investigate season of birth in a Multiple Sclerosis population

Methods: The study was performed in Santa Fe Province (Argentina). Our MS database of over 200 patients is very representative of our MS overall population. We designed a descriptive study. 123 definite MS patients were included. The control group was the siblings of these patients to rule out any bias. Season’s definition used was the standard for the south’s latitudes. At enrollment, birth date and MS clinical course (relapsing-remitting, secondary-progressive, primary-progressive) were assessed. For the evaluation of statistical significance we used chi-square test ($\chi^2$).

Results: Of the 123 MS patients studied, we found that 20.3% were born in autumn; 48% in winter; 14.6% in spring and 17.1% in summer ($\chi^2=2=31.4$, p=0.0000007). The number of winter births was significantly higher in the MS population than in control group ($\chi^2=29.4$, p=0).

Conclusions: MS patients in Santa Fe Province, showed a greater tendency to be born in winter as compared with other seasons and with their own siblings used as control group. 48% of our MS patients were born in winter (p=0.0000007). They were exposed to certain environmental conditions typical of this season (viral agents, less sunlight hours, adverse weather conditions) in a moment of their life, when their immune system (IS) was still immature. In a disease process like MS, where IS (especially T cells) plays such an important role, this finding could be of great implication.

Disclosure: G Luetic has nothing to disclose.

P62
BIRTH WEIGHT IN MULTIPLE SCLEROSIS
Luetic GG
Multiple Sclerosis, Sanatorio Britanico, Rosario, Santa Fe, Argentina

Background: Early in life, in predisposed individuals, environmental factors seems to play an important role in the ethiopathogenesis of MS. Myelin is the target of the immune mediated damage in MS and as we know myelination is an essentially post-birth process. There are many diseases linked with birth weight variations.

Objectives: To study birth weight (BW) in a Multiple Sclerosis population.

Methods: This study took place in Santa Fe Province (Argentina). Our local database is very representative of our MS population. We designed a descriptive study to compare birth weight of definite MS patients with that of the general population (GP). Data from those patients with diabetic mothers was excluded. We considered: sex, clinical course of MS, season of birth, number of siblings and birth weight. 86 patients were included in this study. For statistical analysis we used t-Student, ANOVA or Kruskall Wallis test.

Results: Of the 86 patients studied we found that MS male (n=34) had significantly higher birth weights than MS women (n=52): 3720.6 ± 146 grs and 3361 ± 92 grs respectively (P=0.03 t-Student’s test). When we compared BW of MS patients with those of the GP, we found that MS male patients showed significantly higher BW. This difference was evident from 30th percentile of the BW curve and was greater between 50th and 90th percentile of weight curve (P=0.0003 t-Student’s test). Differences of 500 grs in favour of MS patients were noted (p=0.0003). We couldn’t reproduce this finding when comparing birth weight in women groups.

Conclusions: Birth weight in MS male patients was significantly higher than in general population in our area. Myellogenesis is an active and essentially post-birth process that depends on both intrinsic properties of the cells (oligodendrocytes) and extracellular signals. Environmental changes could affect myelin synthesis. Some studies have shown that dietary fatty acids can be positively involved in the control of CNS myelogenesis. One possible explanation for this finding could be that myelin from these patients was different in its constitution, maybe varying in the amount or quality of its components in a subtle way. This could make it more susceptible to latter immuno-mediated damage when joined to other environmental factors.

Disclosure: G Luetic has nothing to disclose.

P63
USE OF ALTERNATIVE PROVIDERS IN MULTIPLE SCLEROSIS
Marrie R*, Cohen JA*, Hadjimichael OP, Vollmer T*
*Mellen Center, Cleveland Clinic Foundation, Cleveland, OH, Ohio, USA; Neurology, Yale University Medical School, New Haven, Connecticut, USA

Background: Alternative providers are used by MS patients. There are few studies exploring the predictors of use of these therapies. These studies have been small, or limited to a single geographic region, and have identified few predictors.

Objectives: To determine frequency of use of alternative providers (AP) among MS patients, and factors which predict such use, using data from the North American Research Committee on Multiple Sclerosis (NARCOMS) Patient Registry.

Methods: We examined the cohort of MS patients enrolled in the NARCOMS registry residing in the U.S. We used self-reported data on demographics, disability, disease status and use of AP to create a binary multivariate logistic regression model for the use of AP.

Results: Data regarding the use of AP was provided by 17018 patients. Lifetime use of AP was 55%, and current use was 18%. Chiropractors (42%), massage therapists (37%), and nutritionists (16%) were the most commonly used AP. Current use of any AP was statistically significantly associated with being female (OR=1.23), having had a relapse in the last year (OR=1.17), income over $50,000 (OR=1.39), living in the Western U.S. (OR=1.3), having at least some college education (OR=1.24), and being unmarried (OR=1.20). Predictive power of the model was poor (c-index=0.57), despite being a good fit for the data.

Conclusions: Demographic factors play a minimal role in predicting the use of AP in this MS population. There must be other factors involved that may include cost of therapy, accessibility, social acceptability and cultural factors. Given the frequency of use of AP by this patient population, characterization of these factors is important.

Disclosure: R Marrie has nothing to disclose.

P64
THE PREVALENCE OF MULTIPLE SCLEROSIS IN IRELAND
McGuigan C, McCarthy A, Hutchinson M
Dept of Neurology, St Vincents, Dublin, Co. Dublin, Ireland

Background: Ireland has been recognized as a high risk area for MS since the work of Allison and Millar in the 1950s. Recent studies have indicated a high and rising prevalence in the north of the island. A study in County Antrim, northeast Ireland, reported a prevalence rate of 168.7 per 100,000 in 1998. By contrast, prevalence figures for the south of Ireland have remained relatively low with published figures ranging from 48.4 - 73 per 100,000.

Objectives: To establish the prevalence of MS in two Irish counties: Donegal in northwest Ireland and Wexford in the southeast.

Methods: Patients with clinically definite or probable MS (Poser criteria) who were resident within the county borders on the 1st of January 2001 were considered prevalent cases. Sources of case ascertainment included a postal survey...
of General Practitioners, County Physicians, Consultant Neurologists, Respite Facilities and local MS charities. Hospital coding lists and interferon prescription lists were also reviewed. Patient examination and / or review of clinical case records confirmed the diagnosis of MS.

**Results:** In County Donegal, 217 prevalent cases were identified resulting in a prevalence rate of 166.9 per 100,000 (95% confidence limits: 145.5 - 190.7). In County Wexford there were 126 cases giving a prevalence figure of 120.7 per 100,000 (95% confidence limits: 100.5 - 143.7).

**Conclusions:** The southeast of Ireland has a higher prevalence of MS than previously reported, however, the rate remains significantly lower than that for northern counties. This is possibly due to genetic variations within the background populations of the two regions. HLA typing of the background populations in Donegal and Wexford is ongoing to test this hypothesis.

Disclosure: C McGaugan has nothing to disclose.

**P65**

UNCONVENTIONAL THERAPY USE AMONG MULTIPLE SCLEROSIS PATIENTS

Sastre-Garriga J, Manteis E, Rio J, Pericot P, Tellez N, Tintore M; Montalban X

*Clinical Neuroimmunology Unit, University Hospital Vall d’Hebron, Barcelona, Catalonia, Spain; €Neurology Service, Hospital del Mar, Barcelona, Catalonia, Spain*

**Background:** Use of unconventional therapies (UT) is growing in western countries. Prevalence studies as well as knowledge of why they are used in MS are needed.

**Objectives:** To assess the prevalence of UT use among MS patients and to explore associations with clinical, demographical and other variables.

**Methods:** Structured self-administered questionnaires were given to 380 consecutive patients seen at two hospital-based MS clinics. At the time of questionnaire dispensation, clinical data were recorded: duration of disease, time from diagnosis, clinical course of MS, EDSS, and use of disease-modifying treatments. The questionnaire recorded socio-demographical features (including education and income) and inquired on use of UT for MS.

**Results:** The response rate was 50.78%. Clinical and demographical features were not statistically different between patients who responded and those who did not respond. Of the patients who answered the questionnaires 40.9% admitted to using UT in the previous year. Lower degrees of satisfaction with medicine in general, lower degrees of satisfaction with the results of medicine for MS and higher EDSS scores were associated (p<0.05) with higher use of UT in the last year.

**Conclusions:** We found a 40% prevalence of UT use by MS patients in the Barcelona area (similar to that found in a USA survey). UT users have higher EDSS scores and are less satisfied with conventional medicine than non-users. We cannot demonstrate an association with income or educational levels as found in other surveys. Cultural and public health system organisation differences could explain these different findings.

Disclosure: X Montalban has nothing to disclose.

**P66**

ACUTE DISSEMINATED ENCEPHALOMYELITIS (ADEM) IN ADULTS: COMPARISON WITH AN ADEM PEDIATRIC POPULATION


*Neurology, Hospital Ramos Mejia, Buenos Aires, Argentina; €Neurology, Hospital Garrahan, Buenos Aires, Argentina*

**Background:** ADEM is an usually monophasic inflammatory condition of the CNS, typically occurring after infections or vaccinations. Young adults and children are more frequently affected.

**Objectives:** To assess the prevalence of ADEM in an adult population of a Clinic for demyelinating diseases. To analyze the clinical data, the results of complementary studies and prognosis comparing with another pediatric ADEM population (PAP) in the same area.

**Methods:** 590 clinical records registered between 1989-2002 in the Demyelinating Clinic were reviewed. 9 cases were selected with the diagnosis of ADEM (prevalence of 15.2 cases per 1000 clinical records), 8 of these were analysed. The PAP was of 79 patients with a mean age of 5.6 years (0.4-16); 60% were male, 90 % of them experienced complete recovery. We analyzed: preceding infections or vaccinations; symptoms at onset, results of MRI and CSF, treatment and evolution.

**Results:** The mean age at ADEM onset was 32.8 (20-54), 50% were male with a mean time of follow up of 4 months (6-120). 8 cases (87.5%) had a preceding infection (2 of them were EBV; 2 nonspecific viruses and another 3 of bacterial origin), 2 to 30 days (mean time 16) before the neurological manifestation. The symptoms at onset were disturbed consciousness in 6 cases (75%); motor involvement 6 (75%); headache 4 (50%); cranial nerve involvement 4 (50%); sensory disturbances 3 (37.5%); menigism 1 (12.5%), seizures 1 (12.5%). In 4 cases brain MRI showed small and multiple lesions; 3 cases large and multiple and 1 patient no lesions. Gray matter involvement was found in 50 %: CSF pleocytosis in 3 cases. Oligoclonal bands in 20%; 6 cases showed a monophasic pattern, 2 of them had no sequel, 2 had nondisabling symptoms and 2 had moderate deficits. One patient developed a clinical definite Multiple Sclerosis (CDMS) and another a multiphasic course (recurrant ADEM).

**Conclusions:** The prevalence of ADEM in this adult population was significantly lower than in a PAP in the same area. Only one patient developed CDMS and another one recurrant ADEM. According to these data ADEM in adults could have a worse prognosis in terms of sequel.

Disclosure: C Papayannis has nothing to disclose.

**P67**

MULTIPLE SCLEROSIS IN BOTUCATU, BRAZIL - A POPULATION STUDY

Rocha FC*, Herrera LC*, Morales RR*, and the Brazilian Committee for Z

*Neurology Department, Medical School of Unesp, Botucatu, Sao Paulo, Brazil*

**Background:** Population target: Urban people of Botucatu, Brazil’s southwest small city (22.5°S latitude), who had fulfilled multiple sclerosis (MS) diagnostic criteria.

**Objectives:** Study of prevalence and clinical presentation of MS patients in that population.

**Methods:** All patients with symptoms suggestive of MS were personally examined by authors and submitted to paraclinical tests: MRI, CSF, visual evoked potential (VEP), HIV, HTLV, syphilis serology; collagen, inflammatory, infectious and vascular disorders were excluded. Only patients between 15 and 59 years at onset of disease, who had filled diagnostic criteria (McDonalcl et al. 2001), were included.

**Results:** 17 patients had diagnostic of MS in 103,793 inhabitants (prevalence day: 2001 June 1st). Female: 14; male: 3 (46.1: 1). Caucasians: 16; Negro: 1. 13 (76.5%) have European ancestral. Age at onset: 19 to 59 years (mean: 34.9). Evolution forms: relapsing-remitting (RR) (9 patients), primary progressive (PP) (3 p), secondary progressive (SP) (3 p), benign (2 p). Percentage of initial presentation: sensory (29.4%), pyramidal motor (29.4%), diplopia or vertigo (29.4%), paraparesis (23.5%), ataxia (17.7%), optic neuritis (17.7%). Spinal cord syndrome was prominent in PP type and optic neuritis in benign form. EDSS range 1.0 to 7.0.

**Conclusions:** Prevalence of MS in Botucatu - 22.5°S (17/100000) is similar of Sao Paulo - 23.5°S, Brazil (15/100000 - Callegaro, 1999) and Buenos Aires 34°S, Argentina (16.5/100000 - Cristiano, 1997) both in South America. This suggests that these cities have higher risk to MS than reported in literature (Kurtzke, 1998). Frequency of disease is close to south of USA and southern countries of Europe. Factors like geographical location and ethnic background (Caucasians with European ancestry) may be relevant. Clinical distribution corresponded to that described by others in our country (Lana-Peixoto et al, Moreira et al, Tilbery et al). Spinal and cerebellar presentation were associated with worse prognostic, otherwise optic neuritis was associated with better
Imaging (Part 1)

P69

LONGITUDINALLY DIFFUSION TENSOR IMAGING TO MONITOR MULTIPLE SCLEROSIS COURSE

Cassol E1, Ibarrola D2, Ranjeva J3, Manelfe C4, Clanet M4, Berry B5

1Biophysics and Nuclear Medicine, University Hospitals Toulouse, Toulouse, 31, France; 2Neuroradiology-MRI, University Hospitals Toulouse, Toulouse, 31, France; 3Neurology, University Hospitals Toulouse, Toulouse, 31, France; 4Neurologie A, Hôpital Neurologique, Lyon, France

Background: In Multiple Sclerosis (MS), a lack of specificity has been previously reported in derived imaging parameters (such as lesion load quantified on T2-weighted images and Gadolinium enhancement) in order to predict the disease course.

Objectives: Our aim is to determine the reproducibility of Diffusion Tensor Imaging (DTI) in healthy volunteers and to evaluate its capability to monitor patients with Multiple Sclerosis over one year of follow-up.

Methods: Diffusion Tensor Imaging was performed at 1.5 tesla (Magnetom Vision; Siemens Erlangen, Germany). Six non colinear directions of gradients were acquired to obtain the whole diffusion tensor with an Echo-Planar Imaging (EPI) single-shot sequence (b-factor = 506 s.mm-2). Seven MS patients (3 with a Relapsing Remitting form, RR-MS and 4 with a Secondary Progressive, SP-MS) underwent three-monthly MRI examinations over one year of follow-up. All were chosen with a limited cerebral lesion load on T2-weighted images. Seven age and sex matched normal controls underwent three examinations and thirteen controls were also studied once. The mean diffusivity is assessed with the Trace of the tensor (Tr=λ1+λ2+λ3) and the directionality is described with an index of anisotropy (Ani=3λ1/Tr).

Results: DTI is reproducible and sensitive to modifications in cerebral diffusion in MS patients. The Anisotropy parameter seems to be more sensitive to assess the longitudinal modifications over one year of follow-up than mean diffusivity and may better reflect the progressive phase of the disease.

Disclosure: I Berry has nothing to disclose.

Funding: Supported by Association de Recherche contre la Sclérose En Plaques (ARSEP).

P70

MACROPHAGE CELLULAR IMAGING TO MONITOR ANTI-VLA4 ANTIBODY TREATMENT

Dousset V, Deloire-Grassin M, Toull T, Petry KG, Brochet B
EA 2966 (Neurobiology of Myelin Disorders Laboratory), University Victor Segalen, Bordeaux, Bordeaux Cedex, France

Background: Antibodies against adhesion molecule VLA4 have been shown to reduce clinical and histological signs of experimental autoimmune encephalomyelitis (EAE) most probably by reducing blood-brain-barrier (BBB) damage. Anti-VLA4 antibodies are under testing in MS patients (Natulizumab). The new contrast agent ultra-small-particle-iron-oxide (USPIO) is able to disclose. by MRI, in vivo, macrophage containing lesions in EAE. USPIO have been successfully applied to detect active lesions in MS patients.

Objectives: To evaluate the usefulness of USPIO imaging in EAE rats treated by anti-VLA4 antibodies or placebo.

Methods: Acute EAE was induced in Lewis rats by immunization by guinea pig cord homogenates in complete Freund adjuvant. 8 rats were treated by a monoclonal anti rat VLA4 antibody (CD49d) (gift of Biogen, Cambridge, MA) and 8 rats by a placebo. MRI was performed on a 1.5 T magnet. T1 weighted images were performed before and after gadolinium-DTPA (Gd) infusion. T2 and T2* weighted images were also acquired. After the first MRI, rats received USPIO infusion (sinerem®, gift of Guerbet, France) and a second MRI was performed 24 hours after this infusion to detect cellular enhancement. Rats were sacrificed after this MRI to study EAE histological signs and macrophage recruitment by immunohistochemistry.

Results: 3 out of 8 anti-VLA4 antibody treated rats and 7 out of 8 placebo treated rats developed EAE (p<0.03). Mean clinical scores were significantly reduced in the active treatment group (0.24±0.44) compared to the placebo group (1.7±1.1) (p<0.01). The disease was delayed by the treatment (13.5±0.9 days).
P71

COMPARATIVE STUDY OF CELLULAR IMAGING WITH SINEREM® AND USPIO 7228 IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

Deloire-Grassin M, Douset V, Touil T, Petry KG, Brochet B
EA 2966 (Neurobiology of Myelin Disorders Laboratory), University Victor Segalen, Bordeaux, Bordeaux Cedex, France

Background: Phagocytic activity of cells within inflammatory lesions of the central nervous system can be visualized in vivo by MRI using new contrast agents like Ultra small particle iron oxide (USPIO). This has been shown in both experimental autoimmune encephalomyelitis (EAE) and Multiple Sclerosis (MS) using Sinerem®, a first generation USPIO.

Objective: To compare a newly available USPIO, called 7228 to sinerem® for the detection of EAE lesions.

Methods: Acute EAE was induced in 8 Lewis rats by immunization by guinea pig cord homogenates in complete Freund adjuvant. MRI was performed at the peak of EAE clinical signs on a 1,5 T magnet. T1, T2 and T2* weighted images were acquired. Diffusion was measured by obtaining axial 5 mm thick SE echo-planar images (EPJ) (TE/TR = 105.3 ms/ 10000 ms) with matrix resolution of 95 x 128 and with two different b values: 0 and 1000 s/mm². Apparent diffusion coefficient (ADC) maps were generated off-line assuming a monoexponential decrease of signal intensity with b value. From these maps diffusion histograms were calculated after removal of the extracerebral tissue and CSF. TLL was measured off-line on PD weighted images. After applying a threshold, lesions were selected manually on each image by two radiologists and the area was automatically calculated by the software Scion (NIH, USA).

Results: Mean lesion load was 1261±1545 mm² (range 20 to 5564 mm²). From diffusion histograms following parameters were calculated: mean ADC 1000±44 x10⁻⁶ mm²/s (range 945 to 1075 x10⁻⁶ mm²/s), mean ADC peak position 803±31 x10⁻⁶ mm²/s (range 757 to 877 x10⁻⁶ mm²/s) and mean ADC peak height 43.9±6.1 (range 34 to 52). Mean ADC and ADC peak height showed a strong and significant correlation with TLL (0.7355; p = 0.002 and ± 0.7678; p < 0.001 respectively), but the correlation between ADC peak position and TLL was not significant.

Conclusions: Whole brain diffusion histograms can be a reliable and fast method to control disease evolution in MS patients.

Disclosure: D Callegaro has nothing to disclose.

P72

CORRELATION BETWEEN DIFFUSION MAGNETIC RESONANCE IMAGING HISTOSTRUCTURAL ANALYSIS AND TOTAL LESION LOAD IN MULTIPLE SCLEROSIS.

Callegaro D¹, Otaduy M², Lacerda M², Costa M², Bachesi L³, Leite C⁴
¹Neurology, Medical School of the University of São Paulo, São Paulo, Brazil; ²Radiology, Medical School of the University of São Paulo, São Paulo, Brazil

Background: Diffusion MRI is sensitive to the microscopic random translational motion of water molecules in biological tissue. Pathological tissue in MS is commonly characterized by an increase in diffusion. The histogram analysis of diffusion images provides global parameters for the patient, which measure both the lesions and NAWM. It is important to address what is the relation between these parameters and the lesion load measured on conventional images.

Objective: To correlate whole-brain diffusion MR histogram results to total lesion load (TLL) in MS patients.

Methods: Fifteen patients (mean age = 32 ± 8 years) with MS were examined with conventional and diffusion-weighted MR imaging. Studies were performed on a 1.5 T MRI scanner. Axial 5 mm thick T2, T1 and PD weighted images were acquired. Diffusion was measured by obtaining axial 5 mm thick whole brain diffusion histograms. The correlation between these parameters and the lesion load measured on conventional images was assessed.

Results: Mean T1 relaxation time was 669.5 ms in MS subjects (standard deviation (SD) 22.8 ms) and 630.8 ms in controls (SD 15.2 ms), p<0.001. No significant change in T1 was observed during follow up although at baseline, values were significantly related to disease duration. Results suggest that, assuming a linear relationship, the T1 relaxation times increase by 1.14 ms per month (range 12-36 months). Dual fast spin echo (FSE) sequences were obtained along with a gradient echo data set (PD and T1 weighted images) permitting the calculation of a T1 map. Lesions were contoured on the FSE image following registration of this image to the T1 image. Using these regions and SPM derived masks, NAWM segments were generated from the T1 map. Remaining CSF was removed with a 1000ms threshold and 2 sequential erosions. Normalized histograms were generated from the NAWM segments of the T1 map and mean T1 was extracted from the histogram. A hierarchical regression model was used to assess the difference in mean T1 between MS subjects and to look for a gradient of increase in T1 with time. Gender differences were accounted for.

Results: The mean T1 relaxation time was 669.5 ms in MS subjects (standard deviation (SD) 22.8 ms) and 630.8 ms in controls (SD 15.2 ms), p<0.001. No significant change in T1 was observed during follow up although at baseline, values were significantly related to disease duration. Results suggest that, assuming a linear relationship, the T1 relaxation times increase by 1.14 ms per month between disease onset and the first data point. Projecting back from these observations suggests that at the time of disease onset, abnormalities are already present.

Conclusions: This study confirms the presence of diffuse T1 relaxation time abnormalities early in the clinical course of MS and suggests that these are present prior to clinical onset.

Disclosure: G Davies has nothing to disclose.

Funding: Supported by MS society programme grant (MS society of Great Britain and Northern Ireland).
P74
LONGITUDINAL STUDY OF PROGRESSIVE BRAIN ATROPHY IN MULTIPLE SCLEROSIS. PRELIMINARY DATA.

Durand Dubief F1, Pachai C2, Vukusic S3, Gignoux L4, Renoux C5, Cotton F6, Froment J6, Confavreux C7
1Neurologie A, Hôpital Neurologique, Lyon Cedex 03, France; 2Therapy, Lyon, France; 3Neuroradiologie, CH Lyon Sud, Lyon, France; 4Neuroradiologie, Hôpital Neurologique, Lyon, France

Background: Numerous studies have been done to quantify the severity and the evolution of brain tissue damage in multiple sclerosis (MS). Brain atrophy measured by MRI techniques is a potential way to monitor disease progression in MS.

Objectives: The objective of the study was to determine the evolution of the whole brain atrophy in MS patients by annual MRI acquisitions.

Methods: 3D MRI T1 weighted sequences covering the whole brain from the foramen magnum to the vertex, pre and post-Gadolinium enhancement were performed on a yearly basis (matrix 256.256.170 ; resolution : 1 mm). Serial 3D data sets were spatially registered for each patient using a fully automatic algorithm. Intracranial volume, brain parenchyma volume and ventricular volume were automatically segmented. A mask representing artery and vein structures was obtained for each patient by segmenting Gadolinium enhanced acquisitions. These vascular structures were removed from the final brain mask. Two parallel anatomical planes at anterior/posterior commisural level and internal auditory meatus level were interactively defined. These planes were considered as the quantification cut-off. We quantified different volumes of interest such as the volume above the anterior/posterior commisural plane, volume above the internal auditory meatus, whole brain volume and Brain Parenchymal Fraction.

Results: Seventeen patients with clinically definite MS have been included in the study. Eight patients had a relapsing-remitting course and nine a secondary progressive one. Mean disease duration at the first MRI was 13 years (Range : 0 - 34). Mean follow-up duration was 3.8 years (Range : 3 - 5). Mean number of MRI assessments for each patient was 3.5. The different volumes were compared to identify the more informative one. The percentage of brain atrophy was evaluated for each patient over at least 3 years.

Conclusions: Analysis is still in progress

Disclosure: F Durand Dubief has nothing to disclose.

P75
SERIAL MAGNETIZATION TRANSFER IMAGING IN OPTIC NEURITIS

Hickman SJ1,2b, Toosy ATa,b, Miszkiel KA2, Jones SJ2,3, Altman D2, MacManus DG2, Barker GJ, Plant GTb, Thompson AJ, Miller DH3
1NMR Research Unit, Institute of Neurology, University College London, United Kingdom; 2Dept. of Neuro-Ophthalmology, Moorfields Eye Hospital, London, United Kingdom; 3Lysholm Radiological Dept., The National Hospital for Neurology and Neurosurgery, London, United Kingdom; 4Dept. of Clinical Neurophysiology, The National Hospital for Neurology and Neurosurgery, London, United Kingdom; 5Institute of Neurology, University College London, United Kingdom

Background: In multiple sclerosis (MS) reductions in magnetization transfer ratio (MTR) are thought to be due to demyelination and axonal loss.

Objectives: To follow serial changes in MTR in optic neuritis lesions to further validate optic neuritis as a model for MS relapses.

Methods: 26 patients were recruited with acute unilateral optic neuritis. Their optic nerves were imaged a mean 14 days since the onset of visual symptoms with a fat saturated fast spin echo (FSE) sequence and a 3D gradient echo magnetization transfer sequence. 17 of them had serial imaging after 2, 4, 8, 12, 26 and 52 weeks. A blinded observer segmented the optic nerves from the MTR maps. Lesions were then defined on the acute FSE images and from the coordinates the mean lesion MTR was calculated for each time point.

Results: The mean lesion MTR at baseline was 47.3pu compared with 48.2pu in the contralateral healthy nerve (p=0.03). The serial lesion data were analysed using a hierarchical model with a quadratic term which gave the best fit. In the acute phase mean lesion MTR fell rapidly. The coefficient in the linear term was -0.029pu per day (standard error [SE] 0.005), 95% confidence intervals (CI) -0.039 to -0.019, p<0.0001. The quadratic term coefficient was 0.000063 (SE 0.000014), 95% CI 0.000033 to 0.000093, p<0.0001. The rate of decrease lessened over time with the suggestion of a nadir of mean lesion MTR at about 200 days and a small increase in MTR subsequently.

Conclusions: The early fall in MTR is consistent with demyelination and acute axonal damage. The subsequent late rise in mean lesion MTR suggests that this technique may be able to demonstrate remyelination.

Disclosure: S Hickman has nothing to disclose.

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P76
CORTICOSTEROIDS AND OPTIC NERVE ATROPHY FOLLOWING OPTIC NEURITIS

Hickman SJ1,2b, Kapoor R, Jones SJ2,3, Altman D2, Plant GTb, Miller DH3
1NMR Research Unit, Institute of Neurology, University College London, United Kingdom; 2Dept. of Neuro-Ophthalmology, Moorfields Eye Hospital, London, United Kingdom; 3Dept. of Neurology, The National Hospital for Neurology and Neurosurgery, London, United Kingdom; 4Dept. of Clinical Neurophysiology, The National Hospital for Neurology and Neurosurgery, London, United Kingdom

Background: Optic nerve atrophy has been shown to develop following optic neuritis. There has been recent interest in the use of corticosteroids as neuroprotective agents. Pulsed corticosteroid treatment has been shown to reduce the development of cerebral atrophy in multiple sclerosis.

Objectives: To assess whether corticosteroids prevent the development of optic nerve atrophy following optic neuritis.

Methods: Optic nerve short tau inversion recovery images from a recent randomized placebo controlled trial of intravenous methylprednisolone in acute optic neuritis were evaluated. Imaging was performed before randomization and six months later. Mean optic nerve area was measured by an observer blinded to image identity, acquisition order and treatment status from two consecutive orbital slices using a semi-automated contouring technique.

Results: At baseline optic nerve mean area was 18.4mm2 in affected optic nerves and 17.8mm2 in unaffected optic nerve (n=45, p=0.3). After six months optic nerve mean area was 16.4mm2 in affected optic nerves and 17.4mm2 in unaffected optic nerves (n=59, p=0.019). The mean area of affected optic nerves in the corticosteroid group was 15.9mm2 (n=30) compared with 16.9mm2 (n=29) in the placebo group (p=0.29). The mean measurement coefficient of variation was 3.8%.

Conclusions: This technique was able to demonstrate optic nerve atrophy following optic neuritis. There is no evidence from these data that intravenous methylprednisolone prevents the short-term development of optic nerve atrophy following optic neuritis.

Disclosure: S Hickman has nothing to disclose.

Funding: SJH is Supported by The Wellcome Trust. The NMR Research Unit is Supported by The Multiple Sclerosis Society of Great Britain and Northern Ireland.
Background: Triple dose gadolinium (Gd) increases the sensitivity for detecting enhancing lesions in multiple sclerosis.

Objectives: To assess whether the use of triple dose Gd in optic nerve imaging can help in predicting the prognosis for visual recovery following optic neuritis.

Methods: 28 patients were examined a median 13 (range 8-21) days after onset of their first episode of acute unilateral optic neuritis. After administration of intravenous triple dose Gd the patients’ optic nerves were imaged with a fat saturated T1-weighted spin echo sequence. A blinded observer identified and measured the length of any enhancing nerve lesions on the images. Serial imaging was performed on 15 of the patients and the duration of enhancement noted. A clinical assessment was carried out at baseline and again after one year.

Results: The symptomatic lesion was identified in 27/28 cases. The median lesion length at baseline was 30mm (range 0-39mm). Patients with longer lesions (≥30mm) had worse initial 30-2 Humphrey visual field mean deviations (p=0.009) and central field visual evoked potential amplitudes (p=0.05) compared with those patients with shorter lesions. The median duration of enhancement was 63 days (range 0-113 days). Neither the length of the initial lesion nor the duration of enhancement were correlated significantly with final visual outcome.

Conclusions: The initial lesion length in optic neuritis appears to be important in determining the amount of initial visual impairment. The extent and duration of the initial inflammatory lesion does not, however appear to affect prognosis.

Disclosure: S Hickman has nothing to disclose.

Funding: SJH is Supported by The Wellcome Trust. ATT is Supported by Action Research. The NMR Research Unit is Supported by The Multiple Sclerosis Society of Great Britain and Northern Ireland.
often done to determine if this is due to a secondary diagnosis. We report a woman with clinically definite MS for 9 years who had unexpected worsening due to a large CNS mass.

**Objectives:** To investigate possible causes of unexpected deterioration in a patient with MS using both standard and novel MRI techniques.

**Methods:** A 53 year old woman with MS who presented with a 3 month history of anorexia and vomiting had a standard neurologic exam. Prior to biopsy, gadolinium enhanced MRI, proton MR spectroscopy (MRS) and a 48 echo T2 relaxation measurement were performed using a 1.5T GE scanner.

**Results:** MRI demonstrated a large frontal mass (>60cm^3) with mixed signal intensity, edema, significant mass effect and irregular ring enhancement. (One year prior, the patient had a large frontal plaque extending into the corpus callosum. No mass effect was evident at that time). The lesion spectrum was dominated by large peaks around 1ppm, likely due to lipid. T2 measurements showed heterogeneous decreases in myelin water and prolonged relaxation times. At least 3 T2-distinguishable volumes were found within the mass, with intermediate mean T2 times (~130ms) and myelin signal remaining in the central core, while the periphery tended to have almost no myelin signal and longer relaxation times (250-450ms). Histopathology showed characteristics typical of a giant cell glioblastoma (grade IV astrocytoma).

**Conclusions:** MRI is indicated for MS patients with unexpected deterioration to exclude a secondary diagnosis. For more pathologically specific information, MRS and T2 measurements are valuable.

Disclosure: C Lasle has nothing to disclose.

**Funding:** MS Society of Canada.

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**GUIDELINES FOR A STANDARDIZED MRI PROTOCOL FOR THE DIAGNOSIS AND FOLLOW-UP OF MULTIPLE SCLEROSIS**

Li D* Traboulsi A†, Paty D‡, Work Group on Standardized MRI Protocol C

*Radiology, University of British Columbia, Vancouver, British Columbia, Canada; †Neurology, University of British Columbia, Vancouver, British Columbia, Canada; ‡Consortium of MS Centers, Tcaneck, New Jersey, USA

**Background:** The use of magnetic resonance imaging (MRI) has become routine in the diagnostic workup of patients with multiple sclerosis (MS). There are however no guidelines for the use of MRI in practice. Standardized MRI protocols would help maximize the value of individual scans, as well as allow systematic data collection for clinical and comparison studies.

**Objectives:** To develop consensus guidelines for standardized MRI protocols and indications for their use in the diagnosis and follow up of patients with MS.

**Methods:** An expert consensus meeting on ‘MRI Protocols for the Diagnosis and Follow Up of MS’, sponsored by the Consortium of MS Centers (CMSC) was convened November 3–4, 2001 in Vancouver, Canada. Participants included MS neurologists and radiologists from North America, Europe, and New Zealand, with representation from the American Academy of Neurology, American Society of Neuroradiology, and Radiological Society of North America.

**Results:** 10 guidelines were recommended with specific proposals concerning baseline evaluation, diagnosis and follow-up, indications for spinal imaging, use of gadolinium, appropriate requests, reports and archiving. The guidelines proposed standardized MRI protocols to image the brain and spinal cord, with specified required and optional features. Scans should be obtained, if possible, on a 1 Tesla or higher machine, using a slice thickness of 3mm or less (1.5mm or less for 3D sequences), without gaps, with scans oriented along the subcallosal line using a 3 plane localizer if available. Routine follow up MRI’s in practice were not recommended, until standardization. A prototype radiology report was suggested using standardized and consistent common language, describing such features as lesion number, location, size, shape, and character. A copy of the MRI should be retained permanently for future comparison.

**Conclusions:** Consensus guidelines will be posted for review on the CMSC website (www.mscare.org) for discussion and comment. The development of these recommendations is only the beginning of what has to be a continuing process, requiring modification, implementation, evaluation and continued updating and improvement.

Disclosure: D Li has nothing to disclose.

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**THE HYPOINTENSE LESION (“BLACK HOLE”) ON T1-WEIGHTED MAGNETIC RESONANCE IMAGING IN SECONDARY PROGRESSIVE MS: OBSERVATIONS WITH T1-WEIGHTED SPIN-echo, MPRAGE AND MAGNETIZATION TRANSFER IMAGING**

redmond Jr†, Tench CR‡, Blumhardt LD§

*Neurology, Hospital de la Princesa, Madrid, Spain; ‡Centro MEG, Complutense University of Madrid, Madrid, Spain

**Background:** Magnetoencephalography (MEG) is a non-invasive technique that allows us to detect the magnetic dendritic activity and the anatomical localization in MRI images.

**Objectives:** The objective of this study is to detect and measure the abnormal low frequency magnetic activity (ALFMA) and epileptogenic-like activity in multiple sclerosis patients and evaluate their clinical applications as a complementary diagnostic technique.

**Methods:** Twenty one patients were selected according to the followin criteria: twenty one relapsing-remitting (RR) MS patients. All the RR patients were recently diagnosed with at least 2 relapsing episodes within the last two years. Mean age 31.6 (range 22-45). Mean EDSS 1.5 (range 0-3.5). None of them were on corticoid therapy in the last 3 months before the MEG or were on immunosupressor treatment. Spontaneous awake simultaneous MEG-EEG recording was measured using a 148 channel biomagnetic system with a MAGNES 2500W device, performing an equivalent current dipole analysis and superimposing on a corresponding 3D weighted MRI.

**Results:** The results reveal that on all the patient MEG recordings appear focal slow waves (theta & delta) activity; in 17 patients (81%), of wich had slow waves in rolandic areas (frontal and parietal ascendant circumbolations), in addition of the same group, 52% had slow waves in other parietal areas. Others frequently affected areas were frontal and temporal lobes. Also, epileptogenic-like activity was found independently in MEG records (simultaneous EEG was normal in all patients) in 12 patients (57%). The most affected areas were frontal and parietal ascendant circumbolations (52%);and other parietal areas (43%). Other areas with infrequent epileptogenic-like activity were frontal and temporal lobes.

**Conclusions:** The MEG detects the presence of abnormal activities in well limited areas of the brain cortex. Epileptogenic-like activity in a high percent of patients (57%) has been found. This activity was not found in conventional simultaneous EEG records. This sharp MEG activity has no correlation with clinical features, degree of lesional load, degree of motor disability and multiple sclerosis evolution time. The presence of this activity is related with brain cortex affection. This results could be explained in future studies.

Disclosure: D Martin C. receives a grant from Serono laboratories.

**Funding:** Supported by Serono laboratories.
ent Echo (MPRAGE), that provide greater T1 contrast will reduce the pathological specificity of black holes.

**Objectives:** To investigate black hole volumes using moderately T1-weighted T1 SE and heavily T1-weighted MPRAGE, and, to investigate the pathological specificity of such lesions as quantified using the Magnetization Transfer ratio (MTR).

**Methods:** For 12 patients with secondary progressive MS, T2 SE and MTR images, and post contrast T1 SE and MPRAGE images, were acquired. MPRAGE images were reformatted to the same voxel dimensions as the spin echo sequences and co-registered using image correlation. Black hole and T2 lesion volumes were estimated using a seeded region growing technique.

**Results:** Median lesion loads were: T2 10146mm3, T1 1178mm3, MPRAGE 4491mm3. The mean MTR of lesions identified on T1 SE was 0.316, significantly lower than those identified on T2 (0.344, p=0.0004) or MPRAGE (0.33, p=0.03). The mean MTR of MPRAGE lesions was significantly lower than for T2 lesions (p=0.03). Individual lesions were significantly larger on MPRAGE (207mm3) than T1 SE (159mm3, p=0.005). MPRAGE signal intensity (normalised to signal intensity of normal appearing white matter) of lesions visible on MPRAGE but not T1 SE was 0.68, significantly higher than those visible on both T1 SE and MPRAGE (0.88, p=0.05).

**Conclusions:** Heavily T1 weighted Gradient Echo sequences yield greater black hole volumes than T1 SE. Lesions visible on MPRAGE but not T1 SE are smaller and less hypointense than lesions detected on both sequences. MTR values of MPRAGE lesions are higher than T1 (but lower than T2) suggesting that the greater sensitivity of MPRAGE is at the expense of pathological specificity. MPRAGE lesions may not be as pathologically heterogeneous as T2 lesions.

**Disclosure:** i redmond has nothing to disclose.

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**P84**

**STEREOTAXIC CO-REGISTRATION OF MRI AND HISTOPATHOLOGY IN POST-MORTEM MS BRAIN SLICES**

Schmierer K1, Scaravilli F2, Parker GJ3, MacManus DG3, Miller DH3


**Background:** Several groups have examined the pathological correlate of MRI in post-mortem (PM) MS brain. However, no standardised method has been developed so far to accurately match the areas of changes visible on MRI with the tissue specimens.

**Objectives:** To investigate whether a stereotaxic navigation system is a useful tool to co-register lesions on MRI and pathologically in PM brain slices of patients with MS.

**Methods:** PM tissue specimens of seven patients (4 women, 3 men; age: 59.4 +/- 18.7 years) with MS were studied. Formalin fixed coronal slices (thickness: 1 cm) of one cerebral hemisphere were placed on a grid that was attached to a stereotaxic navigation system. Spin echo T2-weighted MRI and FLAIR scans of 5 mm slice thickness were obtained. Lesions visible on MRI were matched with previously obtained scans of the same, but fresh, specimens. Guided by the target points calculated from coordinates of the localizer frame, the dissection of the brain slice was performed. Blocks were cut in halves (thickness: 5 mm) with the cut surface corresponding with the imaging plane. After processing of the blocks for embedding in paraffin, sections were stained with H & E and Luxol-Fast blue.

**Results:** Of 33 lesions detected on MRI, 19 could be analysed whereas 14 could not be matched with the specimens. Normal appearing white matter (NAWM) of each brain slice served as an intraindividual reference. Lesions were chronically inactive, three were classified as remyelinated. Reduced MTR correlated strongly with axonal loss and demyelination as reflected by high LT values in Bielschowsky- and LFB-stained slices. MTR and LT also correlated strongly with T1 hypointensity whereas T1-R did not. The latter may be due to an increase of T1-R in PM brain (mean T1-R of NAWM, 740 ms). No difference for any measure of MRI or DIA was detected between demyelinating and remyelinating lesions, probably due to the small sample size in this study.

**Conclusions:** These preliminary results show that DIA is a promising tool to quantify specific components of MS pathology in PM brain tissue. Its exact role needs to be confirmed in a larger sample and in combination with other quantitative MRI measures (e.g. fractional anisotropy, bound water fraction).

**Disclosure:** Dr. Schmierer is Supported by Serono Pharma GmbH, Unterschleissheim, Germany.

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**P85**

**QUANTITATIVE ASSESSMENT OF LESION PATHOLOGY IN POST-MORTEM MS BRAIN: A CORRELATIVE MRI/HISTOLOGY STUDY**

Schmierer K1, Scaravilli F2, Griffin CM3, Barker GJ3, Miller DH3


**Background:** Recent studies have shown that there has been considerable overlap between lesions at various stages with regard to measures of MRI and histopathology in post mortem (PM) MS brain. To better quantify the pathological components of MS, there is a need to improve the ability of MRI to depict these features more specifically.

**Objectives:** To quantify and correlate the myelin content and axonal density in MS brain using MRI and digital image analysis (DIA) of histological sections.

**Methods:** Five fresh coronal PM MS brain slices (1 cm thick) were studied. T1-, T2-, PD-weighted, and FLAIR scans were performed. T1-relaxation time (T1-R), and magnetisation transfer ratio (MTR) maps were produced. After scanning, the specimens were cut so that the cut surface resembled the imaging plane. Tissue blocks were processed for embedding in paraffin and sections were stained (H & E, Luxol-Fast blue (LFB), Bielschowsky). Immunohistochemistry included glial fibre associated protein (GFAP), CD68, and anti myelin basic protein (MBP). We used a Leica Q500MC image analyser for DIA of the slides. Light transmittance (LT) was obtained separately for LFB and Bielschowsky stained slices.

**Results:** Of 33 lesions detected on MRI, 19 could be analysed whereas 14 could not be matched with the specimens. Normal appearing white matter (NAWM) of each brain slice served as an intraindividual reference. Lesions were chronically inactive, three were classified as remyelinated. Reduced MTR correlated strongly with axonal loss and demyelination as reflected by high LT values in Bielschowsky- and LFB-stained slices. MTR and LT also correlated strongly with T1 hypointensity whereas T1-R did not. The latter may be due to an increase of T1-R in PM brain (mean T1-R of NAWM, 740 ms). No difference for any measure of MRI or DIA was detected between demyelinating and remyelinating lesions, probably due to the small sample size in this study.

**Conclusions:** These preliminary results show that DIA is a promising tool to quantify specific components of MS pathology in PM brain tissue. Its exact role needs to be confirmed in a larger sample and in combination with other quantitative MRI measures (e.g. fractional anisotropy, bound water fraction).

**Disclosure:** Dr. Schmierer is Supported by Serono Pharma GmbH, Unterschleissheim, Germany.

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**P86**

**T2 LESION BURDEN AND T1-HYPOINTENSE (T1-BLACK HOLE) MRI RESULTS FROM THE IMPACT TRIAL**

Simon JP1, Cohen JA1, Goodman A1, Heidenreich F1, Kooijmans M1, Sandrock A2, Tsao EC3, IMPACT Investigators

1Radiology/MRI, University of Colorado HSC, Denver, Colorado, USA; 2Biogen, Inc, Cambridge, Massachusetts, USA; 3Neurology, University of Rochester, Rochester, New York, USA; 4Mellon Center, Cleveland Clinic, Cleveland, Ohio, USA; 5Neurology, Hannover Medical School, Hannover, Germany

**Background:** The IMPACT Trial evaluated the effect of treatment in patients with secondary progressive MS with EDSS 3.5 to 6.5 randomized to treatment
with interferon beta-1a (IFN beta-1a; AVONEX) or placebo by weekly intramuscular injection (60 mcg) for two years. Prior MRI analyses have shown that new or enlarging T2 lesions and gadolinium enhancing lesions (inflammatory activity) are significantly reduced in treated patients at one and two years (p < 0.001).

**Objectives:** To determine the effect of treatment and to define the natural history of T2 burden of disease (T2 lesion volume) and more destructive tissue changes based on T1-hypointense lesions (T1-black holes) in secondary progressive MS.

**Methods:** T2 lesion volume and T1 hypointense lesion volume were determined at baseline, year one and two years for all evaluable studies. Differences between treatment groups were determined based on nonparametric analysis of variance, stratified by baseline EDSS and number of enhancing lesions on baseline MRI.

**Results:** The T2-lesion volumes and T1-lesion volumes were well balanced at baseline. A treatment effect in favor of treatment with IFN beta-1a was seen with a significant reduction in change from baseline T2 volume at both one and two years (p < 0.001 and p < 0.001, respectively). A similar treatment effect was seen with T1 hypointense lesion volume with group differences favorable for treatment for change from baseline at both one and two year intervals, with a significant change over the two year timepoint (p = 0.02).

**Conclusions:** These results extend the positive MRI observations from previous analyses of the IMPACT Trial to include measures of T2 burden of disease and the more destructive and likely irreversible injury indicated by T1 black holes, and are supportive of the clinical findings of positive effects of treatment on MS Functional composite progression, relapses and quality of life.

Disclosure: J Simon and several co-authors have received honoraria as consultant or for speaking for Biogen, Inc. M. Kooijmans, E Tsao, A Sandrock are employees of Biogen, Inc.

Funding: Supported by Biogen, Inc. Cambridge, MA.

### P87

**NEURONAL TRACT DEGENERATION PATTERNS BASED ON DIFFUSION TENSOR VERSUS T2 MRI CHANGES IN THE CORPUS CALLOSUM IN MS**

**Coombs BP, Corboy P, Simon JP**

*Radiology/MRI, University of Colorado HSC, Denver, Colorado, USA; Neurology, University of Colorado HSC, Denver, Colorado, USA*

**Background:** Prior studies have revealed that acute focal MS lesions may be the source of secondary abnormalities observed on high resolution T2 weighted (T2W) MRI images including transcallosal bands and corticospinal tract patterns that traverse long white matter pathways. We have hypothesized that these are the result of neuronal tract degeneration.

**Objectives:** To test the hypothesis that diffusion tensor derived measures (mean diffusivity and fractional anisotropy) are sensitive to abnormality in the corpus callosum independent of the visually detected and defined tract degeneration patterns detected by high resolution T2W images.

**Methods:** Diffusion tensor analyses based on mean diffusivity (MD) and fractional anisotropy index (FA) calculated in a pilot study with 10 consecutive MS patients (EDSS 1.5 to 8) compared to normal controls. MR acquisition included spin-echo echo-planar (EP) navigator-echo phase-corrected series with effective b-values of 0 and 1000 mm^2/s with six diffusion encoding gradient directions. Regional analysis of corpus callosum based on five midline sagittal slices per subject with EP data mapped by a bilinear geometric transformation onto T2-weighted images used as the basis of lesion, ie abnormal appearing white matter (AAMW) versus normal appearing white matter (NAWM) segmentation.

**Results:** In established MS transcallosal bands in some cases involved a substantial fraction of the midline callosum on T2W imaging. Abnormal increased MD was detected in both the NAWM (mean, 95% CI) (0.93, 0.84-1.02) and AAMW fractions (1.24, 0.91-1.57). These abnormalities corresponded to abnormal decreased FA in NAWM (0.70, 0.65-0.75) and AAMW 0.56, 0.48-0.65 fractions.

**Conclusions:** This pilot data suggests that the diffusion tensor derived MD and FA measures are sensitive to pathology in the NAWM in MS in the corpus callosum suggesting the possibility of several classes or degrees of abnormal tissue, some potentially the result of secondary tract degeneration. The corpus callosum provides a volume of relatively homogeneous white matter well-suited for the detailed study of tract degeneration in early MS.

**Disclosure:** J Simon has nothing to disclose.

**Funding:** Supported by National MS Society (RG 3307-A-1).
ponents and functions of the immune system. In vivo and in vitro studies show that mitoxantrone reduces T cell numbers and suppresses humoral immunity.

Objectives: Our objective was to report the early changes in immune cell counts following mitoxantrone treatment in severely progressive MS patients.

Methods: In our study, four relapsing progressive and two secondary progressive MS cases not responding to other treatments (IV methylprednisolone, interferons, plasma exchange, IV immunoglobulin or azathioprine) were included. Before enrollment, patients underwent a general medical history, physical and neurologic examination. Screening blood tests, urinalysis, cardiac tests were performed. The mean age of the patients was 36.6 years (range: 23-61), mean duration of the disease was 13 years (range: 4-30) and mean EDSS was 5.7 (range: 4.0-6.5). According to our protocol, most of the patients were treated in the Oncology outpatient clinic. Blood samples were withdrawn immediately before first and second mitoxantrone infusions (12 mg/m²). The interval between two infusions was 4-8 weeks. Peripheral immune typing including white blood cell, lymphocyte, monocyte, CD4, CD8, CD3, CD2, CD19, CD16, CD4/CD45RA, CD4/CD45RO were done by using flow cytometry.

Results: While total lymphocyte and CD4 count did not show any prominent change, CD8, CD3, CD16, CD4/CD45RA and CD4/CD45RO cell counts showed a tendency to decrease after a single IV mitoxantrone infusion.

Conclusions: Our preliminary study is limited. Detailed longitudinal immunologic studies could be helpful to understand better the mechanism of action of mitoxantrone in MS patients.

Disclosure: A Altintas has nothing to disclose.

P90

EFFECT OF 24 MONTHS INTERFERONβ1A TREATMENT ON CD4, CD8, CD4CD45RO AND CD4CD45RA CELLS IN MULTIPLE SCLEROSIS

Belniak E, Bartosik-Poujek H, Mitosek-Szewczyk K, Stelmasiak Z
Neurology, Medical School, Lublin, Poland

Background: Multiple sclerosis is a chronic, autoimmune, demyelinating disease of the central nervous system. The pathological process underlying MS involves dysregulation of the immune system and it is predominantly T-cell mediated immune disorder.

Objectives: The aim of our study was evaluation of some select T-cells subpopulations: CD4, CD8, CD45RO and CD45RA cells in patients with MS treated with INFβ1a (30 µg im once a week). Methods: We investigated 15 patients (6 men and 9 women aged 32.3±8.4) with RRMS. Peripheral blood samples were collected before therapy and after 9 and 24 months after therapy initiation. Expression of C4, C8, CD45RO and CD45RA were evaluated by flow cytometry method. Comparisons were made with 20 control patients matched in age.

Methods: We investigated 15 patients (6 men and 9 women aged 32.3±8.4) with RRMS. Peripheral blood samples were collected before therapy and after 9 and 24 months after therapy initiation. Expression of C4, C8, CD45RO and CD45RA were evaluated by flow cytometry method. Comparisons were made with 20 control patients matched in age.

Results: Compared to the controls the MS patients showed statistically significant (p<0.01) lower expression of CD8, higher (p<0.001) expression of memory cells (CD4+CD45RO+) and they had statistically significant higher (p<0.001) index CD4/CD8. Expression of CD4+CD45RO+ cells increased after 9 months and was still increased after 24 months after therapy initiation. Compared to controls expression of CD4+CD45RA+ cells didn’t differ and it didn’t show any significant fluctuations during therapy.

Conclusions: Our findings suggest that after 24 months of INFβ1a therapy there are no normalisation of CD8 and CD4+CD45RO+ cells disturbances in MS patients.

Disclosure: E Belniak has nothing to disclose.

Funding: Drug supply supported by Schering Plough Disclosure: Ewa Belniak has nothing to disclose.
mediated, at least in part, by matrix metalloproteinase activity. Other processes central to CNS inflammation, such as ICAM-1 upregulation and TNF-α expression in CNS tissues, are evidently dependent on these initial peroxynitrite-dependent and urate sensitive permeability changes. The likelihood that similar mechanisms contribute to the pathogenesis of MS is Supported by recent evidence that serum urate levels in relapsing-remitting MS patients are inversely correlated with disease activity and BBB dysfunction. This suggests that elevation of urate levels in MS patients may have therapeutic benefit.

Disclosure: D Hooper has nothing to disclose.

Funding: Supported by the National Multiple Sclerosis Society and by a grant from the Commonwealth of Pennsylvania to the Biotechnology Foundation Laboratories.

P93
T-CELL REACTIVITY IN MULTIPLE SCLEROSIS; PREDICTIVE VALUE FOR EFFICACY OF INTERFERON-BETA
Killestein J, Hintzen Rb, Utdehaug Br, van Lier R; Polman C°
°Neurology, VU Medical Center, Amsterdam, NL, Netherlands; °Neurology, EMCR, Rotterdam, NL, Netherlands; °Experimental immunology, AMC, Amsterdam, NL, Netherlands

Background: Measuring proliferative responses of T lymphocytes in whole blood is a simple, reproducible and widely used assay of immune competence. Evidence suggests a role of T-cell reactivity in several infectious and autoimmune diseases.

Objectives: We aimed to compare proliferative T-cell responses in the different clinical subgroups of MS. In addition, it was assessed whether this assay could serve as a predictor of clinical responsiveness to immunomodulatory therapy with Beta-interferon(IFN).

Methods: Proliferative responses of T lymphocytes were measured in whole blood of 189 MS patients (65 RR, 70 SP and 54 PPMS) and 249 healthy controls (HC). Forty-eight relapse-onset patients started treatment with IFN. Based on EDSS progression and number of relapses and steroid interventions in 2 years before initiation of treatment compared to 2 years under treatment, patients were classified as either clinical responder or non-responder to IFN.

Results: T-cell proliferation in samples from both relapse-onset and PPMS patients was increased compared to HC. Furthermore, the proliferative response of whole blood T lymphocytes to phytolaemagglutinin (PHA), correctly predicted 83% of the clinical responders and non-responders to IFN.

Conclusions: Compared to HC, MS patients show increased peripheral blood T-cell reactivity. The level of T-cell proliferation was related to the likelihood of a favorable response to IFN. If these findings are confirmed, baseline proliferative T-cell responses to PHA stimulation have the potential to become a clinically useful prognostic marker for responsiveness to IFN therapy in MS.

Disclosure: J Killestein has nothing to disclose.

P94
INTERFERON-β LEADS TO STABILIZATION OF THE BARRIER FUNCTION IN BOVINE, MURINE AND HUMAN BRAIN CAPILLARY ENDOTHELIAL CELLS IN VITRO
Kraus J°, Ling AK, Hamm S, Kinn KS, Voigt K, Oechmann P°, Engelhardt B°
°Vascular Cell Biology, Max-Planck Institute, Bad Nauheim, Hesse, Germany; °Neurology, Justus-Liebig University, Giessen, Hesse, Germany; °Infectious Diseases, John Hopkins University School of Medicine, Baltimore, Maryland, USA; °Vascular Biology, Max-Planck Institute, Muenster, Westfalia, Germany

Background: BBB breakdown is an early event in the pathogenesis of inflammatory CNS diseases like MS or its animal model EAE. Besides clinical bene-

fits, serial MRI scans from interferon-β (IFN-β) treated MS patients show a reduction of Gadolinium-enhancing lesions. This indicates an IFN-β associated stabilization of the BBB in these patients.

Objectives: To test the influence of IFN-β on the paracellular permeability in an in vitro BBB model.

Methods: We applied a co-culture BBB model consisting of bovine brain capillary endothelial cells (BBCEC) and rat astrocytes. In this model, we investigated the influence of human recombiant IFN-β on the paracellular permeability for H-inulin and 125I-sucrose in different conditions. Furthermore, we assessed the paracellular permeability for H-inulin and 125I-sucrose in immortalized cell lines (human brain microvascular endothelial cells [HBMEC], murine brain endothelomas [b.End 5]).

Results: In their vitro BBB model, co-culture of BBCEC and astrocytes leads to the induction of a barrier, whereas removal of astrocytes results in a breakdown of this permeability barrier function. However, addition of IFN-β to their in vitro BBB model maintains the barrier even in the absence of astrocytes. In the HBMEC and b.End 5 systems, pre-incubation with IFN-β significantly ameliorated the increase in the paracellular permeability after histamine challenge.

Conclusions: In our in vitro BBB model, application of IFN-β prevents the breakdown of the permeability barrier. However, the molecular mechanisms of IFN-β induced BBB stabilization remain to be investigated.

Disclosure: J Kraus has nothing to disclose.

P95
FINE SPECIFICITY OF ANTIBODY RESPONSES TO MYELIN SEQUENCES IN ASSOCIATION WITH HLA CLASS II ALLELES IN THE SERUM OF BRAZILIAN PATIENTS WITH MULTIPLE SCLEROSIS
Carvalho A, Lien AM°, Santos CC°, Sant’Anna G, Frugulhetti F, Leon SV°, Quirico-Santos T°
°Neurology, Hospital Universitário Clementino Fraga Filho - UFRJ, Rio de Janeiro, Rio de Janeiro, Brazil; °Neurology, Universidade do Rio de Janeiro; °Department of Cellular and Molecular Biology, Universidade Federal Fluminense, Niteroi, Rio de Janeiro, Brazil

Background: Multiple sclerosis (MS) is a chronic inflammatory disease of the human central nervous system characterized by demyelination and pronounced B-cell responses to myelin component MBP and proteolipid (PLP). Susceptibility to MS is associated with class II allele expression that confers preferential binding to specific immunodominant myelin epitopes.

Objectives: To determine IgA and IgG antibodies to myelin epitopes associated to HLA class II alleles.

Methods: We determined by ELISA, the levels of IgG and IgA antibodies to myelin epitopes associated to the following alleles DR*1501 (MBP 86-95 and PLP 95-116), DR*0401 (MBP 89-98), DR*0301 (MBP 90-98) and MBP 86-98. It was included 34 patients with clinically definite MS and 59 age-matched healthy individuals. Genomic analysis was performed by amplification of DNA isolated from peripheral leukocytes using PCR followed by SSOP hybridization.

Results: MS patients showed strong (p< 0.001) association with DQA1*0102 and DQB1*0602. Regardless stage of the disease MS patients showed a marked specific oligoclonal IgG production to MBP and PLP fragments. Yet, higher IgA levels was observed for MBP 86-98 sequence with promiscuous overlapping 20 aminoacids which encompass the binding frame recognized by DR molecules *1501, *0401, *0301 and PLP 95-116 with DRB1*1501.

Conclusions: Since MS patients showed low frequency of DRB1*1501 and DRB1*1503 expression, the results suggest that epitope specificity of B cell response to MBP and PLP in this MS population may be influenced by immune recognition associated to DRB1*0401, DRB1*0301 in association with DQB1*0602 and DQA1*0102.

Disclosure: S Leon has nothing to disclose.

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P96
INTERFERON GAMMA SECRETION IN MULTIPLE SCLEROSIS PATIENTS TREATED WITH INTERFERON BETA AND GLATIRAMER ACETATE
Lochnanova A*, Dolezil D*, Zapletalova O*, Hradilek P*
*Immunology, Regional Institute of Hygiene, Ostrava, Czech Republic; **Neurological Clinic, University Hospital, Ostrava, Czech Republic

Background: Multiple sclerosis (MS) is considered to be a T-cell mediated autoimmune disease with various degrees of myelin and axonal damage in the central nervous system (CNS). The expansion of autoreactive T cells targeting the CNS is essential element in inflammatory demyelination. The disease is primarily mediated by T cells that secrete the inflammatory cytokines IL-2 and IFN-γ referred as Th1 cells. The therapies which decrease T cells producing IFN-γ or increase IL-4 production would be expected to have an ameliorating effect on MS.

Objectives: Cytokoids and immunosuppression remain the main therapy of MS. More subtle strategies of immunomodulation such as the use of interferon-b (IFN-b) and glatirameracetate in the treatment of MS are well accepted.

Methods: In order to better understand the mechanism of action of IFN-b and glatirameracetate 14 MS patients treated with these drugs were studied. The number of CD3, CD4, CD8, T cells, CD19 B and NK cells, CD4CD45RA, CD4CD45RO cells and secretion patterns of IFN-γ and IL-4 examined before starting the therapy and in month 1,3,6 and 12.

Results: We did not find any significant changes in the number of T, B and NK cells. IFN-γ secretion after the treatment both with IFN-b and glatirameracetate was reduced. IFN-γ secreting cells and decline kinetics fluctuated a lot among patients. No change in the frequency of IL-4 secreting cells was seen. The high number of IFN-γ producing cells accompanied with slight decline during the therapy was observed in patients with high number of memory CD4CD45RO cells.

Conclusions: The study demonstrates that both IFN-b and glatirameracetate can induce variations in cytokine secretion levels and it also stresses the role played by TH1 cells in the ethiopathogenesis of MS.

Disclosure: A Lochnanova has nothing to disclose.

P97
A ROLE FOR CD1C IN MULTIPLE SCLEROSIS
Lyons P*, Yeager M*, Luecking L*, Wang Q*, Porcelli S*, Trotter P*
*Neurology, Washington University, Saint Louis, Missouri, USA; **Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York, USA

Background: CD1 expression by antigen presenting cells has been recognized for years. Recent data has demonstrated CD1 molecules are capable of presenting lipid antigens to T cells. Autoreactive T cells specific for CNS myelin are thought to mediate MS pathology, and CNS myelin is 70% lipid.

Objectives: The role of CD1C, particularly CD1C in relapsing/remitting MS (RRMS) was investigated. The phenotype of CD1C-expressing cells isolated from the peripheral blood over the course of RRMS was characterized and compared to healthy controls. Also investigated was the CD1-restricted response to myelin lipids of peripheral blood mononuclear cells (PBMC) isolated from MS patients and healthy controls.

Methods: Heparinized whole blood was collected by venipuncture. PBMC were isolated by Ficoll density gradient centrifugation. When necessary, monocytes/macrophages and T cells were purified from gradient-purified cells by magnetic cell separation. The phenotype of CD1C-expressing cells was characterized by flow cytometry. The lymphocyte response to myelin lipids was characterized by ELISA, ELISpot, and proliferation assays.

Results: Flow cytometry of PBMC revealed changes in the phenotype of CD1C-expressing cells during RRMS. In patients experiencing a clinical relapse, a decrease in CD1C+ CD4+ B cells as compared to normal controls and stable MS patients was observed. This was accompanied by a relative increase in CD1C+ CD4+ cells. Flow cytometry revealed this population to be CD1C+ CD4+ CD11c+ monocytes/macrophages. In vitro assays of reactivity to galactocerebroside, sulfatide and total CNS myelin lipids by PBMC isolated from MS patients and normal controls revealed myelin lipid-reactive T cells in both populations, with a higher precursor frequency detected in MS patients.

Assays performed with CD1-transfected HeLa cells demonstrated that at least a portion of this response was restricted to CD1 molecules.

Conclusions: CD1-mediated presentation of myelin lipids may be important to MS pathology.

Disclosure: J Lyons has nothing to disclose.

Funding: Supported by National Multiple Sclerosis Society USA, (RG-3032-A-7).

P98
MOLECULAR TRACKING OF MYELIN BASIC PROTEIN - SPECIFIC T CELL EXPANSION IN MULTIPLE SCLEROSIS
Muraro PA, Wandinger K, Bielekova B, McFarland HF, Martin R
Neuroimmunology Branch, National Institutes of Health, Bethesda, Maryland, USA

Background: Current evidence suggests that T cell responses to myelin antigens such as myelin basic protein (MBP) may be important in the pathogenesis of MS. However, the kinetics of frequency of MBP-specific T cell clones during the course of disease, particularly during clinical exacerbations, is still poorly understood.

Objectives: Our goal was to track candidate pathogenic MBP-specific T cell clones in patients with relapsing-remitting MS and assess a potential relationship of their frequency with the course of disease.

Methods: We developed a highly specific and sensitive technique to measure the frequency of single T cell clones by real-time PCR quantification of clonal T cell receptor (TCR) CDR3 transcripts. We used this novel method to track in a patient with MS a CD4+ T helper 1 clone, P2-10, that recognized the immunodominant myelin epitope MBP(83-99) with high functional avidity. We measured the clonal frequency in PBMC obtained before and during a clinical trial with an altered peptide ligand of MBP(83-99). As controls, we tracked the frequency of other antigen-specific T cell clones in PBMC from a healthy donor and from patients with other neurological disorders.

Results: The frequency of T cell clone P2-10 in PBMC increased 5-fold compared to baseline at one week before the clinical onset of a severe exacerbation, reaching an absolute frequency of 1 cell in ~1,800 PBMC. Clonal frequency had already decreased at baseline levels on the day of onset of new clinical symptoms, when brain MRI showed a sharp increase in the number of contrast-enhancing lesions. In contrast, tracking of control T cell clones in peripheral blood from a healthy subject and from a patient with HAM/TSP showed very stable clonal frequencies over time.

Conclusions: The dynamics of frequency of an autoreactive Th1 clone in a clinical relapse of MS closely resembled the kinetics of ecephalitogenic T cells in animal models, that reach their highest frequency before the onset of disease and rapidly decrease in the peripheral compartments following massive migration into the CNS. These data suggest that significant increases of the frequency of autoreactive effector T cells in MS might be only detectable during a short period of time preceding clinical exacerbations.

Disclosure: P Muraro has nothing to disclose.

P99
NITRIC OXIDE AS AN ACTIVITY MARKER IN MULTIPLE SCLEROSIS
Demir Acar G*, Ȳd̄ıman F*, Ȳd̄ıman E*, K̄ırkalı G*, Çakmakç̄ı H*, Özakbas S*
*Dept. of Neurology, Dokuz Eylül University Faculty of Medicine, İzmir, Turkey; **Biochemistry, Dokuz Eylül University School of Medicine, İzmir, Turkey; †Radiology, Dokuz Eylül University School of Medicine, İzmir, Turkey

Background: Multiple sclerosis (MS) is an autoimmune disease which is the most common cause of morbidity in young adults. Toxic damage on myelin,
Multiple Sclerosis

There was preferential induction of IFN-\(\gamma\)-epitopes that induced cytokine responses, the magnitude of the response, and if PBMC in response to 9-mer overlapping peptides that span the entire PLP and DR15, and DR17. (DRB1*0301) expression was associated with significantly elevated MBP- and \(\gamma\)-0.019 and 0.02, respectively. MS patients showed a significant negative correlation in cranial magnetic resonance imaging (MRI) and increased immunoglobulin (Ig) G index.

**Results:** Nitrite and nitrate levels (NNLs) measured in CSF was 11.6+/−8.6 micromol/ml during relapse, 6.72+/−3.50 micromol/mol during remission; in serum 12.89+/−7.62 micromol/ml during relapse and 12.35+/−6.62 micromol/ml during remission. In healthy subjects NNL measurements in serum and CSF were 4.37+/−1.63 micromol/ml and 7.42+/−2.81 micromol/ml, respectively. Non-parametric Wilcoxon test was used for statistical analysis.

**Conclusions:** Nitrite and nitrate levels during relapse period were very significantly higher than remission period and also healthy subjects (p=0.000). There was no significant difference in serum NNLs between relapse and remission. As a result nitrite and nitrate measurement test is a highly specific (94 %) marker of the disease, however it’s sensitivity is mild (62.5 %). Increased NNLs are also significantly correlated with active lesion existence in cranial MRI and increased IgG index (p=0.05).

**Disclosure:** S Özakbasa has nothing to disclose.

**P100**

**LONGITUDINAL CYTOKINE RESPONSES TO MYELIN PEPTIDES IN MS: PERSISTENCE AND SPREADING OF IMMUNE RESPONSES**

Pelfrey CM, Moldovan IR, Cotlear AC, Born SE, Karaffa M, Lee J, Fisher E, Rudick RA

Cleveland Clinic Foundation, Cleveland, Ohio, USA

**Background:** Myelin-specific immune responses can be found in both MS patients and healthy controls. Consequently, the relevance of these immune responses to MS disease progression is not clear. Few studies have examined which features of the immune response to myelin relate specifically to MS disease progression over time.

**Objectives:** To examine longitudinal cytokine responses to myelin antigens in MS patients and healthy controls in order to understand the role of persistence and spreading of immune responses in disease progression.

**Methods:** We performed a 1 yr longitudinal study measuring cytokine responses every 3 mo in 20 relapsing-remitting MS patients and 27 age/gender matched controls. We determined ex-vivo IFN-\(\gamma\) and IL-10 production by PBMC in response to 9-mer overlapping peptides that span the entire PLP and MBP molecules. At each time-point, we obtained the number and location of epitopes that induced cytokine responses, the magnitude of the response, and if there was preferential induction of IFN-\(\gamma\) or IL-10. We examined possible correlations between cytokine expression patterns and clinical/MSRI parameters.

**Results:** At baseline, MS patients had significantly higher PLP-induced IFN-\(\gamma\) responses and MBP-induced IFN-\(\gamma\) responses, compared with controls (p = 0.019 and 0.02, respectively). MS patients showed a significant negative correlation between both PLP- and MBP-IFN-\(\gamma\) responses and clinical disability (as measured by the MS Functional Composite, MSFC)\(p\) = 0.01 and \(p\) = 0.004, respectively). No correlation was observed in controls. HLA DR17 (DQB1*0301) expression was associated with significantly elevated MBP- and PLP-induced cytokine secretion. Persistent PLP responses (present at more than one time-point) were associated with MS-associated HLA alleles DR4, DR15, and DR17.

**Conclusions:** Our results suggest that clinical disability correlates with IFN-\(\gamma\) responses. For certain myelin antigens/cytokines, we can observe significant differences between MS patients and Controls using the ELISPOT assay. These findings suggest that persistent cytokine responses to the same myelin epitopes over time may distinguish MS patients’ responses from controls. This is an ongoing study that will include several more longitudinal time-points with corresponding clinical and MRI data.

**Disclosure:** C Pelfrey has nothing to disclose.

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**P101**

**FATIGUE AND INFLAMMATION IN PATIENTS WITH OPTIC NEURITIS**

Roed H*, Olsen D*, Langkilde A*, Frederiksen J*, Sellebjerg F*

*Neurology, university hospital Glostrup Denmark, Glostrup, Denmark; †University hospital of Hvidovre, Hvidovre, Europe, Denmark

**Background:** Fatigue is a common and often incapacitating symptom in multiple sclerosis. No clear correlation with markers of inflammation or MRI lesions has yet been established.

**Objectives:** We hypothesized that relationships between fatigue, inflammation or MRI disease activity would be most apparent at onset of disease, and studied these variables in a series of patients with clinically isolated optic neuritis (ON).

**Methods:** We studied 40 patients with ON, 25 patients with secondary progressive MS (SPMS) and 20 healthy controls. Fatigue was measured by the fatigue severity scale FSS, and a FSS score of 5 or more was considered abnormal. ON patients underwent lumbar puncture and MRI studies. MRI studies comprised T2-weighted sequences and T1-weighted sequences before and after the administration of Gd-DTPA. T cell activation in blood and CSF was measured by studying T cells expression of CCR2, CCR5, CD25, CD26, CD122, CD154, CXCR3, and HLA-DR by flow cytometry.

**Results:** FSS scores of 5 or more were observed in 40% of patients with ON (n=40), 68% of patients with SPMS and 0% of healthy controls (n=20; p<0.001 compared to ON and SPMS). FSS scores did not differ significantly in ON and SPMS. In ON there was no correlation between FSS scores and the number of lesions on T2-weighted MRI or the number of Gd-enhancing lesions. Neither did FSS scores correlate with the CSF leukocyte count, The IgG index of T cell activation on CSF or blood as assessed by flow cytometry studies.

**Conclusions:** Fatigue is significantly more frequent in patients presenting with ON than in healthy controls and is not significantly less common than in patients with SPMS. However even in this patient group we found no evidence of an association between fatigue, inflammation or even the presence of MRI lesions in patients with ON.

**Disclosure:** The study was funded by unrestricted research grants by the danaish multiple sclerosis society, Biogen, Serono and Schering.

**Funding:** Supported by unrestricted research grants from the Danish Multiple Sclerosis Society, Biogen, Serono and Schering.
involved in the pathogenesis of multiple sclerosis (MS). The commitment of T lymphocytes to die is partly regulated by the Bcl-2 family proteins, which act as a checkpoint upstream of mitochondrial dysfunction. These proteins include the death antagonists Bcl-2 and Bcl-XL, and death agonists Bax and Bad. Recent studies suggest that altered expression of Bcl-2 family proteins in T lymphocytes is involved in promoting cellular resistance to apoptosis in patients with MS.

**Objectives:** In this study we examined the relationship between alterations in Bcl-2 family proteins expression and clinical disease activity in patients with MS.

**Methods:** We analyzed the expression ratios of pro-apoptosis (Bax and Bad) to anti-apoptosis (Bcl-2 and Bcl-XL) proteins in peripheral T cells from patients with clinically active MS and compared the results with corresponding ratios in patients with stable MS and relevant control groups.

**Results:** We observed a significant reduction in the expression ratios of pro- to anti-apoptosis Bcl-2 members in peripheral lymphocytes from patients with active MS when compared to corresponding ratios in patients with stable MS or other controls. This imbalance in the cellular expression ratios of pro- and anti-apoptosis proteins was functionally active in reducing cellular susceptibility to apoptosis in active MS. It also correlated with clinical features of disease activity, such as the number of gadolinium enhancing MRI lesions and clinical relapses.

**Conclusions:** Our findings indicate that dysregulated expression of Bcl-2 family proteins in peripheral lymphocytes is a feature of clinically active multiple sclerosis.

**Disclosure:** MK Sharief has received honoraria from Serono International.

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**P103**

**THE EXPRESSION OF APOPTOSIS REGULATORY PROTEINS IN B LYMPHOCYTES FROM PATIENTS WITH MULTIPLE SCLEROSIS**

**Sharief MK, Seidi OA**

 Dept of Neuroimmunology, GKT School of Medicine, London, UK, United Kingdom

**Background:** The pathogenesis of multiple sclerosis (MS) is thought to involve T and B lymphocyte-mediated autoimmune phenomena. However, the mechanisms that regulate lymphocyte activity in MS are poorly understood. In normal circumstances, programmed cell death (apoptosis) contributes to the maintenance of lymphocytes homeostasis and the deletion of autoreactive cells. Cellular commitment to apoptosis is partly regulated by the cell death receptor Fas, and the anti-apoptosis proteins Bcl-2 and FLIP.

**Objectives:** Although there is emerging evidence that dysregulations of apoptotic pathways play a role in T cell autoimmunity in MS, the expression of apoptosis-regulatory proteins in B cells from MS patients is largely unknown. In this study, we sought to examine the expression of several apoptosis regulatory proteins in peripheral B lymphocytes from patients with MS.

**Methods:** In this study, we used a combination of dot-blot immunoassays and Western blotting to analyze the expression profiles of Fas, Bcl-2, and FLIP proteins in peripheral B lymphocytes from patients with relapsing remitting and progressive MS, and from appropriate controls.

**Results:** We observed a significant upregulation of Bcl-2 and FLIP proteins in B cells from relapsing remitting MS when compared to corresponding expression in progressive MS, or in non-inflammatory neurologic controls and healthy individuals. This cellular overexpression of Bcl-2 and FLIP proteins was not affected by treatment with interferon-beta, but was also observed in B cells from patients with systemic inflammatory diseases.

**Conclusions:** Our findings suggest that cellular overexpression of the apoptosis-inhibitory proteins in patients with relapsing MS may promote apoptotic resistance of potentially pathogenic, autoreactive B lymphocytes and consequently, may allow for continuing autoimmune tissue destruction.

**Disclosure:** MK Sharief has received honoraria from Serono International.

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**P104**

**INTERFERON-BETA THERAPY DOWNREGULATES SURVIVIN EXPRESSION IN T LYMPHOCYTES**

**Sharief MK°, Zoukos Y°**

°Dept of Neuroimmunology, GKT School of Medicine, London, UK, United Kingdom; °Neurology Dept, St Thomas Hospital

**Background:** Treatment with interferon beta reduces clinical exacerbations in MS through several immunomodulatory mechanisms that involve the attenuation of programmed cell death (apoptosis) of peripheral T lymphocytes. The expression of survivin, a cell cycle-regulated antiapoptosis protein, is up-regulated in mitogen-stimulated T lymphocytes from patients with MS, and this expression correlates with MS disease activity.

**Objectives:** To evaluate the effect of interferon beta on the expression of survivin in B cells and other apoptosis regulatory molecules in peripheral T lymphocytes from patients with MS.

**Methods:** In a prospective, clinical and immunologic study, we evaluated the expression of survivin, Bcl-2 protein, and the death receptor Fas in mitogen-stimulated T lymphocytes from 26 patients with MS, before and serially after interferon-beta-1a therapy. We also investigated the long-term effects of interferon beta-1a on cellular expression of these proteins and T-lymphocyte apoptosis in a cross-sectional study of 19 patients with MS receiving long-term interferon beta-1a therapy.

**Results:** Treatment with interferon beta-1a reduced the expression of survivin in vitro stimulated T lymphocytes. This reduced expression correlated with augmented T-cell susceptibility to apoptosis and with clinical response to treatment. In contrast, interferon beta-1a therapy did not alter cellular expression of Bcl-2 protein. This down-regulatory effect of interferon beta-1a on cellular expression of survivin was maintained after long-term therapy.

**Conclusions:** Our observations suggest that interferon beta exerts a regulatory effect on peripheral T lymphocytes through an antiapoptosis mechanism that involves the down-regulation of cellular survivin expression.

**Disclosure:** MK Sharief has received honoraria from Serono International.

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**P105**

**CHEMOKINE RECEPTOR EXPRESSION ON CEREBROSPINAL FLUID T-Cells IN PATIENTS WITH RELAPSING REMITTING MULTIPLE SCLEROSIS**

**Sindern E, Patzold T, Ossege LM, Gisevius A, Malin JP**

Neurology, Kliniken Bergmannsheil, Bochum, Germany

**Background:** There is accumulating evidence that chemokines are important in trafficking, retention and activation of T-cells in active multiple sclerosis (MS) lesions. In immunohistochemical studies of autopsy brain sections containing active MS lesions CXCX3-1 and interferon-g-inducible protein 10- (IP-10) positive cells were found in the perivascular space around nearly all inflamed vessels within MS lesions.

**Objectives:** To evaluate the chemokine CXCX3-1 expression on T-cells and levels of its ligand IP-10 in blood and cerebrospinal fluid (CSF) of patients with relapsing remitting MS (RR-MS) in association with magnetic resonance imaging (MRI) disease activity.

**Methods:** 22 newly diagnosed definite RR-MS patients underwent Gd-enhanced-MRI examinations and diagnostic lumbar puncture in a short time interval, ranging from 1-24 hours. Chemokine expression was measured by flow cytometry and levels of IP-10 were determined by ELISA.

**Results:** IP-10 was strong intracellularly released, but did not change in association with MRI activity. CXCX3-1 positive T-cells were enriched in the CSF compared with peripheral blood and more than 75% of T-cells in the CSF expressed CXCX3-1 receptor (p<0.0001). CXCX3-1 expression was lower in the peripheral blood of RR-MS patients compared to healthy controls (p=0.005) and was increased in the CSF of RR-MS patients undergoing acute attacks, as illustrated by Gd-enhancing lesions on MRI, compared to patients without enhancing lesions (p=0.005).

**Conclusions:** Our results suggest that MRI documented disease activity is associated with an increase of CXCX3-1 positive T-cells in the CSF of patients with RR-MS.
with RR-MS, possibly due to the migration of activated T-cells from the circulation into the CSF.

Disclosure: E. Sindermann has nothing to disclose.

P106
DETECTION OF OLIGOCOCLAL FREE KAPPA CHAINS IN ABSENCE OF OLIGOCOCLAL IGG IN THE CSF OF CLINICALLY SUSPECTED MS PATIENTS.

Sophie Gb, Christian Sb, Myriam Sb, Thierry Db, H. Hf
 abductionary Laboratory, Cliniques Universitaires Saint-Luc, Brussels, Belgium; a Neurology, Centre Hospitalo-Universitaire Vaudois, Lausanne, Switzerland; bNeuroradiology, Cliniques Universitaires Saint-Luc, Brussels, Belgium; cClinical Chemistry Laboratory, Centre Hospitalo-Universitaire Vaudois, Lausanne, Switzerland

Background: In a previous study, we reported a similar frequency in the occurrence of either CSF-specific oligoclonal free kappa or CSF-specific oligoclonal IgG (92%) in a group of 48 MS patients. In the present work, we specifically studied the presence of CSF free kappa bands in a group of 33 clinically suspected MS patients with no or only a single IgG band. CSF data were compared with the clinical characteristics and MRI scans.

Objectives: To evaluate the relevance of free kappa chains in MS.

Methods: Oligoclonal IgG and free kappa bands were determined by an immunoaffinity blot technique. Brain MRI was performed within one month after lumbar puncture in all patients; spinal cord MRI was done only in the case of a relevant clinical picture. Positive MRI for MS fulfilled the criteria of Barkhof et al. (2) and Tintore et al. (3). MS suggestive MRI revealed 2 or more lesions consistent with MS, either in the brain or in the spinal cord.

Results: Oligoclonal free kappa bands were observed in 18 cases out of 33 (55%), especially in patients with motor dysfunction (5/6, 83%), optic neuritis (7/11, 64%) and sensory impairment (3/7, 43%). Positive MRI was found in 6 cases, all with oligoclonal free kappa bands. The latter were present in 7/11 (64%) patients with MS suggestive MRI, but only in 5/16 (31%) of patients with normal or aspecific MRI.


Disclosure: G. Sophie has nothing to disclose.

P107
FUNCTIONAL CHARACTERIZATION OF THE CD28-RELATED MOLECULE ICOS IN MULTIPLE SCLEROSIS: A POSSIBLE TARGET FOR SELECTIVE IMMUNE INTERVENTION?

Wiendl Hb, Neuhaus Ob, Mehling Mb, Wintterle Sb, Schreiner Br, Wessert Rb, Weller Mb, Hartung P-Hb, Toluoa Fa, Melms Ab
 aNeurology, Uni.Tuebingen, Tuebingen, BW, Germany; bNeurology, KFU, Graz, –, Austria; cNeurology, HHU, Düsseldorf, –, Germany

Background: ICOS is an inducible CD28-related costimulatory molecule with relevance for T cell differentiation and effector function and a possible role in autoimmune diseases like Multiple Sclerosis (MS).

Objectives: To characterize the expression and functional relevance of ICOS in healthy donors and patients with MS.

Methods: FACS staining, proliferation assays, real time PCR, ELISA

Results: After nonspecific or antigen-specific stimulation, ICOS was preferentially expressed on CD4+ Th2 T cells. Blockin experiments with antigen-specific T cell lines as well as SAg-stimulated CD4 T cells demonstrated that ICOS-costimulation affected the production of both Th1 and Th2 cytokines whereas T cell proliferation, expression of T cell activation markers or chemokine receptor expression were unaffected. Interestingly, the effects on cytokine secretion were observed in the absence or presence of B7-1/2, suggesting that ICOS costimulation modulates cytokine secretion also independently of CD28 costimulation. Levels of constitutive and inducible ICOS expression on human T cell subsets from peripheral blood were quantified in healthy donors (n=16) and MS patients (n=10). Constitutive expression of ICOS varied between 0.12 and 12.35%. No significant differences were noted between both groups concerning the constitutive expression or inducibility of ICOS on T cells. Cells constitutively expressing ICOS did not represent the CD4+CD25+ regulatory T cell subset. ICOS expression on CSF T lymphocytes in patients with acute MS relapses or acute neurobehavioral showed that the frequency of ICOS-positive cells was not elevated compared with peripheral blood. Whereas neither IFN-β nor glatiramer acetate altered baseline expression of ICOS on T cells, antiinflammatory cytokines such as IL-10 or TGF-β both slightly downregulated ICOS on PHA-stimulated T-cells.

Conclusions: In summary, ICOS costimulation affects both Th1 and Th2 cytokine production in the presence and absence of B7-costimulation. Since ICOS is rapidly induced on T cells after antigen-specific stimulation, this molecule qualifies as a suitable target aimed at specifically modulating T cell cytokine responses in CNS inflammation.

Disclosure: H. Wiendl has nothing to disclose.

P108
MODULATION OF NEURONAL ACTIVITY AND MOG-INDUCED EAE BY THE ENDOGENOUS PENTAPEPTIDE QYNAD, A PUTATIVE MEDIATOR OF NEUROLOGICAL DYSFUNCTION IN HUMAN DEMYELINATING DISEASES

Wiendl Hb, Meuth Sb, Duyar Hb, Elbs Mb, Landgraf P, Weller Mb, Wessert Rb, Budde Ta
 aDepartment of Neurology, Eberhard-Karls-University Tübingen, Tübingen, Baden-Württemberg, Germany; bInstitute of Physiology, Otto-von-Guericke-University, Magdeburg, Sachsen-Anhalt, Germany; cDepartment of Cell Biology, Eberhard-Karls-University, Tübingen, Baden-Württemberg, Germany

Background: Demyelination and inflammation both contribute to the neurological deficits characteristic of multiple sclerosis (MS). Although conduction deficits attributable to inflammatory demyelination are well known, soluble factors may also contribute to neurological dysfunction. QYNAD is a pentapeptide detected in the CSF of patients with MS that has been proposed to influence symptomatic changes or the disease course in MS by putative blockade of sodium channel action.

Objectives: To further clarify the potential relevance of QYNAD for MS, we characterized the in vitro and in vivo properties of this pentapeptide.

Methods: We performed experiments in 1) acutely isolated thalamic neurons and 2) in a model of myelin oligodendrocyte glycoprotein (MOG)-induced experimental allergic encephalomyelitis (EAE).

Results: QYNAD blocks sodium channels in acutely isolated neurons by shifting their steady-state inactivation to more negative potentials. QYNAD putatively acts via direct action at the sodium channel as demonstrated by confocal laser scanning microscopy using fluorescently labeled, bioactive QYNAD together with a type II sodium channel specific antibody. We explored the influence of QYNAD in MOG-induced EAE in DA/00Hsd rats. QYNAD itself was not encephalitogenic. QYNAD neither altered the onset of disease nor disease severity when given repeatedly (qid) from day 0 to 15 after MOG immunization. Furthermore QYNAD did not influence the severity or frequency of relapses when given after disease onset.

Conclusions: QYNAD specifically blocks neuronal sodium channel action with a limiting concentration at 10 microM. Taking into consideration published values on CSF QYNAD concentrations in MS (9 to 34 microM), our in vitro and in vivo experiments make a correlation of QYNAD concentrations with the clinical status unlikely. Although QYNAD might still be a contributing factor causing rapidly waxing and waning symptoms in MS, a role for permanent axonal damage and thus relevance for overall disease severity in MS is not Supported by this work.

Disclosure: H. Wiendl has nothing to disclose.
Experimental Allergic Encephalomyelitis (Part 1)

P109

α-LIPOIC AND DIHYDROLIPOIC ACID SUPPRESS EAE IN SJL MICE AND INHIBIT MMP-9 ACTIVITY IN VITRO

Marracci G*a, Jones R*b, Meckon Gc, Winter R*d, Riscoe M*e, Bourdette D*f

*Portland VAMC, Portland, Oregon, USA; bNeurology, Oregon Health & Science University, Portland, Oregon, USA; cBiochemistry, OHSU, Portland, Oregon, USA; dChemistry, PSU, Portland, Oregon, USA

Background: Oxygen and nitrogen free radicals are important to the pathogenesis of MS and EAE. α-lipoic acid (ALA) and its reduced form dihydrolipoic acid (DHLA) are antioxidants. ALA treated and suppressed EAE in SJL mice by inhibition of T cell trafficking into the CNS and directly inhibited matrix metalloproteinase-9 (MMP-9) activity (manuscript submitted). As MMP-9 facilitates T lymphocyte transmigration across the subendothelial basement membrane, its inhibition may be especially relevant to the therapeutic benefit of ALA.

Objectives: To elucidate the mechanism of action of ALA in suppressing EAE.

Methods: SJL mice were immunized with PLP139-151 peptide in CFA and given daily injections of ALA, DHLA or SS-dimethyl lipoic acid (Me2LA) commencing on day 7 to suppress EAE. Supernatants were collected from PLP-specific T cells after 48 hours in culture with ALA, DHLA or Me2LA (10-1000 µg/ml). Zymographic analyses were performed in the presence of ALA, DHLA or Me2LA (10-1000µg/ml) in buffer or buffer alone.

Results: The mean maximum EAE score and mean 10-day CDS after suppression with ALA and DHLA were reduced significantly (p=0.01 and 0.02, respectively) below those of saline, while Me2LA did not suppress EAE. Zymographic analyses revealed direct, dose dependent suppression of MMP-9 activity by ALA and DHLA (up to 99%), while Me2LA did not. DHLA was more potent than ALA in directly inhibiting MMP-9. ALA and DHLA, but not Me2LA, inhibited the production of MMP-9 in vitro by up to 94%, in a dose dependent fashion, suggesting that ALA and DHLA can indirectly suppress T cell production of MMP-9.

Conclusions: DHLA is a more potent inhibitor of MMP-9 than ALA, but it has an equal ability to suppress EAE. Since ALA is rapidly reduced to DHLA in vivo, these data suggest that DHLA is the therapeutically active molecule in vivo. Me2LA, unlike DHLA, lacks free sulfhydryl groups and did not inhibit EAE or MMP-9, suggesting that DHLA functions by reducing MMP-9. ALA and DHLA are capable of indirectly inhibiting T cell production of MMP-9, perhaps by inhibiting activation of nuclear transcription factor NF-κB. ALA and DHLA appear to suppress EAE through direct and indirect inhibition of MMP-9 activity.

Disclosure: G Marracci has nothing to disclose.

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P110

NEUROPROTECTIVE EFFECTS OF GLATIRAMER ACETATE IN A MOUSE MODEL OF CHRONIC EXPERIMENTAL AUTOMMUNE ENCEPHALOMYELITIS

Offen B*a, Gilgun-sherki Y*b, Hodengebreher V*c, Panet H*d, Melamed E*ea

aDept Neurological Sciences, Ospedale Maggiore Policlinico, Milano, Italy; bDept of General Pathology, University of Verona, Verona, Italy; cBiosell, DBBIT, Milano, Italy; dDept of Pathology, University of Michigan, Ann Arbor, Michigan, USA

Background: Lymphocyte recruitment into the central nervous system is a critical event in the pathogenesis of multiple sclerosis and experimental autoimmune encephalomyelitis (EAE). Alpha(1,3) fucosyltransferases (FucT) catalyze glycosylation of glycans that ligate endothelial E- and P-selectin and are responsible for leukocyte recruitment to inflammatory sites. Recent evidences suggest that downregulation of FucT activity may represent an attractive target for therapeutic intervention aimed at blockade of chronic inflammatory diseases.

Objectives: The goal of this study was to determine the effect of different FucT deficiency on lymphocyte recruitment in brain venules and on the induction of EAE.

Methods: FucT-deficient mice (FucT/-) for FucT-IV, FucT-VII and double deficient mice for FucT-IV & FucT-VII were generated on C57/B6 genetic background. Intravital microscopy studies were performed in inflamed brain vessels. EAE was induced in wt or FucT’s deficient mice by using MOG35-55 peptide.

Results: Th1 lymphocytes produced from FucT-IV/-/- mice efficiently tethered and rolled in brain postcapillary venules when compared with cells isolated from wt mice. In contrast, primary adhesion of Th1 lymphocytes obtained from FucT-VII/-/- or FucT-VII-/-/- mice was drastically reduced, suggesting that FucT-VII is critical for the recruitment of Th1 cells in brain microcirculation. The onset of actively-induced EAE in FucT-VII/-/-, but not FucT-IV/-/-, mice was delayed. Disease was significantly inhibited in Fuc-T-IV/-/- & FucT-IV+-/- mice when compared with wt mice. Immunohistochemistry studies revealed interesting differences in the composition of the inflammatory infiltrates between wt mice and FucT-deficient mice.

Conclusions: Our data unveil a critical role for FucT-VII in the recruitment of Th1 lymphocytes in brain venules, and suggest that FucT-VII together with FucT-IV play a significant role in the pathogenesis of EAE.

Disclosure: B Offen has nothing to disclose.

Funding: Supported in part by Teva Pharmaceutical Industries
P112

TYPE IV PHOSPHODIESTERASE INHIBITION REDUCES MATRIX METALLOPROTEINASE 9 EXPRESSION AND IMPAIRS NUCLEAR FACTOR-KAPPA B TRANSLATION IN EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

Puerto C, Sánchez A, Baranda P, Ortiz P, García-Merino A
Neuroimmunology Unit, Clínica Puerta de Hierro, Madrid, Spain

Background: Experimental allergic encephalomyelitis (EAE) is a useful model of multiple sclerosis (MS). Members of the NF-κB family are transcription factors involved in the regulation of proinflammatory cytokine and matrix metalloproteinase 9 (MMP-9) genes, that are closely related to the extravasation of immune cells and tissue destruction during EAE. Rolipram (a type IV phosphodiesterase inhibitor) ameliorates EAE by inducing a delay in the entry of inflammatory cells into the CNS, but the precise mechanisms of this drug remain elusive.

Objectives: To investigate the expression of MMP-9 and the in vivo regulation of NF-κB activation after treatment of EAE with rolipram. To determine if rolipram can modulate NF-κB-driven promoter activity and MMP-9 enzymatic activity.

Methods: Lewis rats were immunized with guinea pig myelin basic protein, and treated daily with rolipram. MMP-9 gene expression was determined by semiquantitative RT-PCR. The activation of NF-κB was assessed by electrophoretic mobility supershift assay in cells from the lymph nodes (LN) upon anti-CD3 engagement. Jurkat cells were transfected with the κB-pGL3-prom plasmid, which contains the NF-κB binding site of the MMP-9 gene, stimulated with PMA in the presence of rolipram and the transcriptional activity was assayed by luciferase activity. MMP-9 activity was assayed by gelatin zymography in the supernatant of MBP-reactive cells.

Results: In vivo, rolipram reduces the MMP-9 gene expression, both in LNcs and CNS, and impairs the nuclear translocation of NF-κB in lymphocytes from EAE rats. PDE-IV inhibition decreases promoter activity in a construct containing the NF-κB binding sequence of the MMP-9 gene and reduces IL-2 induced MMP-9 enzymatic activity in MBP-specific rat lymphocytes.

Conclusions: Rolipram may be an interesting agent to be used in combination with IFN-β to further improve therapeutic options for MS.

Disclosure: A Sánchez has nothing to disclose.

Funding: Supported by Fondo de Investigación Sanitaria (98/0038), Comunidad de Madrid (08.5/0001/97), and Fundacion Salud 2000.

P113

DISEASE SIGNS IN GENE KNOCKOUT MODELS OF MULTIPLE SCLEROSIS DIRECTLY CORRELATE WITH BLOOD-BRAIN BARRIER PERMEABILITY

Scott GS, Brimer CM, Kean RB, Hooper D
Microbiology and Immunology, Thomas Jefferson University, Philadelphia, Pennsylvania, USA

Background: The blood-brain barrier (BBB) maintains central nervous system (CNS) homeostasis. However, a breakdown of the normal function of the BBB has been demonstrated in multiple sclerosis and its animal correlate, experimental allergic encephalomyelitis (EAE). This would not only enhance factor exchange between the blood and the CNS, but could facilitate inflammatory cell infiltration into CNS tissues.

Objectives: The present study uses knockout mice lacking a variety of genes relevant to immune function to examine the relationship between BBB breakdown and the evolution of neurological disease signs following immunization with myelin oligodendrocyte glycoprotein (MOG).

Methods: Groups of mice were immunized with MOG and examined for disease signs. BBB permeability was assessed by measuring the uptake of sodium fluorescein into spinal cord tissue.

Results: Mice with targeted disruptions in the genes for IFN-γR receptors, IFN-γ receptors, NF-κB and iNOS developed neurological signs of EAE which varied in incidence, onset and severity between the groups. Nevertheless, all MOG-immunized animals exhibited some degree of increased BBB permeability and, irrespective of the strain, the extent of BBB permeability directly correlated with disease signs.

Conclusions: Our results indicate that the appearance of clinical signs of EAE is critically dependent on BBB breakdown, regardless of the immunological status of the animal. BBB breakdown could not only contribute to disease development by permitting communication between the circulation and the CNS, but may also induce fluid phase shifts resulting in edema formation and neurological dysfunction.

Disclosure: G Scott has nothing to disclose.

Funding: This investigation was supported in part by the National Multiple Sclerosis Society and by a grant from the Commonwealth of Pennsylvania to the Biotechnology Foundation Laboratories.

P114

This abstract was also presented at the platform.

IL-12 DEPENDENT/IFN GAMMA INDEPENDENT EXPRESSION OF CCR5 BY MYELIN-REACTIVE CD4+ T CELLS CORRELATES WITH ENCEPHALITICITY

Bagaeva L, Williams LP, Segal BM

“Microbiology and Immunology, Thomas Jefferson University School of Medicine, Philadelphia, USA; aMicrobiology and Immunology, University of Rochester School of Medicine, Rochester, New York, USA"

Background: The administration of neutralizing antibodies against the Th1 polarizing monokine IL-12 protects otherwise susceptible mice from clinical EAE and the development of inflammatory infiltrates in the central nervous system (CNS). Paradoxically, mice deficient in the signature Th1 cytokine, IFN gamma, remain highly susceptible to EAE; however disease is also suppressed in these animals by IL-12 blockade. Collectively these results suggested that IL-12 might stimulate myelin-reactive T cells to home to the CNS by an IFN gamma independent pathway.

Objectives: To test our hypothesis IL-12 directly stimulates myelin-reactive T cells to upregulate chemokine receptors that are critical for CNS migration.

Methods: We performed RNAse protection assays to measure the expression of a panel of chemokine receptors by lymph node cells (LN cells) that had been primed in vivo with a peptide fragment of proteolipid protein (PLP) emulsified in incomplete Freund’s Adjuvant (IFA) and challenged in vitro with antigen either in the absence or presence of recombinant IL-12 and/or a neutralizing antibody against IFN gamma. In parallel experiments we measured the expression of selected chemokines and chemokine receptors in spinal cords from the adoptive transfer recipients of PLP-specific T cells that were treated as described above.

Results: PLP/IFA primed LN cells only transfer EAE only following ex vivo challenge with antigen and IL-12. IL-12 directly induces expression of CCR5, but not CCR1-4 or CXCR3, by these PLP-specific T cells. Neutralization of IFN gamma does not suppress IL-12 induced CCR5 expression. The chemokines RANTES, MIP-1 alpha, MIP-1 beta and MCP-1 and their receptors CCR1, CCR2 and CCR5 are upregulated in the spinal cords of mice following the injection of IL-12 stimulated effector cells in direct association with the development of inflammatory demyelinating lesions.

Conclusions: Our findings suggest that IL-12 might promote the encephalitogenicity of myelin-reactive T cells by the direct induction of CCR5. Expression of CCR5 most likely facilitates the homing of autoreactive effector cells to the CNS since its ligands are expressed in EAE lesions.

Disclosure: B Segal has nothing to disclose.

Funding: Supported by The National Institutes of Health (RO1 NS41562-1) and the National Multiple Sclerosis Society (Harry Weaver Junior Faculty Award) to BMS
P115
ENGAGEMENT OF TOLL LIKE RECEPTOR (TLR) 9 OR CD40 REVERSES TOLERANCE AGAINST MYELIN ANTIGENS AND PRECIPITATES AUTOIMMUNE DEMYELINATION
Segal BM*, Ichikawa HT*
*Neurology, University of Rochester School of Medicine, Rochester, New York, USA; †Microbiology and Immunology, University of Rochester School of Medicine, Rochester, New York, USA

Background: Myelin-reactive CD4+ T cells, the mediators of experimental autoimmune encephalomyelitis (EAE) and presumably of multiple sclerosis, have been detected in the normal peripheral T cell repertoire. Nonetheless, most individuals do not succumb to autoimmune disease. There is growing evidence that while peripheral APCs stimulate immune responses against foreign antigens in the setting of tissue destruction and “danger”, they actually maintain tolerance against self antigens under steady state conditions. The activation state of APCs is critical in deciding an outcome of T cell priming versus tolerization.

Objectives: To test our hypothesis that tolerance against a candidate myelin autoantigen could be reversed by activation of APCs via CD40 or TLR 9 signaling.

Methods: Adult SJL mice injected i.p. with a peptide fragment of proteolipid protein (PLP) emulsified in Incomplete Freund’s Adjuvant fail to mount lymphoproliferative or cytokine responses and are protected from EAE upon subsequent challenge with the antigen combined with adjuvants. We harvested LN cells from such PLP-tolerized mice and reactivated them ex vivo in the presence of either an agonist monoclonal antibody against CD40, isotype matched rat IgG, a CpG-containing oligonucleotide (ODN) that binds TLR-9, or a control ODN.

Results: Tolerized PLP-specific CD4+ T cells regain the ability to divide, differentiate along a Th1 lineage and transfer EAE only when reactivated in the presence of agonistic antibodies against CD40 or CpG oligonucleotides. The effects of both anti-CD40 and CpG oligonucleotides are dependent upon induction of IL-12. In parallel experiments, we found that systemic injections of mice with anti-CD40 prevent tolerance induction and increase susceptibility to EAE.

Conclusions: Our findings suggest two mechanisms to explain the well documented association between infectious illnesses and flare ups of multiple sclerosis. Microbial pathogens could: (i) release molecules that bind TLRs and/or (ii) stimulate microbe-specific T cells to upregulate CD40 ligand, thereby licensing APCs that bear both microbial and auto-antigens to break tolerance.

Disclosure: B Segal has nothing to disclose.

Funding: Supported by The National Institutes of Health (R01 NS41562-1) and the National Multiple Sclerosis Society (Harry Weaver Junior Faculty Award) to B.M.S.

P116
DIFFERENTIAL ANTIGEN-SPECIFIC PREVENTION OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS WITH NAKED DNA.
Walczak A, Szymanska B, Selmaj K
Dept. of Neurology, Medical University, Lodz, Poland

Background: Vaccination with naked DNA, has been shown by us and others, to generate protective immunity against experimental autoimmune encephalomyelitis (EAE).

Objectives: We have compared the effect of DNA vaccination with plasmids encoding different myelin proteins on the subsequent development of EAE.

Methods: Plasmid vectors, encoding proteolipid protein (pVaxPLP) and myelin oligodendrocyte glycoprotein (pVaxMOG) genes, were used for the study. We assessed the effect of DNA pre-immunization on subsequent EAE course in SJL/J mice, evoked with PLP peptide 139-151 and in C57Bl6 mice, evoked with MOG peptide 35-55. Both clinical and pathologic measures of EAE have been applied to assess the course of the disease.

Results: The EAE course, in mice pre-immunized with pVaxPLP 4 weeks prior EAE induction, more severe disease was observed and only 12 weeks after DNA vaccination, the EAE course was ameliorated. Prevention of EAE was associated with a decrease in T cell proliferation in response to encephalitogenic peptides and with diminished Th1-type cytokine response in both groups of DNA pre-immunized mice in comparison to EAE animals pre-immunized with empty vector (pVax).

Conclusions: These results indicate that kinetic of tolerization of EAE with DNA vaccination, involving T cell unresponsiveness, depends on antigen and/or mouse genetic background. In further studies mechanism of differences in generation of DNA-induced EAE tolerance should be evaluated.

Disclosure: A Walczak has nothing to disclose.

P117
PROFILES OF MATRIX METALLOPROTEINASES (MMPs) IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS: A SIGNIFICANT ELEVATION OF MMP-12
Weaver A*, Pennington CP, Nuttall R*, Hogan A*, Edwards DR, Yong VW*, ‡Neuroscience, University of Calgary, Calgary, Alberta, Canada; †Biological Sciences, University of East Anglia, Norwich, Norfolk, United Kingdom

Background: Matrix metalloproteinases (MMPs) are a family of at least 25 zinc-containing proteolytic enzymes that play an important physiological role in degradation of extracellular matrix (ECM). However, MMPs are also implicated pathologically in cancer and inflammatory diseases such as Multiple Sclerosis (MS). In MS and its animal model, experimental autoimmune encephalomyelitis (EAE), several groups have reported increased expression of specific MMP members (MMP-2, -3, -7, -9 and -19). Nonetheless, an extensive analysis of the involvement of other MMP members has not been conducted.

Objectives: To examine a profile of MMPs and ADAMs (a related metalloproteinase) during the course of EAE, and to use genetically deficient mice to understand the role of a highly expressed MMP.

Methods: Female 129/SvEv mice were induced with EAE using myelin oligodendrocyte glycoprotein in complete Freunds Adjuvant, producing a relapsing-remitting model of MS. Animals were sacrificed at various stages of the disease and spinal cord samples were analysed. Taq-Man Polymerase Chain Reaction (PCR) was used to profile mRNA levels when animals had hindlimb paralysis, while RNase Protection Assay (RPA) was used to evaluate spinal cords over the course of EAE. As a result of the increase in MMP-12 that was observed, MMP-12 null mice were utilized to analyse phenotypic characteristics.

Results: The Taq-man PCR data displayed an increase in MMP-8 (20 fold), -10 (20 fold), 14 (2 fold), -19 (5 fold) and TIMP-1 (150 fold); impressively, MMP-12 was elevated 1000 fold in EAE-afflicted mice compared to healthy animals. RPA further confirmed the dramatic increase of MMP-12 over the course of disease. ADAM-10, -15, -17 and -28 were unchanged in EAE versus controls.

Conclusions: The data demonstrate that specific MMPs, other than previously examined MMP-2, -7 and -9, need to be pursued for their involvement in EAE and MS. In particular, MMP-12 appears to be important over the course of EAE. EAE experiments in MMP-12 knock out mice are currently underway to explore the clinical phenotype. These results will lead to the further understanding of MMPs in EAE and MS and may help to resolve the issue of selective inhibition of particular MMPs.

Disclosure: A Weaver has nothing to disclose.
Neurobiology/Neurophysiology

**P118**

CEREBROSPINAL FLUID ISOELECTROFOCUSING IN A LARGE COHORT OF MULTIPLE SCLEROSIS AND OTHERS NEUROLOGICAL DISEASES.

Amina B, J. Dp, R. Gp, B. Op, B. Hp, T. St, D. Fp, P. Vp

*Neurology, CHU H MONDOR, CRETÉIL, île de France, France; †Neurology, CHRU Lille, LILLE, Nord, France; ‡Biochemistry Laboratory, CHRU Lille, LILLE, Nord, France

**Background:** The presence of oligoclonal immunoglobulin G bands restricted to CSF is one of the most consistent laboratory abnormalities for the diagnosis of multiple sclerosis (MS).

**Objectives:** To confirm the diagnostic value of isoelectrofocusing (IEF) in a large cohort and to evaluate the various neurological diseases likely to present the same profile.

**Methods:** The cerebrospinal fluid of 1000 patients with neurological was studied by IEF. The infectious diseases without microbiological confirmation and/or not fulfilling the classically definite diagnostic criteria for the various neurological diagnoses were excluded. After a follow up of 2 to 36 months, 407 patients were diagnosed as MS and 593 patients as others neurological diseases.

**Results:** The best sensitivity and specificity was obtained with 3 oligoclonal bands. 380 patients had oligoclonal bands including 335 with MS and 50 with others neurological diseases. Our results showed a sensitivity of 83% and a specificity of 92%. The positive and negative predictive values were 87% and 90% respectively. Inflammatory and infectious disorders of the central nervous system represent the main affections associated with oligoclonal bands in particular Sjogren syndrome, HIV encephalitis and Lyme disease.

**Conclusions:** IEF of the CSF is a reliable method for the diagnosis of MS with both good sensitivity and specificity. CSF is therefore of particular interest especially after a clinical isolated syndrome.

**Disclosure:** B Amina has nothing to disclose.

**P119**

PERIPHERAL SENSORY AND MOTOR ABNORMALITIES IN PATIENTS WITH MULTIPLE SCLEROSIS

Anlar Oc, Tombul Tc, Kisli Mc, J. Da, R. Gb, B. Ob, B. Hb, T. Sa, D. Fa, P. Va

*aNeurology, CHU H MONDOR, CRETÉIL, île de France, France; †Neurology, CHRU Lille, LILLE, Nord, France; ‡Biochemistry Laboratory, CHRU Lille, LILLE, Nord, France

**Background:** Peripheral nerve abnormalities are uncommon in multiple sclerosis (MS). When present, they are usually attributed to factors associated with advanced disease, such as malnutrition or cytotoxic drugs or hereditary factors. However, a combination of MS and neuropathy has been reported.

**Objectives:** To evaluate the combination between MS and peripheral neuropathy we studied nerve conduction velocity (NCV) in a group of MS patients and in a group of healthy subjects.

**Methods:** We studied sensory and motor NCV and amplitude in the ulnar, median, tibial, peroneal and sural nerves in one side, right or left, in 20 definite MS patients diagnosed according to the criteria of Poser Scale and in 15 healthy subjects. The total number of studied nerves were 91 in patients group and 69 in control group.

**Results:** The most frequent electrophysiologic abnormalities noted in patients group were low amplitudes of the ulnar and sural nerves and slow conduction velocities of the tibial and sural nerves. Electrophysiologic abnormalities were found in 15 of 91 nerves examined (16.5%). The neurologic disability was not associated with the presence of electrophysiologic abnormalities. The electrophysiologic abnormalities in control group subjects were found in 2 of 69 nerves examined (2.9%). There were a slight slowness in sural nerve conduction in two healthy subjects.

**Conclusions:** Our findings indicate a high frequency of sensory and motor neuropathy in a selected group of MS.

**Disclosure:** O Anlar has nothing to disclose.

**P120**

ABSTRACT NOT AVAILABLE FOR PUBLICATION

**P121**

OLIGODENDROGLIAL EXPRESSION OF EDG-2 RECEPTOR: DEVELOPMENTAL ANALYSIS AND PHARMACOLOGICAL RESPONSES TO LYSOPHOSPHATIDIC ACID.

Bruno Sb, Sonia Bc, Julien Ac, Celine Jp, Gilles Bp, Marie-Stephane Ap, Pierre Sb, Bernard Zb, Catherine Lc

aInserm U495, hôpital de la Salpêtrière, Paris, France; bcentre d’investigation clinique, hôpital de la Salpêtrière, Paris, France; cInserm U 109, centre Paul Broca, hôpital Sainte Anne., Paris, France

**Background:** Edg-2 is a member of the Edg seven transmembrane G-protein coupled receptor family, which, to date, contains 8 members (Edg-1-Edg-8). These receptors are mainly involved in bioactive lipid signaling: Edg-1, Edg-3, Edg-5 and Edg-8 have been proposed to act as sphingosine-1-phosphate receptors, whereas lysophosphatidic acid (LPA) has been proposed as the ligand for Edg-2, Edg-4 and Edg-7.

**Objectives:** To assess the expression of Edg receptors in oligodendrocytes, and the biological effects of Edg-2 putative ligand, LPA.

**Methods:** The expression of Edg-2 receptor in oligodendrocytes was studied in vivo and in vitro.

**Results:** We show that both in vitro and in vivo, Edg-2 receptors are not detected during early stages of oligodendrogial development, but are expressed only in mature oligodendrocytes, shortly before the onset of myelination. We also provide evidence that other members of the Edg family are expressed in oligodendrocytes. Whereas LPA has been reported to be a ligand of Edg-2 receptor in different cell types, it had no effect on either survival, maturation or cytoskeleton organization in oligodendroglial cultures. In myelinating oligodendrocyte-neuron co-cultures, LPA did not influence myellogenesis. In addition, LPA failed to induce Ca2+ mobilization and had no effect on forskolin-induced cAMP accumulation. Phosphorylation of the ERK1/ERK2 MAP kinases was the only response elicited by LPA in oligodendrocytes.

**Conclusions:** Therefore, in contrast to other cell types, in which LPA exerts pleiotropic effects, Edg-2 positive post-mitotic oligodendrocytes display a restricted responsiveness to LPA. Nevertheless it is so far the only G protein-coupled receptor identified to date whose expression is restricted to mature oligodendrocytes and myelin in the post-natal CNS.

**Disclosure:** S Bruno has nothing to disclose.

**Funding:** Supported by INSERM ( institut national de santé et de recherche médicale) and ARSEP association de recherche sur la sclérose en plaques.

**P122**

SLEEP DISORDERS IN MULTIPLE SCLEROSIS PATIENTS. CLINICAL CORRELATIONS

de Andres de Frutos C, Lopez Esteban P, Peraita Adrados R

aEpilepsy Unit, Hospital general Gregorio Marañon, Madrid, Spain; bSleep and Epilepsy Unit, Hospital general Gregorio Marañon, Madrid, Spain

**Background:** The sleep disorders in MS are not well-known

**Objectives:** 1. To determine the presence of sleep abnormalities in patients diagnosed with Multiple Sclerosis (MS) using polysomnography. 2. To detect the relationship between sleep disorders and the level of disability (EDSS), depression (Beck) and fatigue.

**Methods:** We studied 32 patients diagnosed with MS according to Posser’s criteria. The patients had not previously complained about the poor quality of their sleep; 12 men, 20 women; mean age 42.03, 10.6 years; Expanded disability status scale (EDSS) mean 2.95, 1.88; mean time
of outcome of the illness 7.6, 6.43 years. All the patients were evaluated using the following questionnaires: Epworth sleepiness scale, Beck’s depression scale, Fisk’s fatigue impact scale, and general sleep disorder questionnaire (Montpellier). All patients underwent a standard nocturnal PSG recording (EEG, EOQ, EKG, submental and both tibialis anterior EMGs, oro-nasal airflow, thoraco-abdominal effort and oxyhemoglobin saturation). The results of the PSG were compared with those of 14 healthy controls matched for age.

Results: Statistical significant differences were observed in comparison with the controls: longer sleep latency (p=0.016), shorter total sleep time (p=0.001), lower sleep efficiency index (p=0.001), greater WASO (p=0.008) and greater sleep fragmentation index (p=0.079). We found periodic leg movements (PLM) in 48.87% of patients (p=0.019). No evaluable respiratory abnormalities were observed in these patients. A positive relation is observed between the duration of WASO and time of outcome of the disease, regardless of age. The sleep fragmentation index is the parameter with the greatest correlation with the level of depression, according to the Beck scale and the total and social fatigue impact scale. There is a negative correlation between the total sleep time and the degree of disability measured by EDSS.

Conclusions: 1. Sleep disorders are underevaluated in patients diagnosed with MS. 2. There exists a relation between the physical factors and the polysomnography alterations in these patients

Disclosure: C de Andres de Frutos has nothing to disclose.

P123

ELECTRONYSTAGMOGRAPHY FINDINGS IN PATIENTS WITH MULTIPLE SCLEROSIS

Kıfıncı M, Can U, Benli S, Özliolu L, Akkuzu B

*Neurology, Ba\'kent University, Ankara, Turkey; *Ear, Nose and Throat, Ba\'kent University, Ankara, Turkey

Background: Electronystagmography (ENG) records the changes in eye position indicated by the polarity of the cornea-retinal potential relative to each electrode placed beside the eye. Since the vestibular apparatus contributes significantly to the control of eye movements, these movements can be exploited to examine the activity of the peripheral vestibular end organs and their central vestibulo-ocular pathways. Therefore, ENG can be used to reveal small lesions located in the brain stem, and cerebellum.

Methods: Eighteen clinically definite MS patients with or without vertigo were evaluated with neurological and otological examination along with ENG test battery and contrast enhanced cranial MRI. The ENG test battery included saccades, gaze, sinusoidal tracking, optokinetic nystagmus, positional test, Dix-Hallpike maneuver and caloric.

Results: The most prominent abnormality was detected by optokinetic stimulus, 88.8% of the patients had abnormal or asymmetric optokinetic nystagmus. Sinusoidal tracking was abnormal in 55.5%. Abnormalities in saccades were found in 44 4 %, and caloricics in 27.7%. Gaze and positional tests were abnormal in 11.1% of the patients. Nystagmus could be elicited in none of the patients with Dix-Hallpike maneuver. Vertigo was a symptom in 50% of the patients. In cranial MRI 94.4% of the patients had supratelentorial and 77.7% had infratentorial lesions. The lesions were located only in supratentorial regions in 22.2%, and only in infratentorial regions in 5.5%. Four of the patients (22.2%) had abnormal ENG findings although they did not have any infratentorial lesion. Three of these patients did not complain of vertigo, and two of them had no abnormality in brain stem and cerebellar examinations. In one of the patients (5.5%) there was only infratentorial lesions; she complained of vertigo, and she had abnormalities in ENG test results confirming the clinical symptoms and MRI findings.

Conclusions: We suggest that ENG can be used as a laboratory support in diagnosis and/or follow up of probable or definite MS patients with clinically silent and/or MR negative lesions.

Disclosure: M Kifincı has nothing to disclose.

P124

BRAINSTEM AUDITORY EVOKE RESPONSE WITH HIGH CLICK STIMULUS REPETITION RATE IN MULTIPLE SCLEROSIS

Lana-Beixoto MA, Santos MA, Munhoz MS

CIEM INAS UFMG Center for Investigation of Multiple Sclerosis, Brazilian Committee for Treatment and Research in Multiple Sclerosis, Belo Horizonte, MG, Brazil

Background: Abnormalities of the BAER in MS are characterized by prolongation of the interpeak intervals, absence of waves and poor reproducibility. The effect of the increase of the stimulus rate may increase the BAER sensitivity. Results: MS patients and controls were not different regarding conventional auditory tests. BAER was significantly abnormal in the MS group in the following conditions: (1) at 11/s click rate regarding the latency of wave III (in males) and wave V; (2) at 31/s regarding latency of wave V; (3) at 51/s regarding latency of wave III, wave V and I-III interval (only in women); (4) at 61/s regarding latency of wave III, wave V and I-III interval (both in women and men); (5) at 71/s regarding latency of wave V in women. Conclusion: This study shows that high click stimulus rate increases significantly the sensitivity of the BAER and may be more helpful in detecting abnormalities in the auditory pathways in MS patients.

Objectives: To evaluate the effects of high stimulus repetition rate in the wave latencies and interpeak intervals of the BAER in MS patients.

Methods: The cohort comprised 20 women and 9 males with CDMS (mean age 43.8 and 38.2) and a control group of 20 women and 9 men (mean age of 22.6 and 23.1) with no history or objective sign of neurological or otological disturbance. MS patients had no infratentorial MRI lesion. BAER was performed using a stimulus repetition rate of 11 clicks/s, 31, 51, 61 and 71 clicks/s.

Results: MS patients and controls were not different regarding conventional auditory tests, BAER was significantly abnormal in the MS group in the following conditions: (1) at 11/s click rate regarding the latency of wave III (in males) and wave V; (2) at 31/s regarding latency of wave V; (3) at 51/s regarding latency of wave III, wave V and I-III interval (only in women); (4) at 61/s regarding latency of wave III, wave V and I-III interval (both in women and men); (5) at 71/s regarding latency of wave V in women.

Conclusions: This study shows that high click stimulus rate significantly increases the sensitivity of the BAER.

Disclosure: M Lana-Beixoto has nothing to disclose.

P125

NEUROPHYSIOLOGICAL EVALUATION OF EXECUTIVE FUNCTIONS IN MULTIPLE SCLEROSIS: A FOLLOW-UP STUDY

Leocani L*, Martellini Vb, Annovazzi Pa,b, Tickonova Ia, Possa Fb, Comi Ga,b

*Clinical Neurophysiology, Hospital San Raffaele, Milan, Italy; **Neurology, Hospital San Raffaele, Milan, Italy

Background: Cognitive impairment is a frequent complication of Multiple Sclerosis (MS), particularly related to frontal executive functions, reflecting a subcortical dementia. Frontal lobes are involved in motor planning and execution, reflected in the event-related desynchronization (ERD) of the EEG sensorimotor rhythms. Objectives: In order to investigate neurophysiological correlates of executive function in MS, we evaluated ERD to self-paced movement and reaction times to the Stroop test (which evaluates frontal functions) in MS patients with and without frontal neuropsychological deficits.

Methods: Ten MS patients, who failed in three or more frontal neuropsychological tests, were compared to 10 MS patients matched for age, handedness, disease duration and disability, who did not fail at any frontal test. EEG was recorded during self-paced movement paradigm. Manual reaction time to the Stroop test was also measured. Nine frontal patients and nine non frontal patients underwent follow-up examination of both EEG and reaction time measurements.

Results: Stroop reaction times and ERD were significantly delayed in both MS groups compared to normal subjects and in frontal compared to non frontal MS.
patients. At follow-up, RT performance at the Stroop test were worsened in both groups of patients, but mostly in frontal patients. Patients with frontal dys- function also showed changes in EEG parameters.

**Conclusions:** These data suggest that frontal cognitive involvement in MS cor- responds to abnormal bioelectrical activity emerging also during a self-paced movement paradigm. Moreover, changes over time of both EEG and reaction times suggest a potential usefulness of these measurements in monitoring frontal function in MS.

**Disclosure:** L Leocani has nothing to disclose.

**P126**

**PRESENCE OF HERPES SIMPLEX VIRUS 1 IN SAMPLES FROM PATIENTS WITH OPTIC NEURITIS AND MULTIPLE SCLEROSIS**

Christodoulou C, Constantinou A, Koptides D, Paschalidou M, Georgiadi N, Chatzisiotiri A, Milonas P,*

*Department of Molecular Virology, The Cyprus Institute of Neurology and Genetics, Nicosia, -, Cyprus; *2nd University Department of Neurology, Aristotle University, Thessaloniki, Greece; *Department of Ophthalmology, Aristotle University, Thessaloniki, -, Greece

**Background:** Multiple sclerosis is one of the most venerable of neurological diseases and one of the most important by virtue of its frequency, chronicity and tendency to attack young adults. The etiology of MS remains unknown. Several epidemiological studies emphasize the importance of environmental, familial and genetic factors. There are supporting data over the years of research that viruses are possible agents triggering the autoimmune response. The initial event in the genesis of MS could be viral infection of nervous sys- tem, than some secondary factor must be operative in later life to activate the neurological disease and cause exacerbation. It is widely believed that this sec- ondary mechanism is an autoimmune reaction.

**Objectives:** Our group is interested not only in the viral etiology of MS but also in the way the possible viral agent enters the CNS. Our hypothesis concerns the eye as possible entry. To perform a preliminary study, we chose 13 patients with both optic neuritis and MS. Serology and molecular virology studies of Herpes simplex virus 1 (HSV1) were carried out in serum, tears and eye biopsies.

**Methods:** Methodology used for serology study was commercially available classical ELISA for the detection of IgG and IgM antibodies against HSV1. For the molecular study nested PCR was performed using primers specific to amplify a DNA fragment inside the DNA polymerase gene of HSV1. The PCR was followed by Southern blot and hybridization with specific radioactive probes.

**Results:** All of the patients presented IgG antibodies against HSV1. The molecular study showed that 9 out of 13 patients were positive for the DNA of HSV1 in serum. We investigated eye biopsy and tear samples of 5 patients. 4 of the patients presented HSV1 DNA in their tear sample and 3 out of 5 biopsies were positive for the HSV1 DNA.

**Conclusions:** We consider that these preliminary results suggest that the hypothesis of the eye being a possible entry for a neuropetric viral infection is worth to be investigated further.

**Disclosure:** M Paschalidou has nothing to disclose.

**P127**

**HHV-6A ACTIVE INFECTION IN MULTIPLE SCLEROSIS PATIENTS**

Roberto A, Virginia D, Eduardo V, Juan José P, Rafael A,*

*Neurology, Hospital Clínico San Carlos, Madrid, Spain; *Microbiología, Hospital Clínico San Carlos, Madrid, Spain

**Background:** Recent studies have reported a possible association between MS and HHV-6, an ubiquitous infectious agent highly prevalent in the human pop- ulation; however, other studies have not confirmed these results.

**Objectives:** To establish the DNA prevalence and viral load of HHV-6 (and both variants A and B) in MS patients and two control groups: healthy blood donors (HBD) and patients with rheumatoid arthritis (RA).

**Methods:** We analyzed the whole DNA of blood and serum samples of 105 patients with relapsing remitting MS (RRMS), 70 HBD and 52 patients with RA by real time quantitative polymerase chain reaction (PCR) assay with a sensitivity of 1 copy to determine the presence of HHV-6 genomes. An absolute determination of the viral load was made with TaqMan probes and previously quantified HHV-6 DNA.

**Results:** Results. HHV-6 DNA prevalences were as follows: 1) In blood: i) RRMS patients: 57.1% for HHV-6, 21.9% variant A, 32.4% variant B, 2.8% variant A plus B; ii) HBD: 30.6% for HHV-6, 27.1% variant B, 3.5% variant A plus B; iii) RA patients: 34.6% for HHV-6, 9.6% variant A, 25% variant B. 2) In serum: i) RRMS patients: 17.1% for HHV-6, all of them variant A; ii) HBD: 0%; iii) RA patients: 9.6% for HHV-6, 7.7% variant A and 1.9% vari- ant B. The mean HHV-6 viral load of the positive samples in HHV-6 genomes / microgr. of input DNA were: 1) In blood: 5.9 in RRMS patients, 5.6 in HBD and 6.2 g in RA patients. 2) In serum: 18.8 in RRMS patients and 27.4 in RA patients.

**Conclusions:** 1) HHV-6 DNA prevalence in blood was significantly higher in the MS patients group (p=0.01). 2) HHV-6 variant A is the responsible of that statistically significant difference in blood. 3) There is a 17.1% and a 9.6% of MS and RA patients suffering a HHV-6 active replication at the sampling. 4) Almost all the HHV-6 positive samples in serum were variant A, which leads us to think that is the only variant involved in MS. 5) Despite the difference in HHV-6 DNA prevalences, we did not find significant differences in the viral loads.

**Disclosure:** A Rafael has nothing to disclose.

**P128**

**HOW DOES BETA INTERFERON TREATMENT AFFECT TO HHV-6 VIRAL LOAD IN MULTIPLE SCLEROSIS PATIENTS?**

Virginia D*, Roberto A, Eduardo V, Juan José P, Rafael A,*

*Neurology, Hospital Clínico San Carlos, Madrid, Spain; *Microbiología, Hospital Clínico San Carlos, Madrid, Spain

**Background:** Beta interferon (beta-IFN), an approved treatment for MS, may modify the clinical course of the disease, as demonstrate recent clinical trials. However, we do not completely know what immunomodulatory mechanisms may involve in the mediation of its positive effects in the treatment of MS patients.

**Objectives:** To know if beta-IFN treatment affects: 1) the DNA prevalence of the HHV-6; 2) the HHV-6 viral load.

**Methods:** We made a prospective single center cohort study of 76 serum samples of 76 consecutive patients undergoing RRMS. Forty one patients were receiving beta-IFN treatment, but none received steroid treatment prior to blood sampling (14 were suffering an exacerbation at the time of blood draw- ing and 27 were on remission), and 35 did not receive beta-IFN treatment (17 in exacerbation and 18 on remission). To establish the presence of HHV-6 genomes and quantify the viral load, the DNA of these samples was studied by a real time quantitative polymerase chain reaction (PCR) assay.

**Results:** HHV-6 DNA prevalences were: 1) In beta-IFN treated patients: 14.9% for all the patients, 13.8% for MS patients in exacerbation, 15.7% for MS patients in remission. 2) In beta-IFN untreated patients: 19.6% for all the patients, 16.9% for MS patients in exacerbation, 20.9% for MS patients in remission. The viral loads (in HHV-6 genomes / microgr. of input DNA) were: 1) In beta-IFN treated patients: 16.7 for all the patients, 11.4 for MS patients in exacerbation, 18.1 for MS patients in remission. 2) In beta-IFN untreated patients: 20.4 for all the patients, 15.1 for MS patients in exacerbation, 19.3 for MS patients in remission.

**Conclusions:** 1) There is a statistically significant difference (p=0.01) in serum HHV-6 DNA prevalence between patients treated and non treated with beta-IFN; therefore, there is almost five per cent more of MS patients that suffer a HHV-6 active replication when they are not treated with beta-IFN. 2) There is no difference in viral load between MS patients beta-IFN treated and non treated once the virus begin an active replication. 3) beta-IFN treatment reduces in the same proportion the viral loads and the HHV-6 DNA prevalence of MS patients in exacerbation and in remission.

**Disclosure:** A Rafael has nothing to disclose.
New Clinical Trials (Part 1)

P129
DIFFERENT NON-RADIOACTIVE PERMEABILITY ASSAYS IN AN INVITRO MODEL OF THE BLOOD-BRAIN-BARRIER

Voigt KE\textsuperscript{a,b}, Kraus JR\textsuperscript{c}, Oschmann P\textsuperscript{a}, Engelhardt B\textsuperscript{d}
\textsuperscript{a}Department of Neurology, University Hospital Giessen, Hessen, Germany; \textsuperscript{b}Max-Planck-Institute for Physiological and Clinical Research, Bad Nauheim, Hessen, Germany

Background: Increased molecular transendothelial permeability has been suggested to be involved in the breakdown of the blood-brain barrier (BBB) which is part of the pathogenesis of multiple sclerosis.

Methods: As an invitro BBB model, the immortalized endothelial cell line bEnd5 from mouse brain capillaries was grown to confluence in DMEM for two days on semipermeable filters precoated with different matrices. Matrices included rat tail collagen, type IV collagen, and fibronectin dissolved in H2O or PBS. Culture media was replaced with colorless DMEM, and Evans blue dye solution was added to the upper compartment for a final concentration of 0.05%, before filters were successively transferred to other wells containing colorless DMEM at various time points. Spectrophotometric absorbance was measured at 650nm in aliquots from the lower compartments, and the results were calculated as optical density over time elapsed. Permeability was stimulated with either histamine or thrombin and was compared to unstimulated controls.

Results: Filters precoated with rat tail collagen exhibited an increase in paracellular permeability upon both histamine and thrombin stimulus. On type IV collagen coated filters, only thrombin but not histamine increased the permeability. Fibronectin did not lead to an elevated permeability across the endothelial monolayer.

Conclusions: The results indicate that Evans blue dye diffusion across monolayers of brain endothelial cells grown on tissue culture inserts coated with rat tail collagen can be applied as an invitro transendothelial permeability assay of the BBB.

Disclosure: K Voigt has nothing to disclose.

P130
THE USE OF PHARMACOKINETIC (PK) MODELING AND EFFICACY DATA TO ESTABLISH OPTIMAL DOSING OF NATALIZUMAB (ANTEGREN™)

Bennett D\textsuperscript{a}, Ludden T\textsuperscript{b}, Shah J\textsuperscript{c}, Floren L\textsuperscript{c}, Beckman E\textsuperscript{d}
\textsuperscript{a}Biogen, Inc; \textsuperscript{b}Globomax LLC, Hanover, Maryland, USA; \textsuperscript{c}Elan, San Diego, California, USA

Background: The interaction of \(\alpha4\beta1\) integrin (VLA-4) with VCAM-1 appears to be important for autoreactive T cell migration across the blood brain barrier into MS lesions. Natalizumab is a monoclonal antibody in the class of \(\alpha4\)-integrin selective adhesion molecule inhibitors (SAM-inhibitors) directed against the \(\alpha4\)-subunit of VLA-4. Binding of natalizumab to VLA-4 and the resulting inhibition of binding to VCAM-1 may be a key element of the immunomodulatory effects of natalizumab. A clinical trial in which 213 relapsing MS patients were randomized to receive placebo, 3 mg/kg or 6 mg/kg of natalizumab monthly demonstrated that natalizumab treatment led to significant decreases in the formation of new Gd-enhancing lesions on brain MRI scan and in clinical relapses. There were no significant differences in tolerability or efficacy between the two dose groups.

Objectives: To establish the optimal dosing regimen, in terms of safety and efficacy, for natalizumab in phase III MS trials.

Methods: Population PK modeling was employed using PK and \(\alpha4\)-integrin binding data from Phase II studies of natalizumab.

Results: The results of the population analysis indicate that there are only small changes in natalizumab clearance across a broad range of body weights. An analysis of serum natalizumab concentration and \(\alpha4\)-integrin saturation levels demonstrated that \(\alpha4\)-integrin saturation levels increase with serum natalizumab concentration, which was observed over a wide range of body weights. Significant overlap was seen between dose groups. The serum concentrations of natalizumab achieved maintained adequate receptor saturation throughout the treatment period corresponding with similar efficacy seen in both MRI and clinical endpoints. Pharmacokinetic modeling demonstrated that fixed dosing of natalizumab every 4 weeks achieves more consistent serum concentrations across a wide range of body weights than mg/kg dosing. The result is adequate \(\alpha4\)-integrin saturation required for efficacy while keeping drug exposure levels within the defined safety ranges established in prior studies.

Conclusions: The PK features of natalizumab with its unique targeted mechanism of action correlated with efficacy data from the Phase II studies enabled fixed monthly dosing in the on-going Phase III natalizumab clinical trials.

Disclosure: D Bennett E Beckman Biogen staff T Ludden Globomax staff J Shah L Floren Elan staff

P131
SAFETY AND TOLERABILITY DOSE COMPARISON OF INTERFERON BETA-1A IN RELAPSING-REMITTING MULTIPLE SCLEROSIS: THE EVIDENCE STUDY

Bever CT, for the EVIDENCE Study Group

Background: The EVIDENCE study showed that the efficacy of subcutaneous (sc) interferon (IFN) beta-1a 44 mcg (Rebif\textsuperscript{®}) thrice weekly (tiw) was superior to intramuscular (IM) IFN beta-1a 30 mcg (Avonex\textsuperscript{®}) once weekly (qw) at 24 and 48 weeks of treatment in patients with relapsing-remitting multiple sclerosis.

Objectives: We reviewed detailed 48-week safety data to determine whether the improved efficacy was achieved at the cost of lower safety or tolerability.

Methods: 677 patients with clinical or laboratory-supported definite MS were randomized to either IFN beta-1a 44 mcg sc tiw or 30 mcg IM qw. Clinical safety assessments were performed every 4 weeks to week 24 and then every 12 weeks to week 48. Blood tests were done at baseline and months 1, 3, 6, 9, 12 and 24.

Results: 339 patients received IFN beta-1a 44 mcg sc tiw (high dose) and 337 received 30 mcg IM qw (low dose). Fla-like symptoms were common but did not differ significantly between groups either in terms of incidence or severity. Injection-site events were reported in 83% of high dose and 28% of low dose patients (p<0.001). 85% of injection site reactions on high dose were rated as mild and similar numbers of patients on high (n=4) and low (n=3) dose discontinued treatment because of injection site reactions. Overall, therapy was stopped in 25 high dose patients (7.4%) and 21 low dose patients (6.2%). The most common reason was for adverse events (16 high dose patients and 14 low dose patients). Elevations of serum alanine aminotransferase (ALT) was the most common hepatic adverse event (11.5% on high dose vs. 4.7% on low dose; p=0.001). Significant overlap was seen in 6 patients in the high dose group (1.8%) and 4 in the low dose group (1.2%) with 3 dropouts on the high dose and 1 on the low dose for enzyme elevations. Leukopenia (6.2% high dose vs. 0.6% low dose; p=0.001) was the most common white blood cell adverse event. Severe leukopenia was not seen in either group. Depression (16% vs. 18%) and headache (38% vs. 32%; p=ns) were approximately equal. Only one death occurred during the study (a solo plane crash).

Conclusions: The advantage in efficacy seen clinically at 48 weeks with 44 mcg sc tiw compared to 30 mcg IM qw was not offset by clinically significant safety concerns or reduced adherence to therapy.

Disclosure: C Bever is on the speaker’s bureau and has received honoraria from the study sponsor; Serono Laboratories, Inc. In addition, some other members of the EVIDENCE Study Group receive support from the study sponsor.

Funding: Supported by Serono Laboratories, Inc.
P132
THE REGISTRY TO EVALUATE NOVANTRONE® (MITOXANTRONE FOR CONCENTRATE INJECTION) EFFECTS IN WORSENING MS (RENEW): STATUS REPORT SEPTEMBER 2002
Goodkin DE, Flanders K, Leung J, Butine m, Stead R
Immunex Corporation, Seattle, Washington, USA

Background: The RENEW study is a multicenter (N=50), open-label, observational safety study of 500 patients with worsening RR, SP and PR MS who initiate commercially available NOVANTRONE according to guidelines in the package insert.

Objectives: To describe the cumulative dose and tolerability of NOVANTRONE® in the RENEW study.

Methods: Entry criteria: Patients with CD or LSD RR, SP, or PR MS who initiate NOVANTRONE 12mg/m2 within 3 months of site-IRB approval, platelets >100,000 cells/ml, granulocytes >2000 cells/ml, age 18-65 yrs, negative pregnancy test. Exclusion criteria: PPMs, history of CHF, left ventricular ejection fraction (LVEF) <50%, previous treatment with NOVANTRONE® or other anthracyclones or anthracyclines, mediastinal radiotherapy or TLI; AST, ALT, total bilirubin (LFTs) >2x ULN; current UTI or other severe untreated infection, nursing or pregnant women. Procedures: medical evaluation, CBC with platelet count, and LFTs are conducted every third month during treatment and every year thereafter (total 5 years). LVEF is determined at baseline, prior to each dose above a cumulative dose 100mg/m2, and annually after therapy is discontinued.

Results: RESULTS: The first patient was enrolled on 2/9/01. As of 2/11/2002: 261 patients enrolled, (69%) mean age 46 (21-68), mean EDSS 5.9 (1.-9.0), mean LVEF baseline 62% (50-82), mean infusions 2 (range 1-5), mean cumulative dose 24.8mg/m2 (range 9.1-60.5), SAEs, 12, 5 of which are possible therapy-related; patients with CHF 0, LVEF <50% 0, therapy related mortality 0, other deaths 1 (PE, unrelated to therapy). Results through 09/18/02 will be presented.

Conclusions: CONCLUSIONS: NOVANTRONE® has been generally well tolerated by patients enrolled in the RENEW study. Data from the RENEW provide rigorously collected data that reflect the tolerability of NOVANTRONE® as approved for use in clinical practice.

Disclosure: All authors are employees of the Immunex Corporation.

P133
This abstract was also presented at the platform.

THE EFFECT OF INTERFERON B-1B ON QUANTITIES DERIVED FROM MT MRI IN SECONDARY PROGRESSIVE MS

Background: Magnitization transfer transfer magnetic resonance imaging (MT MRI) can provide in vivo markers reflecting the severity of irreversible, MS-related brain damage occurring within and outside T2-visible lesions.

Objectives: To assess the effect of interferon (IFN) b-1b treatment on the accumulation of brain damage in patients with SPMS, measured using MT MRI.

Methods: Eighty-two SPMS patients from five centers participating into a European, multi-center, double-blind, placebo-controlled trial of IFNb-1b in SPMS underwent brain T2-weighted and MT MRI at baseline. Follow-up data were available for 75 patients at 12, 54 and 47 at 36 months. MT MRI scans were post-processed and analyzed to obtain histograms of MTR values from the whole brain. A ROI-based analysis of MTR values from the NAWM was also performed.

Results: In both the treatment arms, there was a decrease of average brain MTR values from baseline to month 24 (mean change=-4.9%) and month 36 (mean change=-4.3%). These changes were statistically significant for the placebo group at both the time-points and for the IFNb-1b group at month 24 only, with no significant treatment effect. A decrease of NAWM MTR was also observed, with no significant difference between the two treatment arms.

Conclusions: In this cohort of patients with SPMS, IFNb-1b as compared to placebo did not show an overall effect on the worsening of MT MRI measures. The data show that change in MTR is a promising tool for monitoring disease evolution in SPMS.

P134
SINGLE CENTRE, DBPC, RANDOMISED TRIAL OF INTERFERON BETA 1B IN PRIMARY PROGRESSIVE AND TRANSITIONAL PROGRESSIVE MULTIPLE SCLEROSIS: AN EXPLORATORY PHASE II STUDY

Background: The beneficial effects of IFN beta have been shown just for patients in the relapsing phase of MS. The role of interferon beta in the treatment of patients with secondary progressive MS still remains a controversial issue. The single phase II randomized controlled trial on PPMS using IFNb-1a (IM) shows no significant effect on EDSS though some effect on T2 lesion load.

Objectives: To investigate safety and hints of efficacy of interferon (IFN) beta1b given to patients with primary progressive (PPMS) and transitional progressive multiple sclerosis (TPMS).

Methods: 73 patients (49 PPMS/24 TPMS) with EDSS scores between 3.0 to 7.0, were enrolled and randomized to receive either 8MIU of IFN beta1b or placebo administered every other day subcutaneously for 2 years. Safety parameters including BDI and Ashworth’s and Krupp’s scales and blood tests were performed every three months. Clinical outcomes (EDSS and MS Functional Composite (MSFC)) were also performed every three months and the Sickness Impact Profile every six months. MRI measures (T2 and T1-weighted brain lesion load, brain parenchymal faction (BPF), T2 active lesions, spinal cord atrophy, MTR and spectroscopy) and neuropsychological assessment (BRNB) were undertaken annually.

Results: Adverse events significantly associated with IFN-beta included injection-site reaction, flu-like symptoms and lymphopenia. One patient on the placebo arm died because of pulmonary infection. In all, 96% of the patients reached study end and 93% completed the treatment period of the study. Treatment groups were comparable on all baseline variables. The proportion of patients with confirmed progression at 6 months was 22.2% in the IFN arm and 32.4% in the placebo arm (p=0.2). Statistically significant differences were found for T2 (p=0.006) and T1 (p=0.01) lesion load and number of active lesions (p=0.0005) in favor of the IFN-treated group. MSFC and BPF data will be presented.

Conclusions: IFN-beta 1b is safe in the treatment of patients with PPMS and TPMS. Our study seems to point to a beneficial effect of IFNb1b on MRI parameters in this group of patients.

Disclosure: X Montalban has nothing to disclose.
Funding: Supported by Schering España, SA.
P135

IFN BETA CHRONIC TREATMENT: HOW TO MANAGE THE DOSE AND THE FREQUENCY OF ADMINISTRATION IN PATIENTS WITH ABSENCE OF DISEASE ACTIVITY

Pipieri A, Barbero P, Bergui M, Verduin E, Clerico M, Durelli L
Turin University, Torino, Italy

Background: The chronic administration of a drug on alternate days may affect patient compliance and encourage the decision to reduce IFN beta dose. A study of clinical and MRI effects associated with the reduction of IFN beta dose is needed

Objectives: To evaluate the MRI-effects of reducing IFN beta dose in patients with RRMS in chronic treatment with IFN beta-1b and with clinical-MRI stabilization

Methods: Prospective one year follow-up of 27 RRMS patients randomised to gradually switch from on-alternate-day IFN beta-1b to once-a-week IFN beta-1a (13 patients), or to continue on IFN beta-1b (14 patients). Before IFN beta reduction the patients had to be on chronic IFN beta treatment for at least 3 years, and without clinical and MRI signs of disease activity during the last 2 years. MRI was performed before randomisation and at the end of the one year follow-up

Results: One year after the reduction of IFN beta dose clinical outcome measures were, for the group of patients switched to once-a-week IFN beta-1a, a mean number of the new PD/T2 lesions 1.92±1.32, of the enlarging PD/T2 lesions 0.69±0.95, of the gadolinium-enhancing lesions 0.92±0.95; for group of patients continually treated with IFN beta-1b, mean number of the new PD/T2 lesions 0.57±0.94, of the enlarging PD/T2 lesions 0.21±0.8, of the gadolinium-enhancing lesions 0.35±0.74. All these outcome measures were significantly lower (p<0.01) for the patients who continued on IFN beta-1b except for the mean number of the enlarging lesions

Conclusions: IFN beta treatment is a chronic one. The reduction of IFN beta-1b dose is not advisable even in patients with years of absence of disease activity

Disclosure: A Pipieri has nothing to disclose.

P136

3 TESLA MAGNETIC RESONANCE IMAGING COMPARISON OF INTERFERON BETA-1B AND GLATIRAMER ACETATE - A RANDOMIZED, SINGLE-BLIND STUDY IN RELAPSING-REMITTING MULTIPLE SCLEROSIS

Wolansky LP, Cadavid DP, Cook SD, Skurnick JF, Biswal B, Pachner AR, Hill J
"Radiology, UMDNJ, Newark, New Jersey, USA; 2Neurosciences, UMDNJ, Newark, New Jersey, USA; 3Preventive Medicine Biostatistics/Epidemiology, UMDNJ, Newark, New Jersey, USA"

Background: Currently approved immunomodulatory therapies for relapsing-remitting multiple sclerosis (RR-MS) include two forms of interferon beta (interferon beta-1a and 1b) and glatiramer acetate (GA, Copaxone®), a synthetic polymer that mimics the structure of myelin basic protein. While recent comparative studies have demonstrated the superiority of higher frequency, higher dose interferon beta formulations in the treatment of RR-MS, there have to date been no direct randomised comparisons of the efficacy and tolerability of interferon beta and GA.

Objectives: The purpose of this presentation is to announce the initiation of a randomised, single-blind, 48-week study to compare the effects of interferon beta-1b vs. GA on MRI and clinical disease measures in patients with RR-MS.

Methods: 110 patients will be recruited to the study and receive the approved dose of either interferon beta-1b (250µg [8 million international units] subcutaneously [sc] every other day) or GA (20 mg sc daily). MRI scans will be conducted 4 weeks before baseline, at baseline, and then every four weeks thereafter for a period of 48 weeks, while clinical assessments will be performed at baseline, week 4 and every 12 weeks thereafter. All MRI scanning will be carried out using ultra-high magnetic field strength (3 Tesla) and triple-dose gadolinium (Gd).

Results: The primary endpoint variable will be the total number of combined-active lesions (Gd enhancing + new long TR lesions) over the twelve, monthly scans with patient as the unit of analysis. Secondary end-points will include volumetric analysis, diffusion tensor imaging, neuro-cognitive function and clinical disease activity (relapses and progression of disability).

Conclusions: Enrolment is expected to be complete by August 2003, with the results expected in 2005.

Disclosure: L Wolansky has nothing to disclose.

Genetics (Part 1)

P137

A SCANDINAVIAN GENOME-WIDE LINKAGE DISEQUILIBRIUM SCREEN IN MULTIPLE SCLEROSIS PATIENTS INDICATES ASSOCIATION AT 1Q (D1S1601) AND 1Q (D1S1986)

1Multiple Sclerosis Research Unit, Copenhagen University Hospital, Copenhagen, Denmark; 2Institute of Immunology, Oslo National Hospital, Oslo, Norway; 3Clinical Immunology, Copenhagen University Hospital, Copenhagen, Denmark; 4University of Cambridge Neurology unit, Addenbrookes Hospital, Cambridge, CB2 2QQ, United Kingdom; 5Department of Neurology, Huddinge University Hospital, Huddinge, Sweden; 6Department of Neurology, Ullevål University Hospital, Oslo, Norway; 7Department of Neurology, Lund University Hospital, Lund, Sweden; 8Department of Neurology, Haukeland Hospital, Bergen, Norway; 9Department of Neurology, Gothenburg University Hospital, Gothenburg, Sweden

Background: Multiple sclerosis (MS) is a chronic inflammatory disease. Unknown genetic and environmental factors contribute to the disease. Objectives: The present study is based on a genetic homogenous Scandinavian population and part of a Genetic Analysis of Multiple sclerosis in EuropeanS study (GAMES).

Methods: Two independent Scandinavian genome-wide screens for linkage disequilibrium have been performed, using pooled DNA and a dense map of 6000 microsatellite. In the first screen, 199 cases were compared with 200 controls; in the second, a further 201 cases were compared with a second set of 200 controls.

Results: Statistical data were achieved from 4041 markers in the first and 4228 markers in the second screen. Results for both screens were available in the same 3360 markers. Thirteen markers showed statistically significant differences between case-control allele image patterns (AIP) in both screens, among these the HLA marker D6S2447. When additional AIPs were generated for the most promising of these markers, statistical significance was retained for two markers - the D1S1601 marker (1q42) and the D1S1986 marker (1q23). These two novel genomic regions may contain susceptibility genes for multiple sclerosis.

Conclusions: Two novel genetic regions possibly contributing to the genetic susceptibility to MS among Scandinavians has been identified. Further work is needed to dissect which genes are actually responsible for the observed association.

Disclosure: P Datta has nothing to disclose.

Funding: The project received financial support from the European Commission (project number CT97-2422); Norwegian Foundation for Health and Rehabilitation (project number 1998/273); The Norwegian Research Council, Norway; Odd Fellow MS Society, Norway; Medinnova, Norway; The Danish Multiple Sclerosis Society (project number 01/001), Denmark;
Gerda and Aages Foundation, Denmark; and the Wellcome Trust, United Kingdom (grant 022549).

P138

-384 INTERLEUKIN-2 POLYMORPHISMS IN MULTIPLE SCLEROSIS

Fernández V¹, Leyva L², Mayorga C³, Mateasanz F¹, Fedetz M¹, Alcina A¹, Guerrero M¹, León A¹, Luque G¹, Fernández O¹
¹Neurology, Hospital Regional Universitario Carlos Haya, Málaga, Spain; ²Research Unit, Hospital Regional Universitario Carlos Haya, Málaga, Spain; ³Immunology and Cellular Biology, Instituto de Parasitología y Biomedicina Lopez Neyra, Granada, Granada, Spain; ⁴Neurology, Hospital Clínico San Cecilio, Granada, Granada, Spain

Background: Interleukin-2 (IL-2) is an immunoregulatory cytokine with a key role in maintenance of self-tolerance and in CNS. Recent findings suggest that IL-2-2RB locus contributes to the genetic susceptibility in some multiple sclerosis (MS) patients. So far, only two il-2 polymorphisms have been identified, at position -384 and 114 and it would be interesting to analyse their association with susceptibility to MS

Objectives: To investigate the association of -384 polymorphisms in the IL-2 with the susceptibility to MS and its clinical characteristics (clinical form, gender, age at onset, symptoms at onset, disease duration, EDSS score at the moment of study, response to interferon therapy) as well as with HLA class II alleles.

Methods: DNA was collected from 90 patients with clinically definite MS (56 with relapsing-remitting (RR) and 34 with secondary-progressive (SP) clinical form) and 153 controls, and was extracted by standard procedures. The sequence containing -384 polymorphisms was amplified with oligonucleotide IP46 modified to create a restriction site for the Bfa-1 enzyme with the G allele. The forward primer was IP47. Amplification yielded a band of 131 bp that, after digestion with Bfa-1, gave products of 110 and 21 bp, that were separated on 12 % polyacrylamide gel electrophoresis, stained with ethidium bromide and visualized with ultraviolet light. The HLA class II subregions DRB1, DQA1 and DQB1 were investigated by commercial molecular biology methods.

Results: No significant differences in the genotype frequencies between MS group and controls were found. A positive association between the heterozygous -384 genotype (G/T) and the SP clinical form (57% vs. 43%, p = 0.009) was detected. There was a trend of association (p = 0.07) between the homozygous -384 genotype (T/T) and the RR clinical form (66% vs. 47%). No association was detected. There was a trend of association (p = 0.07) between the homozygous -384 genotype (G/T) and the SP clinical form (57% vs. 43%, p = 0.009) and the SP clinical form (57% vs. 43%, p = 0.009).

Conclusions: The fact that different alleles of the same polymorphism show a positive association in different populations would suggest that these polymorphisms do not influence susceptibility but that another as yet untested loci is involved.

Disclosure: V Fernández has nothing to disclose.

P139

CTLA-4 GENE POLYMORPHISMS AND THEIR INFLUENCE ON SUSCEPTIBILITY TO MULTIPLE SCLEROSIS IN N. IRELAND

Hegarty SV¹, Silversides J², Vandenvreoke K², McDonnell G², Hawkins S², Graham C²
¹Regional Genetics Centre, Belfast City Hospital Trust, Belfast, Co. Antrim, United Kingdom; ²School of Pharmacy, Queens University of Belfast, Belfast, Co. Antrim, United Kingdom; ³Dept. of Neurology, Royal Victoria Hospital, Belfast, Co. Antrim, United Kingdom

Background: Polymorphisms within the cytotoxic T lymphocyte associated (CTLA-4) gene have been implicated in a number of autoimmune diseases ranging from type 1 diabetes mellitus, Graves disease as well as multiple sclerosis. The CTLA-4 and CD28 molecules are co-stimulatory T cell receptors that are involved in the control of T cell activation. CD28 upregulates cellular activity by its interaction with the B7 ligand of the antigen presenting cell while CTLA-4 provides the necessary down regulatory signal to reduce the ongoing immune response. CTLA-4 is therefore a good candidate gene for investigation in autoimmune disorders.

Objectives: The human CTLA-4 gene is located on chromosome 2q33. From literature the CTLA-4 gene contains the polymorphisms at positions -318 (CT/T) in the promoter (Genbank/MT4363), +49(A/G) of exon 1 coding for threonine or alanine respectively (Genbank/MT4363), and a dinucleotide (AT)n repeat sequence in the 3'-untranslated region of exon 4 at position 642 (Genbank/MT37243). A case control study was proposed to determine the influence of these polymorphisms in the N. Ireland population on MS risk.

Methods: Alleles of the polymorphism within the promoter (-318CT/T) were identified by PCR using sequence specific primers, the exon 1 (49A/G) polymorphism was typed using PCR/RFLP while the microsatellite was genotyped using fluorescently labelled PCR primers and sized on an ABI3100. Statistical analysis was carried out by the Chi-squared test.

Results: The promoter and exon 4 polymorphisms showed no significant differences between case and control populations however a strong association to the A allele of the +49A/G polymorphism of exon 1 of the gene was demonstrated. There were significantly more homozygous A allele patients (42%) in comparison to the control population(29%; p = 0.0031).

Conclusions: The fact that different alleles of the same polymorphism show a positive association in different populations would suggest that these polymorphisms do not influence susceptibility but that another as yet untested loci is involved.

Disclosure: S Hegarty has nothing to disclose.

Funding: Supported by MS Society of Ireland.

P140

ASSOCIATION STUDY OF FAS AND FASL POLYMORPHISMS WITH MULTIPLE SCLEROSIS

Kantarcı OH¹, Hebrink DD², Achenbach SP², Elizabeth AP², McMurray CT², Weenschenker BP²
¹Neurology, Mayo Clinic & Foundation, Rochester, Minnesota, USA; ²Department of Health Sciences Research, Mayo Clinic & Foundation, Rochester, Minnesota, USA; ³Departments of Pharmacology, Biochemistry and Molecular Biology and Molecular Neuroscience Program, Mayo Clinic & Foundation, Rochester, Minnesota, USA

Background: Fas and FasL expression leads to antigen activation-induced apoptosis of T-cells. Mutations in Fas and FasL cause autoimmunity and lymphoproliferation in mice and humans. Others have reported association of polymorphisms adjacent to or in Fas and FasL with MS susceptibility.

Objectives: To study the association of Fas and FasL polymorphisms with susceptibility to, gender bias, age at onset, course and severity (based on EDSS and duration) of MS.

Methods: Three single nucleotide polymorphisms (SNPs) per gene were selected from the SNP database to establish haplotypes that span the genes. Genotyping was performed in a population-based sample of 122 cases and 244 gender, age and ethnicity-matched controls using restriction fragment length polymorphism methodology. Results were not corrected for multiple comparisons in this preliminary analysis.

Results: There was linkage disequilibrium (LD) between 5’(-670) and Exon7(74) SNPs of Fas in both cases and controls (p = 0.00001). Homozygosity for 5’(-670*A) (p = 0.034; OR: 1.78, 95%C.I: 1.04-3.03) and for Exon7(74)*C (p = 0.019; OR: 1.84, 95%C.I: 1.10-3.07), the two specific alleles in LD, was associated with increased risk of MS in women but not men. There was a trend to association of the haplotype defined by these alleles with increased risk of MS in women but not men. There was a trend to association of the haplotype defined by these alleles with increased risk of MS in women but not men. There was a trend to association of the haplotype defined by these alleles with increased risk of MS in women but not men.

Disclosure: S Heggarty has nothing to disclose.
being primarily due to a difference in the frequency of heterozygotes. None of the Fas polymorphisms was associated with age of onset, severity or course. 

Conclusions: Two SNPs of Fas in LD with one another and a 5’ region SNP of FasL may be associated with susceptibility to MS. In the case of the Fas SNPs, the effect was evident only in homozygous women. 

Disclosure: O Kantarci has nothing to disclose. 

Funding: National MS Society (RG-2870-A-2 ) 

P141 

ANALYSIS OF A NOVEL INTRAGENIC SINGLE-NUCLEOTIDE POLYMORPHISM OF THE FAS GENE IN RELAPSING MULTIPLE SCLEROSIS 

Lucas M*, Zayas MD*, Costa AF*, Solano F*, Durán E*, Izquierdo G* 

*aMolecular Biology, University Hospital, Sevilla, Andalucía, Spain; *bNeurology, University Hospital, Sevilla, Andalucía, Spain

Background: Interaction between Fas and FasL is a crucial mechanism for clonal deletion and immune tolerance and privilege, control of T cell expansion during immune responses and killing by cytotoxic T lymphocytes. We have recently published the association of a CA repeats polymorphism of the FasL gene to relapsing multiple sclerosis. 

Objectives: The purpose of the present work was the search of intragenic markers of Fas since a polymorphic marker could be very useful in the study of the hypothetical association of the Fas death system to multiple sclerosis. 

Methods: The groups consisted of 172 healthy unrelated and 195 relapsing MS patients of Caucasian origin. The unknown intronic sequence of intron IV of the Fas gene was amplified with primers designed from the flanking sequences and the sense and antisense strands of the amplified DNA were sequenced. SNP is detectable by endonuclease restriction analysis with Mael that easily allowed the screening of a large number of DNA. 

Results: The sequencing of both the sense and antisense strands of a 907 bp stretch, within the unknown sequence of intron IV of the Fas gene, identified a novel single-nucleotide polymorphism (SNP) A/T(735)G/C. By the endonuclease restriction procedure, we determined the allele frequencies in 344 chromosomes of healthy individuals and 390 chromosomes of relapsing MS patients. We found a higher frequency of the heterozygous TC genotype in MS patients than in healthy controls. The Fas/Mael polymorphism was not associated to the Class II HLA DRB1*1501 and DQB1*602 alleles. A small but significant association was detected among the intronic polymorphism Fas/MaeI and the Class II HLA DRB1*1501 and DQB1*602 alleles. 

Conclusions: This polymorphism could be useful for examining latent associations between specific alleles and MS susceptibility. 

Disclosure: M Lucas has nothing to disclose. 

Funding: Supported by FIS 01/0108-04. 

P143 

RANTES AND CHEMOKINE RECEPTOR 5 POLYMORPHISMS: SUSCEPTIBILITY TO AND OUTCOME IN MULTIPLE SCLEROSIS 

Partridge JM*, Fryer A*, Olier W*, Boggild M*, Strange R*, Hawkins C* 

*MS Research Group, School of Medicine, Keele University, Stoke-on-Trent, ST4, United Kingdom; *ABC Epidemiology Unit, Manchester University, Manchester, M13, United Kingdom; *Walton Centre for Neurology and Neurosurgery, Liverpool, L9, United Kingdom

Background: MS is a T cell dependent inflammatory disease of the CNS. Chemokines are molecules involved in leucocyte recruitment and activation of inflammation in the CNS. Chemokine receptor 5 (CCR5), a major receptor for the chemokine RANTES, is expressed in normal CNS tissue. The receptor is overexpressed in infiltrating lymphocytes, particularly the proinflammatory Th1 type important in MS pathogenesis. RANTES is a chemoattractant for Th1 cells in MS patients due to CCR5 overexpression. This effect can be blocked using anti-CCR5 antibodies. Both RANTES and CCR5 demonstrate functional polymorphisms; the CCR5 Δ32 is a truncated allele of the gene that encodes a non-functional receptor while the –403 G-A substitution in the RANTES promoter is associated with an 8-fold increase in transcriptional activity. 

Objectives: We performed candidate gene association studies to determine whether: (a) CCR5 Δ32 confers protection from MS or reduced severity, (b) RANTES –403 G-A is associated with MS susceptibility and outcome and, (c) combinations of these genotypes are important. 

Methods: 346 patients and 204 controls of Northern European origin were recruited. DNA was extracted from leucocytes and PCR-based assays were used to identify the CCR5 and RANTES polymorphisms. Outcome was assessed with Kurtzke’s EDSS. Cases were stratified into mild/moderate (EDSS 0-5.5) and severe disability (EDSS 6-10) after disease duration of 10 years. Results were analysed using logistic regression to correct for independent covariants of age of onset, gender and disease duration. Significance levels were set at p<0.05. 

Results: Significant association was found between possession of the –403 A allele of RANTES and MS (OR = 1.54, 95% CI 1.03-2.29, p = 0.03). No association was seen between CCR5 Δ32 and MS when considered alone. A com-
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been performed, showing no role for this polymorphism. However only few studies focused on the role of the ApoE genotype in disease severity and progression and these studies have shown contradictory results.

**Objectives:** To study the relation of the ApoE genotype with disease characteristics in a large number of MS patients, using both clinical and MRI measures.

**Methods:** In a group of 422 patients with clinical definite MS, demographic and clinical findings were recorded and related to the ApoE genotype. In a large sub-group of these patients both cross-sectionally as well as longitudinally obtained MRI parameters were related to the ApoE genotype. In addition the ApoE genotype was determined in a group of 144 healthy controls.

**Results:** No significant difference was found in the distribution of ApoE genotypes in MS patients and controls. Disease characteristics (including age of onset and onset type), disease severity (progression index, time to reach EDSS 6) and MRI findings (lesion volumes and atrophy measures) were not significantly influenced by the ApoE genotype.

**Conclusions:** In this cohort the lack of impact of the ApoE genotype on disease susceptibility was confirmed. Moreover we did not find a relation between the ApoE genotype and disease characteristics, disease severity and progression as determined both clinically and by follow-up MRI scans.

**Disclosure:** J Aoaolu has nothing to disclose.

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**Immunotherapy (Part 1)**

**P148**

**MITOXANTRONE THERAPY IN PROGRESSIVE MULTIPLE SCLEROSIS**

Afdholu J, Ermir C, Gur M, Morali S, Tank O

**SSK Okmeydanı**, Istanbul, Turkey

**Background:** Mitoxantrone (MTX) is an immunosuppressive agent recently approved by the FDA for worsening multiple sclerosis (MS) patients.

**Objectives:** The aim of this study was to evaluate the clinical efficacy and safety of MTX in progressive MS patients.

**Methods:** 19 patients (13 females, 6 males, 24-60 years of age range) with a clinical and/or MRI active disease and a confirmed increase of disability in the previous year were included into the study. And we are still continuing the study. All the patients had complete blood count, hepatic function tests and echocardiographic evaluations before the first and third administrations.

**Results:** All the patients are treated with single administrations of MTX at the dose of 12 mg/m² of body surface. The mean interval of administrations is 6 weeks and mean follow up is 12 weeks (range 4-24 weeks). The treatment was well tolerated in most of the patients. Until now EDSS was stable in all patients, and neither improvement nor progression was observed in baseline EDSS.

**Conclusions:** Until now our results seems to confirm the safety of and tolerability of MTX in progressive MS patients. At least clinically no progression was observed; this seems promising to us.

**Disclosure:** J Zwemmer has nothing to disclose.

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**P149**

**This abstract was also presented at the platform.**

**P150**

**BIOAVAILABILITY OF THREE INTERFERONβ PREPARATIONS: A 96 HOURS TIME-COURSE STUDY.**


**Neurological Clinic H. S. Luigi, Regional Multiple Sclerosis Centre, Orbassano, Turin, Italy**

**Background:** MxA is an antiviral protein induced by type I IFNs. Quantification of MxA mRNA in PBMC of MS patients is an appropriate method to measure IFNβ bioavailability.

**Objectives:** To assess the bioavailability of the 3 IFNβ formulations available for the treatment of MS, during 96 hours time-course, considering NABs presence and regimen administered.

**Methods:** A new qe-PCR method was used to quantify MxA mRNA in PBMC of 99 treatment-naïve and 45 IFNβ-treated MS patients (13 Avonex, 11 Betaseron, 21 Rebif). During 96h time-course study, MxA was evaluated in treated-patients at time 0, 24, 48, 72 and 96h. Avonex was administered at +12, whereas Betaseron and Rebif at +12 and +60. Patients were RR-MS, none of them had switched the type of IFN treatment and no concurrent steroids treatment was administered during the study. Every 3 months, serum NABs were studied by CPE assay. Two categories of pts were identified: NABs+ (n=31), NABs (±2 consecutive positive samples, n=10) and isolated-NABs+ (only 1 positive sample, n=4) pts.

**Results:** Untreated pts showed MxA mean value of 36±32 fgMxA/pgGAPDH; range 1-160 and an upper normal threshold was established (mean±SD=132 fgMxA/pgGAPDH). 31 NABs- pts (9 Avonex, 8 Betaseron, 14 Rebif) showed similar bioavailability at +24 and +48h, but a significant higher bioavailability in Betaseron and Rebif than Avonex at +0, +72 and +96h. Ten persistent NABs+ pts (2 Avonex, 2 Betaseron, 6 Rebif) showed that IFNβ injections always failed to increase MxA levels. NABs+ time-course area under the curve was significantly higher than NABs+ independently from the type of IFN.

**Conclusions:** The more frequent administration of Betaseron and Rebif provided a significant greater 96 hours bioavailability in NABs+ patients compared to patients treated once a week with Avonex. Otherwise, no differences were
observed in persistent NABs+ patients in whom, although a more frequent administration, NABs presence abolished IFNβ biological effect.

Disclosure: A Bertolotto has nothing to disclose.

Funding: Supported by Serono Pharma, Dompe Biotech, Associazione Ricerca Biomedica, Fondazione Cavaleri Ottolenghi.

P151
USE OF INTERFERON BETA 1B IN SECONDARY PROGRESSIVE MULTIPLE SCLEROSIS IN ARGENTINA


Background: The beneficial effects of Interferon β1b (IFN β1b) in the secondary progressive multiple sclerosis (SPMS) were studied in two different trials. The European study showed a significant delay in the Expanded Disability Status Scale (EDSS) progression, the American study could not achieve an statistical difference in this parameter.

Objectives: We evaluated the effect of IFNβ1b in SP-MS and compared our results with published data.

Methods: This was an open, multicentric, retrospective, observational 2 years study. 69 SP-MS patients with EDSS scores of 3.0-6.5 receiving 8 MIU IFN β1b every other day s.c. Primary objective was to assess the effect of IFN β1b therapy on the EDSS progression. The statistical method for the primary objective was paired t test.

Results: Mean age (SD, years): 47.1 ±7.03; female: 61.8%; median disease duration (years): 11.65 (6-16.7); median conversion time to SP-MS: 7.2 (4.3-12.7); median EDSS at baseline: 5.5 (4.5-6.0); median EDSS at endpoint: 6.0 (4.25-6.5). The mean number of relapses 2 years previously was 0.971, 88% of the patients were free of relapses at the first year of treatment and 80% at the second year. 75.4% of the patients presented adverse effects, but 62.3% of them were mild. Non-steroidal anti-inflammatory drugs or paracetamol were used to reduce flu-like symptoms. Systemic steroid treatment was standardised. Reason for dropping out or stopping was analyzed.

Conclusions: The EDSS showed a lower progression after IFN β1b treatment in SPMS patients. The number of relapses also decreased after treatment. These findings are consistent with previous studies and support the usefulness of IFN β1b treatment in SPMS.

Disclosure: Sotelo H. MD is product manager Schering Argentina.

Funding: Supported by Schering Argentina.

P152
RELAPSING REMITTING MULTIPLE SCLEROSIS THERAPY EXPERIENCE IN ARGENTINA


Background: In 1998 Kappos, L. compared retrospectively the four pivotal studies, (IFNβ- 1a: IM (AV), IFN β1-a SC (R44), IFNβ1b (BF) and glatiramer acetate (CO)) therapies for relapsing remitting multiple sclerosis (RR-MS).

Recently Khan, O. reported the first prospective comparative study between Immunomodulating therapies (IMT)

Objectives: To evaluate the effect of IMT (AV, R44, BF and CO) vs. a non-treated (NT) group of patients on the relapse rate in RR-MS patients, and compared our results with published data.

Methods: We included 134 patient(s): (AV: 26p, BF: 2p, R44: 20p, CO: 3p, NT: 38p, with definite RRMS in this retrospective and observational multicenter study. The statistical analysis was performed with Epil6.04 Database.

Results: Treatment data were: Median time of treatment (days): AV: 514(448-808), R44: 503.5(346-754), BF: 800.5(406.5-1352), CO: 1211(743-1488). Median number of relapses: AV: 0(0-1), R44: 0(0-1.5), BF: 0.5(0-1), CO: 0(0-1), NT: 2.2. Annual rate of relapses: AV: 0.32, R44: 0.48, BF: 0.29, CO: 0.20, NT: 0.44. Last EDSS median/ mean EDSS difference: AV: 1.5(1.4-4.0)/28.088), R44: 1.5(1.25-3.25)/0.2(0.5), BF: 2.5(1.25-5)/0.12(0.99), CO: 1.75(1.35-4.2)/0.5, NT: 1.5(1.25-4)/0.2(0.5).

Conclusions: The results indicate that treatment with IMT is beneficial compared to no treatment. Our data are similar to those observed in the pivotal studies involving the four agents after 1 year of therapy. We are aware of the study design limitations, but this is the first report in Latin America.

Disclosure: A CARRA has nothing to disclose.

P153
BBR2778, A NEW NON-CARDIO TOXIC DRUG STRUCTURALLY RELATED TO MITOXANTRONE, REDUCES THE SEVERITY OF RAT ACUTE AND CHRONIC EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS.

Cavaletti Gr, Frigo M, Rota S, Stanzani L, Tredici G, Dassi M, Perseghini P, Lolli F, Mazzanti B, Biagioli T, Cavaletti E, Pezzoni G, Sala F, Crippa L, Di Luccio E, Sala V, Oggioggi N, Riccio P, aClinica Neurologica, Univ Milan Bicocca, Monza, MI, Italy; bGerardo Hosp, Monza, MI, Italy; cUniv Florence, Florence, Italy; dNovapharma, Monza, MI, Italy; eUniv Basilicata, Potenza, PZ, Italy

Background: MS treatment with mitoxantrone (MTX) is often limited by its cardiotoxicity.

Objectives: To examine the effect of the MTX-related non-cardiotoxic drug BBR2778 in EAE models.

Methods: Chronic EAE (induced in Lewis rats after spinal cord homogenate sc injection): 30 rats were iv treated on days 14, 21, 27 and 34 as follows: group 1: saline; group 2: BBR2778 16.25 mg/kg/day; group 3: MTX 1.2 mg/kg/day. Acute EAE (induced in Lewis rats after myelin basic protein [MBP] sc injection): 40 rats were divided into 2 groups and were iv treated with BBR2778 32.5 mg/kg/day on days 7 and 4 or were left untreated. Five rats/group were sacrificed on days 10, 14, 23 and 41.

Results: Chronic EAE: both MTX and BBR2778 induced a marked reduction in total WBC and lymphocytes count vs. untreated EAE rats. During the 72-day observation period the incidence of relapses was significantly lower in the BBR2778 group vs. untreated EAE group (p = 0.028). Anti-MBP Ab were significantly lower in the BBR2778 and MTX groups vs. controls (p = 0.016). In most of the BBR2778 rats the in vitro studies evidenced a reduced proliferation of spleen lymphocytes. Despite each daily dose was equal to 1/4 of the single LD10 of BBR2778 and MTX, histopathology confirmed that MTX was markedly cardiotoxic while BBR2778 did not induce any relevant cardiotoxic change. Acute EAE: hematological determinations evidenced a reduction in CD3+, CD4+ and CD8+ cells vs. untreated EAE rats, but the most impressive result was an extremely marked reduction in CD45+ cells. Accordingly, anti-MBP Ab titers were lower in the BBR2778 group. The clinical signs of EAE were significantly less severe in BBR2778-treated (p = 0.021, day 14). In vitro proliferation tests demonstrated that BBR2778 treatment markedly reduced the reactivity of spleen lymphocytes to MBP.

Conclusions: Although further pre-clinical studies are still needed, our data are a proof-of-concept in favor of a possible future role of BBR2778 in the treatment of MS.

Disclosure: G Cavaletti is a consultant for Novapharma
Mitoxantrone is highly effective in the treatment of active multiple sclerosis (MS). Only few data are available concerning immunological mechanisms.

Objectives: We investigated the effects of mitoxantrone on proliferation and cell death of peripheral blood leukocytes from MS patients.

Methods: Peripheral blood derived mononuclear cells (MNC) were obtained from 29 active MS patients (mean age 38.8 yrs., m: f 1: 1.4) before and immediately after 1h mitoxantrone infusion. Isolated MNC were cultured for 24h with and without phytohemagglutinin activation (PHA, 5μg/ml). Proliferation was measured by 3H-thymidine uptake. Cell death was analysed flow-cytometrically using Annexin-V-propidium double staining (Ann/Pi).

Results: Mitoxantrone decreased proliferation of MNC in 24/26 patients in comparison to control MNC from the same patient before mitoxantrone application (58 ± 22 % of controls, p< 0.001). Mitoxantrone induced late apoptotic/necrotic changes in MNC of 23/28 patients (p< 0.01), increasing the proportion of Ann/Pi + cells by 16% (p< 0.01). In PHA-stimulated MNC this increase was more pronounced (21%, p<0.001). Subpopulation analyses (CD3, 4, 8, 14, 19, 25, 56) performed in 7de novo mitoxantrone-exposed patients so far revealed no significant differences in susceptibility towards apoptosis.

Conclusions: Our data indicate that already a short 1h in vivo exposure to mitoxantrone induces a profound suppression of proliferative responses in MNC of MS patients. This suppression appears to be mediated by induction of apoptotic/necrotic cell death.

Disclosure: A Chan has nothing to disclose.

P155

EPITOPE SPECIFICITY OF NEUTRALIZING ANTIBODIES AGAINST INTERFERON-BETA

Gneiss C, Reindl M, Berger T, Deisenhammer F
Neurology, University of Innsbruck, Innsbruck, Tyrol, Austria

Background: Neutralizing antibodies (NAB) - a subset of antibodies against interferon-beta (IFNb) - are supposed to bind to the receptor binding residues of the IFNb molecule. This has, however, not been proven yet for human NAB.

Objectives: To analyze the epitope-specific binding pattern of NAB and non-NAB to the IFNb molecule.

Methods: Thirty-one 12-mer peptides and one 11-mer peptide with an offset of 5 amino acids representing the amino acid sequence of the human IFNb molecule (Mimotopes, France) were used as antigens in an ELISA antibody assay. Sera of 72 MS patients (37 with NAB and 35 with non-NAB) were investigated.

Results: A significant difference of peptide binding between NAB and non-NAB was found at residues 1-12, 121-132, and 151-162. The strongest binding was observed between NAB and residues 1-12 and there was a highly significant positive correlation between NAB titers and titers against residues 1-12. Binding to residues 1-12 was more pronounced in patients on IFNb-1a than in patients on IFNb-1b.

Conclusions: Although interpretation of the results is limited due to linear epitopes there is a difference between NAB and non-NAB with respect to binding to amino acid sequences at the IFNb molecule. The difference between IFNb-1a and IFNb-1b treated patients supports our observation that residues 1-12 play an important role in NAB binding since IFNb-1b lacks methionin at position 1. Several consequences may be considered: (1) Binding to residues 1-12 might be used as simple screening test for NAB; (2) Substitutions or deletions at residues 1-12 (especially at residues I-5 since there was no binding to residues 6-17) might reduce the antigenicity of recombinant IFNb. If such a molecule was biologically active this would be an interesting opportunity for future IFNb drug designs.

Disclosure: M Eraksoy has nothing to disclose.

P156

IMMUNOMODULATORY THERAPIES IN FAMILIAL MULTIPLE SCLEROSIS

Eraksoy M, Turan N, Kurtuncu M, Kyiat A, Yapiç Z, Akman-Demir G
Neurology, Istanbul University, Faculty of Medicine, Istanbul, Turkey

Background: Although the cause of multiple sclerosis (MS) remains uncertain, recent evidence has revealed that both genetic and environmental factors determine susceptibility. Familial aggregation of MS was found to be 5-20%. There has been some debate whether familial cases are separate entity or unsuitable for entry into clinical trials of therapeutic agents.

Objectives: The aim of this study was to determine the demographics, outcome and benefit from treatment with immunomodulatory drugs of patients with familial multiple sclerosis. The results were compared with 1596 sporadic MS patients in whom 256 have been using immunomodulatory therapies.

Methods: The demographics, clinical findings and responses to immunomodulatory agents (IFNb and glatiramer acetate) of 44 patients with familial MS were evaluated in a hospital-based cohort in Istanbul.

Results: The familial frequency of MS was 5.3% (n=175) of total 1755 patients with MS at April 2002. The familial MS patients were the members of 94 families and this group was divided arbitrarily into the following three groups: sibling pairs, parent-child pairs and second, third relatives. Consanguinity was seen in fourteen families. The age at onset was 29.5 yrs. Female/male ratio was 1.7/1. Most of the patients (45%) were seen from onset of the disease. The most common initial manifestations were sensory (45%), brain stem (20%) and optic neuritis (15%). According to Poser and co-workers’ criteria 94% of the patients was clinically definite MS. Relapsing-remitting course was seen in 56% of 175 patients. The mean duration of disease was 11 yrs, and Kurtzke’s score was 3.0 at the last follow-up. The mean follow-up was 9 yrs. Of 175 (25%), 44 patients have been receiving immunomodulatory therapy at least 1 yr.

Conclusions: In conclusion, familial MS patients resembled those remaining sporadic in both clinical, demographic characteristics, outcome and responses to immunomodulatory therapies.

Disclosure: M Eraksoy has nothing to disclose.

P157

CAMPATH-1H IN THE TREATMENT OF MULTIPLE SCLEROSIS PATIENTS

Le Page E, Amanda Louise C, Coles A, Denys V, Miller D, Hale G, Waldfmann H, Compston A

*NMRI Unit, Institute of Neurology Queen Square, London, united kingdom; 'Therapeutic Antibody Center, Oxford, united kingdom, United Kingdom

Background: Campath-1H, a humanised anti-leukocyte (CD52) monoclonal antibody previously assessed in the treatment of patients with secondary progressive multiple sclerosis (SPMS), did not alter disease progression (Coles et al., 1999).

Objectives: To assess efficacy in patients with early active relapsing-remitting MS.

Methods: This is a retrospective study of 47 MS patients treated by Campath-1H: cohort I 36 SPMS, cohort II 11 cases treated before confirmed progression. Patients were evaluated every 3-6 months to record EDSS,
new relapses and lymphocyte sub-populations to monitor the depletion induced by Campath-1H. Serial MRIs were performed in 20 patients from cohort I.

**Results:** Cohort I: Campath-1H was started 12 and 4 years (means) after disease and progression onset, respectively. The mean duration of follow-up was 6 years. The majority of patients showed progressive disability within the first 18 months or 2-5 years after treatment in those who were initially stabilised. Cohort II: Campath-1H was given 1.7 years (mean) after disease onset. Although cases were selected for active relapsing-remitting disease a progression was subsequently confirmed in 3 patients (onset 4-8 months before treatment). Mean number of relapses was 3/patient in the year before treatment and mean EDSS changed by 2.5 points. The mean duration of follow-up was 12 (3-25) months in the 8 RRMS patients. Three of them had a single relapse 12, 12 and 15 months after treatment (without change in EDSS) representing a change in annualised relapse rate from 2.9 to 0.36. Of the 3 with SPMS, one continued to worsen progressively, one had a single relapse at 18 months with increased disability and the third remained unchanged after 3.5 months.

**Conclusions:** This study strengthens the evidence for greater efficacy of Campath-1H at an early phase of multiple sclerosis, when disease activity is attributable to inflammation. These principles are now being adopted in a multi-centre randomised trial comparing Campath-1H with interferon beta.

**Disclosure:** E Le Page has nothing to disclose.

**P158**

INTERFERON BETA INHIBITS MONOCYTE-DERIVED DENDRITIC CELL MATURATION

McClurg AEa, Fleming EMb, Hawkins SAa, Daddy MEa, McQuaid Sa, Johnston JAc, Armstrong MAa

aMicrobiology & Immunobiology, Queens University, Belfast, Northern Ireland, United Kingdom; bMedicine, Queens University, Belfast, Northern Ireland, United Kingdom; cPathology, Queens University, Belfast, Northern Ireland, United Kingdom

**Background:** MS has a wide range of presentations and a spectrum of clinical courses, which is characterised by multifocal CNS damage, postulated to be mediated by CNS antigen-specific T cells. Dendritic cells (DC) are the most potent antigen presenting cell and are unique in their ability to initiate primary immune responses. They are thought to play a pivotal role in the decision between T cell activation and anergy. We have identified mature CD83+ DC in active MS plaque tissue, and have shown in *in vitro* studies that IFN-β, a disease-modifying drug used in the treatment of MS, can interrupt the differentiation pathway of peripheral blood monocyte-derived DC.

**Objectives:** To investigate the role of myeloid DC in MS. To determine if the efficacy of IFN-β is due to its ability to affect DC maturation.

**Methods:** Monocytes were separated from whole blood and cultured for six days with GM-CSF and IL-4 to induce differentiation to dendritic cells. IFN-β was added at a physiological concentration. Maturation was then induced by addition of LPS, at different concentrations, for varying times. Expression and induction of CD83, CCR3 and CCR7 was studied by flow cytometry. The LPS pathway was examined by western blotting for IκBα activation.

**Results:** The resultant cell after IFN-β addition is intermediate in size between a monocyte and a DC, and expresses CD14++ , CD1a++, CD40+, HLA-DR+ and CD16++, in comparison to immature monocyte-derived DC which are CD14+, CD1a++, CD16, CD40, HLA-DR+. Culture of the intermediate-type cells with different concentrations of LPS, which stimulates immature monocyte-derived DC to mature and upregulate CD83, did not change their phenotype to either mature DC or macrophages.

**Conclusions:** The inability of LPS to stimulate these intermediate cells may mean they are anergic and unable to potentiate the immune response. The ability of this drug to disrupt DC maturation may contribute to its efficacy as a disease-modifying drug in MS.

**Disclosure:** A McClurg has nothing to disclose.

**Funding:** Supported by Biogen.
(4.6%) and 3 (2.8%) patients. Reversible menstrual abnormalities were reported by 2 (3.2%) women while definitive amenorrhea occurred in 4 (6.4%) patients, all over 35 years. Finally, transient hair loss was observed in 2 (1.9%) cases. Lymphopenia occurred in 17 (15.7%), increase of hepatic enzymes in 12 (11%) and hypogammaglobulinemia in 6 (5.6%) patients. A mammary and a basal cell carcinoma were diagnosed in 2 (1.9%) women, both previously treated with azathioprine. Finally, 67% of the patients judged the treatment regimen as acceptable and well tolerated.

Conclusions: Our findings point to a reasonable safety and tolerability of pulse therapy with CPM. Further trials are needed to define the efficacy of CPM as a therapeutic option for the most active forms of MS.

Disclosure: E Portaccio has nothing to disclose.

P161
EXPERIENCES WITH MITOXANTRONE TREATMENT - SIDE EFFECTS IN SECONDARY CHRONIC PROGRESSIVE MULTIPLE SCLEROSIS PATIENTS
Rajda C, Bencsik K, Torok M, Vecsei L
Neurology, University of Szeged

Background: Mitoxantrone an antineoplastic agent, often characterized as an immunosuppressant drug, has been approved for treating patients with secondary chronic progressive multiple sclerosis. Recent studies have been shown an ameliorating effect on disease activity, indicated by MRI and clinical data.

Objectives: Side effect and drug interactions in patients with multiple sclerosis are limited.

Methods: We report on 15 secondary chronic progressive multiple sclerosis patients treated with 20mg mitoxantrone every third month in a prospective 9-month study (Sept., 2001 - April, 2002). The male: female ratio was 4: 11, mean duration of disease was 13 years. The initial EDSS score was between 5.5 and 8.

Results: After the mitoxantrone treatment 9 patients were stabilized, 3 patients showed improvement in the EDSS score, and 3 patients felt amelioration without EDSS score changes. One dropout was because of diffuse myocardial lesion, but none of the other patients exerted significant differences in the LVEF. One patient at the first treatment reported nausea, but the other two administration of mitoxantrone was without side effects. Vomiting was the complaint by 2 patients, and 1 patient complained about mild hair thinning after the 3rd administration. No drug interactions between the earlier administered drugs and mitoxantrone were found.

Conclusions: According to our data mitoxantrone treatment is well tolerated with minor side effects, decreasing disease activity, stabilizing the progression of secondary chronic progressive multiple sclerosis.

Disclosure: C Rajda has nothing to disclose.

P163
A PHASE III STUDY OF ORAL INTERFERON BETA-1A AT TWO DOSES IN RELAPSING REMITTING MULTIPLE SCLEROSIS USING MRI, CLINICAL AND IMMUNOLOGIC MEASURES.
Vollmer TL, Preiningerova J, Markovic-Plez S, Rizzo M, Cutter G
aNeurology, Yale School of Medicine, New Haven, Connecticut, USA; bUniversity of Nevada, Reno, NV, Nevada, USA

Background: Interferon Beta 1a or 1b are the most common disease modifying agents for MS (DMAMS) used today. Biologic effects of orally administered interferon have been demonstrated in animals.

Objectives: We investigated the safety and efficacy of orally administered interferon beta 1a (Avonex®) in multiple sclerosis.

Methods: A randomized, phase IIb trial assessed the safety and efficacy of two doses (0.1 MIU or 6 MIU) of oral interferon beta 1a given daily to RRMS patients. A 3-month baseline phase with monthly brain MRI was followed by a 6-month treatment phase with monthly MRI. Inclusion criteria: age 18 to 65, EDS 0 to 6.0, at least 2 relapses in the previous 3 years and at least 0.67 Gadolinium positive lesions/scan on the baseline MRIs. Primary outcome measures: number and volume of Gd-enhancing lesions. Secondary outcome measures: relapse rate, EDSS, MSFC, MS Performance Scales, Patient Determined Disease Steps, total burden of disease, number of T2 lesions and brain atrophy index. Cytokine (IL-2, INF-gamma, IL-4, TNF- alpha) production following anti-CD3 mab PBMC stimulation were measured.

Results: Twenty one subjects met study criteria (75% female, mean age 41.8 years and mean EDSS 3.7). 19 subjects completed the study. Two subjects withdrew at 3 and 5 months of treatment for reasons other than side effects. There was no treatment effect on number or volume of Gadolinium enhancing lesions in either treatment group at any time point. We observed 5 relapses/63 patient months in the baseline phase, 7 relapses/63 patient months during the first three months of treatment and 0 relapses/59 patient months in the last three months of treatment. There was a significant decrease in the production of INF-gamma (p=0.02) in comparison to the baseline period. Other cytokine responses were variable, but not clearly due to treatment. No clear treatment effects could be identified on secondary MRI or clinical outcome measures. No significant toxicities were noted.

Conclusions: Although oral interferon beta-1a appeared safe at doses of up to 6 million IU/day, we were not able to identify a definite biological or clinical effect in low or high dose groups over a 6-month treatment period.

Disclosure: T Vollmer has research grants from Biogen. Funding: Supported by Biogen.

Multiple Sclerosis
P164
PROLIFERATIVE RESPONSE TO GLATIRAMER ACETATE MAY PREDICT CLINICAL RESPONSE TO THERAPY

Weder CR, Baltarri G, Lienert C, De Libero G, Kappos L, Duda PW
Neurology, University Hospital; Experimental Immunology, University Hospital, Basel, BS, Switzerland

Background: Glatiramer acetate (GA) is effective in the treatment of relapsing-remitting multiple sclerosis (RR MS). We and others have shown GA to induce high GA specific proliferative and cytokine responses in vitro in PBMCs of all individuals tested. Proliferation is decreased and cytokines are shifted towards Th2 during treatment.

Objectives: To investigate whether there is a correlation between in vitro immunological and clinical responses to GA.

Methods: 9 RR MS patients were examined prior to and after 2, 4, 6, 12 and 14 months of standard GA treatment. Proliferative and cytokine (IL-10, IL-5 and IFNγ) responses were tested in primary assays by culturing 150,000 freshly isolated PBMCs with 1-300 µg/ml GA in 96 well plates. 5 untreated RR MS patients were also tested. Without knowledge of the immunological findings, the treated patients were subdivided into responders (R) and non-responders (NR) to GA treatment based on relapse rate, cycles of steroid treatment and EDSS change during the first year of GA therapy as compared to the year prior to treatment.

Results: 2 of 9 patients were considered clinical NR. Proliferative responses prior to treatment were comparable in treated and control patients. Immediately before the first GA injection, proliferative responses were higher in the 2 NR than in the other treated patients at all GA concentrations tested. At the intermediate GA concentration of 30 µg/ml, values were 83.885 and 91.816 cpm in the NR; the range in R was 9.161-73.616, the median 39.209 cpm. NR as well as controls had higher fluctuations of GA specific proliferative responses than R. Around relapses, proliferation was increased in most patients. No clear relation between clinical and cytokine responses was observed.

Conclusions: We hypothesize that high in vitro proliferative responses to GA prior to treatment and around relapses are likely due to a general MS related increase in inflammation. If GA is first injected in this proinflammatory environment, the desired immunomodulatory effects may be delayed or abrogated. Despite the small number of patients we have studied, our results suggest that the immunological status of patients immediately prior to initiation of GA therapy may affect clinical response to the treatment.

Disclosure: C Weder has nothing to disclose.

Funding: Supported by Swiss MS Society and Aventis Pharma.

Pathology

P165
SERUM PARAOXONASE ACTIVITY IN UNTREATED AND IFN-BETA TREATED MS PATIENTS

Neurorehabilitation Clinic General Hospital “Umberto I”, Ancona, Italy; Neurological Clinic General Hospital “Umberto I”, Ancona, Italy; Department of Biochemistry University of Ancona, Ancona, Italy; Rehabilitation Hospital “S. Stefano”, Ancona, Italy

Background: Free radical and oxidative damage have been suggested as a casual factor in the development of MS. Oxidative injury could mediate demyelination and axonal injury in MS subjects and in animal model. IFN-beta has shown to inhibit iNOS activity and suppress endogenous NO production, suggesting a protective effect against oxidative damage. Paraoxonase (PON) is an enzyme associated with plasma HDL and plays an important role against lipid peroxidation of LDL and HDL; its plasma activity is inversely related to the occurrence risk of several disease such as atherosclerosis, hypercholesterolemia and inflammation.

Objectives: To investigate the activity of PON in plasma of MS pts, in order to verify modificatio of PON activity during IFN-beta treatment.

Methods: 30 pts with defined RRMS with EDSS<=3.5 were studied. 20 pts were treated with IFN beta (group A)and 10 did not assume any drug (group B). The activity of PON in plasma of the two groups was compared with the activity of PON in 60 healty subjects (control group) matched for age and sex.

Results: The mean value of plasma PON activity in the control group was 2946 +/- 228 U/ml. PON activity was statistically lower in group B (mean value 1953 +/- 400 U/ml), whereas the difference of PON activity between group A (mean value 3113.5 +/- 425 U/ml)and controls was not statistically significant.

Conclusions: These results show that PON activity is significantly decreased in plasma of untreated pts, whereas PON activity in treated pts is similar to healthy subjects, suggesting that IFN-beta therapy could modulate the PON activity in MS pts.

Disclosure: M Danni has nothing to disclose.

Funding: Supported by Spain’s MEC FISS Program, File 00/0846 to EMMC.
**P167**

ALPHA-SYNUCLEIN IMMUNOREACTIVE DEPOSITS IN MULTIPLE SCLEROSIS LESIONS

Paramo D, Izquierdo G, Seillhaem D

- Neurology, Hospital Macarena, Sevilla, Spain
- Neuropathology, Hospital Pitié-Salpêtrière, Paris, France

**Background:** Current interest in multiple sclerosis pathology is focused in axonal damage since it is supposed to cause progression of the disability in MS patients even when inflammatory activity has diminished or disappeared and there are no relapses. Several mechanisms have been proposed to explain axonal damage, such as axonal transection during inflammatory injury and Wallerian degeneration of the transected neuraxis. We hypothesized that other neurodegenerative processes might be implied. Alpha-synuclein is thought to play a key role in several neurodegenerative disorders, such as multiple system atrophy (MSA), Parkinson disease, Alzheimer disease, etc and it has been recently found in axonal swellings of Neuaxonatxal distrophies.

**Objectives:** To study the presence of alpha-synuclein in multiple sclerosis plaques.

**Methods:** We have studied by immunohistochemistry the presence of alpha-synuclein in white matter of 10 normal subjects and in 30 multiple sclerosis plaques.

**Results:** We have found alpha-synuclein immunoreactive deposits in 80% of MS plaques, but not in white matter controls.

**Conclusions:** Deposition of alpha-synuclein in axonal swellings of plaques may imply just the presence of axonal damage, but it could suggest a new mechanism for disability progression in multiple sclerosis patients.

Disclosure: D Paramo has nothing to disclose.

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**Friday, September 20, 2002**

**Posters**

**Rehabilitation and Quality of Life**

**P168**

PRESSURE PAIN IN MULTIPLE SCLEROSIS PATIENTS

Armutlu K, Kerem M, Bumin G, Akbayrak T, Yigiter K, Keser P, Karabudak R

- School of Physical Therapy and Rehabilitation, Hacettepe University, Ankara, 06100, Ankara, Turkey
- Department of Neurology, Hacettepe University Hospitals, Ankara, 06100, Ankara, Turkey

**Background:** Pain sense is vital. Low levels of pain sense are important during everyday task to tell us that prolonged posture is putting too much strain on our body. The pain sense may be affected in early stages of neurological diseases such as peripheral neuropathy and Multiple sclerosis (MS). These types of neurological diseases, especially MS, may progressively restrict mobilisation of patients. In these patients, loss of pain sense may cause severe pressure sores.

**Objectives:** The purpose of this study was to compare Pressure Pain Threshold (PPT) and Pressure Pain Tolerance (PPTO) in subjects with Multiple Sclerosis (MS) and healthy people. PPT and PPTO in different stages in MS patients were also investigated.

**Methods:** This study included 31 subjects diagnosed as clinically definite MS and 31 healthy subjects with same age range group. Study group including subjects with MS were divided into 3 subgroups. According to Expanded Disability Status Scale (EDSS) scores between 1 and 3.5 (n=12), between 4 and n=10), between 6.5 and above (n=9) were classified as early, mild and late stages, respectively. MS patients who had trigeminal neuralgias, and whose scores below 24 in mini-mental test, were excluded from the study. Measurement of pressure pain was used by algometer. Assessments were performed on masseter muscle, bicipital tendon, thanar area, tip of middle finger and anterior proximal of thigh.

**Results:** MS duration was 8.96±6.41 years and MS types were relapsing-remitting type and primary progressive type of MS patients. There was a significant difference between MS group and control group in PPT and PPTO of masseter muscle, bicipital tendon, thanar area, middle finger tip and thigh (p=0.05). When three MS groups according to the stages compared, there was no significantly difference between the groups in these parameters (p=0.05).

**Conclusions:** Our results showed that PPT and PPTO were significantly increased in MS patients. Possibility loss of pain perception should be taken into consideration when planning physiotherapy rehabilitation programs for MS patients. Patients and caregiver should be informed for this aspect.

Disclosure: K Armutlu has nothing to disclose.

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**P170**

QUALITY OF LIFE, DISABILITY AND DEPRESSION IN EARLY MS

Deloire-Grassin M, Ouallet J, Salont E, Barroso B, Brochet B

EA 2966 (Neurobiology of Myelin Disorders Laboratory), University Victor Segalen, Bordeaux, Bordeaux Cedex, France

**Background:** Health-related quality of life (QOL) assessment seems to reflect better the broad impact of MS than disability and impairment scales (EDSS, MSFC). Little is known about the quality of life at early stage of disease.

**Objectives:** To assess the QOL in patients with MS a few weeks after the diagnosis.

**Methods:** 69 patients have been included by the AQUISEP network less than six months after a diagnosis of MS. All signed a written consent. Their characteristics were: 58 RRMS (mean age 34.62 ± 9.1); 7 PPMS (mean age 44.29 ± 8.22 years, the mean score of Expanded Disability Status Scale(EDSS) was 4.5±1.87. Handicap status was assessed with The London Handicap Scale Questionnaire(LHSQ), QOL assessed with Functional Status Questionnaire(FSQ). Similar items, which were physical activity, mobility, social activities and occupation, were examined. The correlation between EDSS and each associated questions were also examined.

**Results:** It was observed that, there was a correlation between EDSS and items of physical activities of FSQ(p<0.05), while there was no significant correlation with items of physical activities and occupation(p>0.05). When the correlation between EDSS and LHSQs items were examined, it was seen that there was a significant correlation with all items except orientation. It was observed that there was a significant relation between ADL items of FSQ and mobility and physical activities items of LHSQ, there was not a relation between occupation items of FSQ and LHSQ(p>0.05). There was not any significant correlation between FSQs social activities items and LSHQs social integration item(p>0.05).

**Conclusions:** As a result, there was a significant correlation between all handicap status and QOL items of ambulatory MS patients except social activities item. And also we thought that because of cultural differences we could not find any relation according to social activities. It can be concluded that when the handicap status and QOL levels were evaluated, the social activities seen lower than expected.

Disclosure: K Armutlu has nothing to disclose.
P171
AEROBIC TRAINING IN MULTIPLE SCLEROSIS - INFLUENCE ON METABOLIC, ENDOCRINE AND QUALITY OF LIFE PARAMETERS

Heesen Ca, Gold SMb,c, Mladek Mb, Bartsch Kd, Hartmann Sb, Ludwig A,c, Witte Jb, Reer Rb, Braunmann Kc, Schulz Kc

aNeurology, University Hospital Eppendorf, Hamburg, Germany; bMedical Psychology, University Hospital Eppendorf, Hamburg, Germany; cSports Medicine, University of Hamburg, Hamburg, Germany; dSports Medicine, University of Halle, Halle, Thueringen, Germany

Background: In contrast to the earlier view that physical exercise may potentially exacerbate symptoms in MS and should thus be avoided, aerobic training has recently been suggested as a therapeutic intervention. It is however not known which groups of patients may benefit from such programs. Furthermore, metabolic and endocrine responses to acute physical exercise in MS have not been studied.

Objectives: To examine the effects of aerobic exercise on metabolic, endocrine and quality of life parameters in MS.

Methods: We investigated acute effects of a step-by-step bicycle ergometry and a continued exercise with 60% VO2-max in MS patients and healthy controls. Furthermore, MS patients received an eight week low-level training and a continued exercise with 60% VO2-max in MS patients and healthy controls. Furthermore, MS patients received an eight week low-level training.

Results: A reduction of more than 30% of QOL was observed for 10 out of 15 scores of QOL in RRMS patients and all 15 scores in progressive patients at the time of diagnosis. The lowest scores were obtained for the following scores: role limitation physical, energy, sleep, health perception. EDSS and each KFS were correlated with different items of QOL but MSFC is only correlated with physical functions and health distress scores. Regression analysis revealed that the overall QOL is independently associated with MADRS score, KFS mental (reflecting mainly cognitive complaints) and EDSS score which is mainly an impairment scale at the stage of the disease. Overall QOL was not associated with MSFC.

Conclusions: Health-related QOL is strongly affected at early stages of MS. Depression, cognitive complaints and neurological impairment are independent predictors of overall QOL.

Disclosure: B Brochet has nothing to disclose.
Funding: Supported by ARSEP and Schering France SA.

P172
ONE-YEAR CHANGES ON THE MS FUNCTIONAL COMPOSITE AND PATIENT-PERCEIVED DISABILITY

Hoogervorst E, Kalkers N, Uitdehaag B, Polman C

Neurology, VU Medical Centre, Amsterdam, The Netherlands, Netherlands

Background: The MS Functional Composite (MSFC) is an outcome measure comprising three quantitative tests of: leg function (Timed Walk Test [TWT]), arm/hand function (9-Hole Peg Test [9-HPT]) and cognition (Paced Auditory Serial Additon Test [PASAT]). The Guy’s Neurological Disability Scale (GNDS) is a questionnaire divided in 12 subcategories, directed to assess a patient’s disability in the previous four weeks, is based on patient-self report and is driven by patient interview.

Objectives: The aim of this study was to prospectively characterise the relation between one-year changes in functional impairment (MSFC) and changes in the patient’s own perceived disability (GNDS) and their corresponding components.

Methods: Two hundred and ninety MS patients underwent MSFC and GNDS examinations on the same day at baseline and 1-year follow-up. We studied correlations between change in MSFC and GNDS and their corresponding components. In addition we studied the change in total number of GNDS-subcategories with scores 3 or higher (indicating disability for which help by others is required) per quartile of MSFC change.

Results: Mean MSFC at baseline was 0.14 and at follow-up 0.086, median GNDS-sum scores were equal at baseline and follow-up, 14.0. Good cross-sectional correlations were found between MSFC and GNDS at baseline (+0.60) and follow-up (+0.63), whereas no significant correlation was found between change in MSFC and GNDS (+0.06). Weak to marginal correlations were found between change in the corresponding components: TWT vs. lower limb function r = -0.22 and 9-HPT vs. upper limb function r = -0.12. No correlation was found between change in the PASAT and cognition. Analysing the number of GNDS subcategories with scores 3 or higher per quartile MSFC change, indicates that there is a profile of more pronounced worsening on the MSFC being associated with greater increase in number of GNDS subcategories for which help is required and that vice versa those who have improved on the MSFC have the lowest increase.

Conclusions: Our longitudinal data suggest that one-year changes in the MSFC, especially with respect to leg and arm function, are associated with changes in disability as perceived by the patient.

Disclosure: E Hoogervorst has nothing to disclose.

P173
HIGH LEVELS OF ANXIETY AND DISTRESS IN MS PATIENTS AND THEIR PARTNERS IN THE FIRST YEARS AFTER DIAGNOSIS

Janssens C, van Doorn P, de Boer P, van der Meche F, Passchier J, Hintzen R

aNeurology, Erasmus MC, Rotterdam, Netherlands; bMedical Psychology and Psychotherapy, Erasmus MC, Rotterdam

Background: Shortly after diagnosis, MS patients and their partners have to deal with many uncertainties and potential serious disability. This may prolong the uncertain and stressful period that had already started before disclosure of diagnosis. It is unknown how stressful this period is.

Objectives: To evaluate the psychological impact of MS for patients and partners in the first years after diagnosis.

Methods: Quality of life (SF-36), anxiety and depression (HADS) and disease-related distress (IES) were assessed in 101 recently diagnosed (< 2 years)
P174
THE MULTIPLE SCLEROSIS QUALITY OF LIFE INVENTORY AND DISABILITY AS MEASURED BY THE EDSS: EXPERIENCE IN A BRAZILIAN MULTIPLE SCLEROSIS SAMPLE.
Lana-Peixoto MA, Araujo CR, Haase VG, Lacerda SS, Lima EP
CIEM MINAS UFMG Center for Investigation of Multiple Sclerosis, Brazilian Committee for Treatment and Research in Multiple Sclerosis, Belo Horizonte, MG, Brazil

Background: The Multiple Sclerosis Quality of Life Inventory (MSQLI) is a broad outcome assessment battery which includes not only evaluation of physical capabilities but also a variety of quality of life goals. The EDSS on the other hand is a disability scale that predominantly evaluates gait. The relative strength of the physical component of the outcome on the quality of life can be assessed by correlating the MSQLI with the EDSS.

Objectives: To correlate the MSQLI with the EDSS in a sample of Brazilian MS patients.

Methods: The MSQLI consists of a set of separate scales that assesses 10 domains from the patient’s perspective. It was individually administered to a group of 39 CDMS patients. The EDSS score was assigned by the clinic neurologist.

Results: Thirty-nine CDMS patients (27 women and 12 male) were selected at random from our MS Research Center. Their mean age was 42.08 years (sd=9.1), mean formal schooling was 10.23 years (sd=4.25) and the mean disease duration 8.86 years (sd=7.83). There were 23 patients with relapsing-remitting MS, 11 with secondary progressive MS and five with primary progressive MS. The median EDSS was 3.5 and the mean Ambulation Index (AI) 1.9 (sd=2.51). The patients scored poorly in some domains such as visual, bladder and bowel control, cognition, sexual satisfaction and pain (indicating better functioning or less pain). The SF-36 Functional Physical Scale, SF-36 Bodily Pain Scale, SF-36 General Health Scale, Modified Fatigue Impact Scale, Pain Effect Scale, Bladder Control Scale, Bowel Control scale and Impact of Visual Impairment Scale significantly correlated with the EDSS while the Perceived Deficits Questionnaire, the Mental Health Inventory and the Modified Social Support Survey did not correlate with the EDSS score.

Conclusions: As EDSS primarily measures ambulation it shows poor correlation with the scales that measure cognition, mental health, social support, sexual satisfaction and emotional aspects. Although quality of life is affected by physical disability the EDSS fails to give a real account other important domains in human life.

Disclosure: M Lana-Peixoto has nothing to disclose.

P175
PREVIOUS HISTORY OF OPTIC NEURITIS AS A FACTOR FOR DECREASED VISION-SPECIFIC QUALITY OF LIFE IN MULTIPLE SCLEROSIS.
Lana-Peixoto MA, Martins R, Haase VG, Lacerda SS
CIEM MINAS UFMG Center for Investigation of Multiple Sclerosis, Brazilian Committee for Treatment and Research in Multiple Sclerosis, Belo Horizonte, MG, Brazil

Background: Health-related quality of life (HRQOL) is markedly influenced by disturbances of vision and vision-specific quality of life (VSQOL) is decreased in MS patients. Experience of the ONTT revealed that patients with history of optic neuritis have decreased VSQOL.

Objectives: To assess the role of positive history of optic neuritis in the decreased VSQOL in MS patients.

Methods: VSQOL was measured by the National Eye Institute Visual Function Questionnaire 25-item version (NEI-VFQ-25). Mean scores were calculated for each subscale and for the composite score. Vision tests included corrected visual acuity, contrast sensitivity, color vision and automated perimetry. Only patients with CDMS and no history of other ophthalmic disorder were included. The cohort was divided into two groups: Group I comprised MS patients with previous history of optic neuritis; and Group II consisted of MS patients with no history of optic neuritis.

Results: There were 19 patients in Group I and 20 in Group II. MS was relapse-remitting in 23, secondary progressive in 7 and primary progressive in 8. In Group I there were 16 women and 3 men; the median age was 44 years, and median EDSS was 4.0. In Group II there were 10 women and 10 men with median age of 40.5 and median EDSS of 3.0. Visual acuity in Group I ranged from 20/20 to NLP (median 20/20); in Group II it ranged from 20/15 to 20/40 (median 20/20). Twenty patients had color vision disturbance (13 in Group I and 7 in Group II) and contrast sensitivity function was abnormal in 17 patients in Group I and 5 patients in Group II. The NEI-VFQ-25 Composite Score was 82.9 (SD 23.2) (median 92.9) in the whole cohort, 72.9 (SD 28.9) (median 82.8) for patients in Group I, and 92.5 (SD 9.2) (median 96.7) in Group II. Comparison between score in the MS cohort and the Reference Group showed that the composite score and scores in most of the subscales were significantly lower in patients with MS. The NEI-VFQ-25 scores were not significantly different in MS patients with or without previous history of optic neuritis.

Conclusions: Previous history of optic neuritis seems not to play a role in the decreased VSQOL in MS patients.

Disclosure: M Lana-Peixoto has nothing to disclose.

P176
ASSESSING THE VALIDITY OF THE MULTIPLE SCLEROSIS IMPACT SCALE IN A COMMUNITY BASED POPULATION.
McGuigan C, McCarthy A, Hutchinson M
Dept. of Neurology, St Vincents, Dublin, Co. Dublin, Ireland

Background: The Multiple Sclerosis Impact Scale (MSIS) is a validated outcome measure of the physical (MSIS 20) and psychological (MSIS 9) impact of MS. It’s construct validity has not previously been independently examined against recognised measures of physical and psychological impact.

Objectives: To assess the construct validity of the MSIS physical and psychological components in a community based population with MS.

Methods: 73 patients with clinically definite or probable MS (Poser criteria) were enrolled during the course of an epidemiological study. The author individually assessed each patient. A Kurtzke EDSS score was rated, Multiple Sclerosis Functional Composite (MSFC) score compiled and each participant was asked to complete a Beck’s Depression Inventory (BDI-II), MSIS 20 and MSIS 9.

Results: Study group characteristics included: average age 47.5 years (19-72); years since diagnosis 13.4 (1-41); female to male ratio 2.4 : 1 and average Kurtzke EDSS 4.3 (0-9). The MSIS 20 showed a moderate correlation with the EDSS results (r2 = 0.6808) and a weaker but significant correlation with the MSFC
scores (r2 = 0.323). The psychological impact scale, MSIS 9 correlated well with the BDI-II (r2 = 0.6341). There was however an unexpected correlation between the MSIS 20 (physical impact scale) and the BDI-II, significant at p = 0.022.

Conclusions: Our study indicates the the convergent validity of the MSIS 20 and MSIS 9 are acceptable but suggests problems with the discriminant validity of the MSIS 20, as it appears to be significantly influenced by mood. Further longitudinal studies are required to assess the validity of the MSIS before it is widely accepted for clinical trials.

Disclosure: C. McGuigan has nothing to disclose.

P177
THE RELATION OF HEALTH RELATED QUALITY OF LIFE AND SUBJECT DISCONTINUATION IN A PHASE 3 CLINICAL TRIAL
Miller DM, Cohen JA, Tao EC, Kooijmans MF
* Mellen Center, Cleveland Clinic Foundation, Cleveland, OH, Ohio, USA; Biogen, Inc, Cambridge, Massachusetts, USA

Background: Health related quality of life (HRQoL) is increasingly used as an end point in MS trials. The influence of HRQoL on study discontinuation has received far less attention. IMPACT was a randomized, placebo-controlled, double blinded 2-year trial of IFNβ-1a in secondary progressive MS. Benefits of IFNβ-1a were shown on disease progression, relapse rate, MRI, and health related quality of life using the Multiple Sclerosis Quality of Life Inventory (MSQLI).

Objectives: Subject retention has an important effect on trial outcomes; little is known about factors that precipitate subject drop-out. These analyses assess the association of HRQoL and study discontinuation.

Methods: MSQLI includes the generic SF-36 (2 component measures) and 9 disease-specific scales. The MSQLI was administered to English-speaking subjects in IMPACT at baseline, month-12 and month-24. Differences in scores and change scores from baseline to month-12 were examined for completers (COMs) and non-completers (NON-COMs). Between group differences were calculated using the Wilcoxon Rank Sum Test.

Results: 325 subjects provided HRQoL data; 258 (79%) were COMs. 94 (28%) were NON-COMs. At month-12, at baseline, COMs had greater perceived cognitive deficits (P < 0.02) and the IFNβ-1a group (P = 0.047) and worsening bowel control in the IFNβ-1a group (P = 0.015).

Conclusions: There is evidence that HRQoL factors affect subject retention in this study. The pattern of influence appears to change over the course of the study. It is recommended that future investigations of factors that influence retention should include HRQoL.

Disclosure: Drs. Kooijmans and Tao are employed by Biogen, Inc.
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P178
AN OBJECTIVE FOLLOW-UP OF UPPER LIMB FUNCTION IN MULTIPLE SCLEROSIS
Nisipeanu P*, Hocherman S*, Hardan Y*, Inzelberg R
*Neurology, Hillel Yaffe Med Ctr, Hadera, Israel; †Faculty of Medicine, Technion, Haifa

Background: In Multiple Sclerosis Functional Composite the function of the upper limb is assessed by the 9-Hole Peg Test (9HPT). The decondition occurs even in people with relatively low degree of disability. It participates in the origin of fatigue. Significantly decreased values of the expiratory flow, which may reflect muscle weakness, do not participate in the origin of fatigue. Value VText seems to be diagnostic factors associated with dyspnea in MS.

Methods: 54 MS outpatients (14 men, 40 women) without recent clinical relapse and acute respiratory illness were studied. Disability was evaluated according to Expanded Disability Status Scale (EDSS), fatigue according to Modified Fatigue Impact Scale (MFIS), Fatigue severity scale (FSS), respiratory parameters on spirometer by flow-volume method, functional parameters on bicycle spirometer using the anaerobic threshold method. Obtained values were compared with the normal controls. Statistical analyses were carried out using Statistical Analysis System.

Results: EDSS of subjects was 2.8±1.53, illness duration 8.33±6.62, age 37.0±10 yrs. Maximal functional parameters W/kg, HR, VE/kg, MET, VO2/kg/ml, BF, VText (p = 0.001), O2/Hb (p < 0.05) were significantly lower, RQ, EqO2, EqCO2 did not differ from the normal controls. Respiratory parameters PEF (p = 0.001) and MEF75, 50, 25 (p = 0.05) were significantly lower, while VCin, VCex, ERV, FVC, FEV1 did not differ from the normal controls. We found a correlation between fatigue and functional parameters VText, HR, VO2/kg/ml, MET, O2/Hb (p < 0.05), W/kg, VE/kg and relative weight (p < 0.1). We did not find any correlation between fatigue and respiratory parameters, even though a larger percentage (53.71%) of the patients complained of dyspnea. The 10% reduction of value VText increases the probability of respiratory dysfunction 1.353 times.

Conclusions: The decondition occurs even in people with relatively low degree of disability. It participates in the origin of fatigue. Significantly decreased values of the expiratory flow, which may reflect muscle weakness, do not participate in the origin of fatigue. Value VText seems to be diagnostic factors associated with dyspnea in MS. The confirmation of results could help to find an advisable prescription of load and lead to global changes in the approach to rehabilitation of MS patients.

Disclosure: K Nisipeanu has nothing to disclose.
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Multiple Sclerosis
P180
CORRELATION BETWEEN "FUNCTIONAL SYSTEMS" OF EXPANDED DISABILITY STATUS SCALE AND HEALTH RELATED QUALITY OF LIFE: ANALYSIS OF 184 MULTIPLE SCLEROSIS PATIENTS

Idiman E, Özakbas S, Yozbatiran N, Uzunel F, Oguz M, Kürsad F
Dept. of Neurology, Dokuz Eylul University Faculty of Medicine, İzmir, Turkey

Background: Two factors make multiple sclerosis (MS) a disease with important psychological and social implications: First, MS typically affects young adults, thereby limiting their most productive years. Second, MS has an unpredictable nature making it difficult for patients to gain a sense of control over their illness. Over the past decade health related quality of life (HRQOL) instruments have become increasingly popular as end-point tools in clinical studies for measuring patient-assessed health status. Research on quality of life assessment in MS is still limited. Recently the SF-36 was supplemented by 18 additional items (MS-18 mod) to obtain the MS Quality of Life-54 (MSQOL-54) questionnaire specific for MS patients.

Objectives: The aim of the study was to determine the correlation between HRQOL and clinical status.

Methods: We have used MSQOL-54, which was culturally adapted for Turkish MS population, and Expanded Disability Status Scale (EDSS). 184 clinically definite MS patient (139 female, 45 male) were included in the study. They were over 18 years of age. Mean age was 39.2 (16-68 years), mean disease duration was 6.69 (2-29 years), mean EDSS score was 3.06 (0-8). 62.3% have relapsing-remitting, 33% have secondary progressive, 4.4% have primary progressive course.

Results: Mean mental health composite was 63.48 (13.64-100), mean physical health composite was 60.70 (5.08-98.3). The EDSS was significantly associated with both physical and mental health composite (p<0.05). Sensory functional system was significantly associated with physical health composite (p<0.05).

Conclusions: We concluded that pyramidal and cerebellar functional disorders are the main factors to have negative effects on HRQOL in MS, and sensory functional disorder has a marked negative effect on physical health composite of HRQOL in MS.

Disclosure: S Özakbas has nothing to disclose.

P181
CROSS-CULTURAL ADAPTATION AND VALIDATION OF MULTIPLE SCLEROSIS QUALITY OF LIFE QUESTIONNAIRE (MSQOL-54) IN TURKISH MULTIPLE SCLEROSIS POPULATION

Idiman E, Uzunel F, Özakbas S, Yozbatiran N, Oguz M, Callioglu B, Gökce N, Bahar Z
Dept. of Neurology, Dokuz Eylul University Faculty of Medicine, İzmir, Turkey

Background: Multiple sclerosis (MS) is a chronic progressive disease with multiple neurologic impairments. The disease can also dramatically affect the quality of life of the patients.

Objectives: The aim of this study was to investigate the validation of the translated and cross-cultural adapted MS Quality of life-54 (MSQOL-54) on 183 Turkish MS patients.

Methods: One hundred eighty-three of MS patients who met the inclusion criteria were enrolled into the study. Patients were classified into three severity groups according to the expanded disability status scale (EDSS): Group I (EDSS 0-3.5), group II (EDSS 4-5.5), group III (EDSS 6-8). MSQOL-54 questionnaire were translated into Turkish, culturally adapted, and validated in 183 patients. Working, health insurance, marital status and education level were compared with the MSQOL-54.

Results: The mean age of the 183 patients (138 female and 45 male) were 39 years. The questionnaire were well accepted, but small cultural adaptations were required. From the different groups, only in the group I EDSS score was significantly associated with the physical health composite. None of the other groups and parameters showed correlation with physical health composite nor mental health composite.

Conclusions: Assessment of quality of life of MS patients is independent from disease severity, and disability level is important in the general evaluation procedure. The MSQOL-54 was easily administered and well accepted in the Turkish MS population.

Disclosure: S Özakbas has nothing to disclose.

P182
QUALITY OF LIFE FOR MS PATIENTS: REFERENCE STANDARDS FROM A LARGE PANEL OF PATIENTS

Pierre C, Didier V, Laurent G, Maryline L, Dominique A, Aurélien M
NEUROLOGIE, CHU, CLERMONT FERRAND, France

Background: Quality of Life (QoL) assessment for MS patients is a major part of the evaluation of therapies and care. The QoL questionnaires are now applied to a large number of patients, leading to standards reference that may be closer to every type of patients. We have known a valid QoL questionnaire, such as the SEPS9, a French trans-cultural adaptation of Barbara Vickrey’s MS QoL 54.

Objectives: We analyzed a 423 patients large database in order to define the various profiles for QoL and to group the patients in homogeneous clusters according to the main characteristics of their disease.

Methods: We considered patients having a confirmed MS from various cohort surveys at their first assessment. They all have fulfilled the SEPS9qol questionnaire. We have also collected data from their clinical assessment such as defined in EDMUS (European Database for Multiple Sclerosis). Analysis combined one and two ways analysis to select the clinical variables, which permit to create homogeneous QoL group of patients, and multiple linear regressions as well as logistic regressions in order to create the groups and to check their validity.

Results: The main variables related to QoL were the EDSS score (0-3, 3.5 - 6, > 6), the age of the disease, (+ 10 years), a remittent or a progressive form and the period since the last peak of the disease. By combining these variables, Multivariate analysis revealed 8 clusters of homogeneous QoL patients.

Conclusions: It is now possible to compare every patient to his QoL group and we have to assess how this comparison helps to improve both patients’ care and medical decisions.

Disclosure: C Pierre has nothing to disclose.

P183
FRONTAL SYNDROME AND QUALITY OF LIFE IN MS PATIENTS

Pierre C, Didier D, Delphine L, Céline T, Florence B, Laurent G
NEUROLOGIE, CHU, CLERMONT FERRAND, France

Background: Qol assessment is an important issue for the evaluation of therapies and care strategies in MS. But, cognitive disorders, mainly frontal, are frequent in MS patients, and they can modify their response to QoL questionnaires. Those aspects of frontal dysfunctions could be important in relation to their QoL.

Objectives: To determine in MS patients, with stable motor handicap and global cognitive state, if frontal dysfunction impairs their perception of quality of life (QoL).

Methods: The survey was made among 101 MS patients, 21 male and 80 female, 19 - 70 years-old (m=44 +/- 10). EDSS score was less than 7.5 (3.55 +/- 2.18), Mini Mental State more than 24, and the Hamilton depression scale less than 14. Neuropsychological batteries were : WAIS,R, Grober and Buschke, Stroop, Wisconsin card scoring test, and categorial fluency test. QoL assessment used SEP59, a valid Qol questionnaire which is a French trans-cultural adaptation of Barbara Vickrey’s MS QoL 54.

Results: Spearmann rank tests showed 1/ Significant correlations between neuropsychological tests ; 2/ No correlation between the dimensions in QoL scale and neuropsychological tests, whatever the EDSS score was (< 4 ? 4).

Conclusions: A possible dissociation in the frontal syndrome, i.e. cognitive and comportamental dysfunctions, could explain these results.

Disclosure: C Pierre has nothing to disclose.
P184
PREDICTION OF QUALITY OF LIFE IN SEVERELY DISABLED MS-PATIENTS
Ritter SB, Ladurner G, Wranek U
Neurology, Christian-Doppler-Klinik, Salzburg, Salzburg, Austria

Disclosure: S Ritter has nothing to disclose.

Background: Quality of life (QoL) has gained increasing influence as an evaluation criterion in medicine. A number of studies have been published which have examined QoL in patients with MS suffering from low to moderate physical disability (EDSS < 5.5). The investigators found a decrease in various QoL-domains. Little is known about QoL of severely disabled MS-patients and about the predictors of QoL in this population.

Objectives: The sample consisted of 21 MS-patients (11 men) with a mean EDSS-Score of 6.905 (range, 5.5 - 9.0), a mean disease duration of 19.05 years (range, 7 - 38) and a mean age of 51.38 years (range, 35 - 76).

Methods: We assessed QoL with the Euroqol, symptoms anxiety and depression with the Hamilton Anxiety and Depression Scale (HADS), cognitive function with the Paced Auditory Serial Addition Test (PASAT), social support with the MOS Social Support Survey (MSSS), coping with the Fragebogen zur Erhebung von Kontrollüberzeugung zu Krankheit und Gesundheit (KKG) and cerebral atrophy (3VW, SCC).

Results: In a linear regression analysis depression was the by far strongest predictor for QoL (R²= .405; p= .003), followed by cognitive function (R²= .235; p= .026) and fatalistic coping (R²= .188; p= .005).

Conclusions: With the neuropsychological interventions addressing on depression, cognitive performance and coping, QoL of severely disabled MS-patients can be improved. This is an important finding, because this sample does not fulfill the criteria of new disease-modifying drugs.

Disclosure: S Ritter has nothing to disclose.

P185
QUALITY OF LIFE AND COST OF ILLNESS IN MITOXANTRONE TREATED MULTIPLE SCLEROSIS PATIENTS.
Vollmer T
d, Hadjimichael O, Buenconsejo J

aNeurology, Yale School of Medicine, New Haven, Connecticut, USA; bNeurology, VA Conn Healthcare System, West Haven, Connecticut, USA

Background: Progression of disability in MS is associated with deterioration in quality of life (QoL). Mitoxantrone is an FDA approved therapy for aggressive relapsing and secondary progressive MS patients.

Objectives: We are conducting a five year study to evaluate the relationship between disease activity and progression of disability/handicap, cost of disease and the quality of life (QoL study) among patients who are participating in the RENEW (Mitoxantrone Safety Trial) trial. We report on a comparison of demographic characteristics of study patients treated with mitoxantrone (Q&C) to NARCOMS Registry patients also treated with mitoxantrone (NRM) and to NARCOMS patients who are currently treated with interferons or glatiramer acetate (ABC).

Methods: After informed consent, patients complete a set of questionnaires measuring disability (Patient Determined Disease Steps (PDDS)), handicap (Performance Scales), fatigue (Modified Fatigue Impact Scale), QoL (SF12 and Health Utilities Index). The PDDS estimates the EDSS. The Performance Scales measure handicap in ten neurological domains.

Results: The mean age of the 162 Q&C patients currently enrolled and that of 174 NRM participants is similar (46 years, sd=9.7). For the 10,607 ABC patients the mean age is 44 years. Q&C patients include slightly more females and more Caucasians, have higher income and more have private insurance than the other two groups. The two mitoxantrone treated groups have more college and post-college graduates. However, 45% of the ABC group is employed versus 27% of the Q&C and 18% of the NRM patients. Both mitoxantrone treated groups had more relapses within the last year (2.5 and 2.6 vs. 2.0), and both had a higher disability score (PDDS=5.0 and 5.5) than the ABC group (PDDS=3.4).

Conclusions: Patients enrolled in the RENEW study are similar to mitoxantrone treated patients in the Registry, and both these groups have more severe MS than the average ABC treated patients. Mitoxantrone treatment may be more available to those who have adequate insurance and income to cover its cost. Data will be presented on cost of disease, fatigue, depression, and QOL in mitoxantrone treated MS patients.

Disclosure: Authors have received a research grant from Immunex Corporation.

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P186
EVOLUTION OVER A 3 MONTH PERIOD OF GLOBAL, PSYCHOLOGICAL, AND SOCIO-PROFESSIONAL FUNCTIONING IN PATIENTS WITH RELAPSING REMITTING MULTIPLE SCLEROSIS, DURING AVONEX® TREATMENT INITIATION
Warter J, Gentin M

aService de neurologie 2, Hôpital civil de Strasbourg, Strasbourg cedex, France; bBIOGEN, Nanterre, France

Background: This open, non comparative, multicentric study has been performed on patients with Multiple Sclerosis (MS), new to any interferon treatment.

Objectives: Study’s objective was to evaluate the evolution of global, psychological, and socio-professional functioning in these patients during the first months of Avonex® treatment.

Methods: After a 2 week pre-inclusion period, patients were receiving a weekly intramuscular injection of 30 µg of Avonex® for 12 weeks. GFS (global functional evaluation), MADRS (Montgomery and Asberg Depression Rating Scale), CGI-S (clinical global impression, severity) and tolerance were assessed every 4 weeks visit 1(V1) to visit 4 (V4).

Results: 594 patients (71.9 percentage (%) women) aged 39±10 years have been included between May 2000 and May 2001. At V1 and V4 respectively, GFS score was 84.9±10.3 and 85.9±10.4, CGI-S score was 1.86±1.03 and 1.93±1.09, MADRS score was 4.9±4.5 and 4.5±4.6.

Conclusions: There is no significant statistical change between V1 and V4 for the principal efficacy outcome (GFS) which remains stable. In addition, MADRS and CGI-S scores do not show any significant change from baseline. 45 patients (7.5%) were withdrawn from the trial, 36 for adverse events (AE) and 9 for other reasons. Flu-like symptoms have been the main AE reported, by 69.4% of patients at V2, 44.4% at V3 and 35.8% at V4, their frequency and intensity decreasing along with Avonex® treatment continuation. This study, carried out on a large number of patients shows no change in global, psychological, and socio-professional functioning assessed by GFS in MS patients during the 3 first months of treatment with Avonex®. In addition, there is no change in depressive mood assessed by MADRS nor in global psychological functioning assessed by CGI-S.

Disclosure: J M Warter, MD, PhD, C H U Strasbourg, Strasbourg cedex, France; cBIOGEN, Nanterre, France

Funding: Supported by BIOGEN.

P187
SITUATION OF MEDICAL CARE OF PATIENTS WITH MULTIPLE SCLEROSIS IN NORTH-EASTERN GERMANY
Zettl ULP, Krüger T

aNeurology, Christophorus-KKH, Ueckermünde, M/V, Germany; bNeurology, University of Rostock, Rostock, M/V, Germany

Background: Until now the state of medical care, rehabilitative medical care and socio-medical care of multiple sclerosis (MS) patients in Germany has not been subject of a study.

Objectives: This investigation gives an overview of the situation of MS patients in north-eastern Germany.

Methods: The study is based on a structured questionnaire containing 64 items in the following categories: epidemiological-medical, psychological and
Disclosure: BR Brooks has served as a consultant to Teva Neurosciences, was a statistically significant difference (p=0.0196) between pre- and post-fatigue did not get fatigue much during 30-40 seconds of fatigue task. However, there worsened in each group after fatigue task (pre-GA=0.0107g vs post-GA=0.0132g; non-GA-treated MS patients (N=96;GA=52,non-GA=44). The amplitude of PT was determined offline using standard algorithms for each patient to determine the effect of fatigue on PT amplitude. Each patient underwent a standard computerized tremor assessment before and after 30-40 seconds of fatigue task. The accelerometer was attached to the dorsal of the hand. The patient was instructed to keep the accelerometer attached arm out-stretched in front for five seconds of data collection at 200 samples per second. There was a five seconds of relaxation period after each repetition. In order to induce fatigue in tested arm, the patient was instructed to keep his/her arm in front out-stretched for 30-40 seconds. Three consecutive records were collected without rest period in between. The amplitude and frequency of bilateral hand PT was determined offline using standard algorithms (FFT) employing uniaxial accelerometer input for computer analysis. The distribution of ratio of post-fatigue/pre-fatigue PT amplitude data was obtained for each patient to determine the effect of fatigue on PT amplitude. Results: The amplitude of PT worsened after fatigue tasks in both GA-treated and non-GA-treated MS patients (N=96;GA=52,non-GA=44). The amplitude of PT worsened in each group after fatigue task (pre-GA=0.0107g vs post-GA=0.0132g; pre-non-GA =0.0241g vs post-non-GA=0.0725g g=9.81 m/s/s). Paired t-test statistic indicated that there was no significant difference (p=0.132) between pre- and post-fatigue GA-treated patients group indicating GA-treated MS patients did not get fatigue much during 30-40 seconds of fatigue task. However, there was a statistically significant difference (p=0.027) between pre- and post-fatigue non-GA-treated patients group indicating fatigue made PT amplitude much worse. Conclusions: GA treatment has a beneficial effect on ameliorating the post-exercise fatigue-induced PT amplitude seen after 30-40 seconds of sustained posture in MS patients.

Disclosure: T Krüger has nothing to disclose.

Symptomatic Management

P188

GLATIRAMER ACETATE [GA] TREATMENTS HAVE SIGNIFICANT EFFECTS ON CONTROLLING FATIGUE-INDUCED TREMOR AMPLITUDE IN MULTIPLE SCLEROSIS PATIENTS

Dogan S, Konopacki R, Brooks BR
Neurology, University of Wisconsin-Madison, Madison, Wisconsin, USA

Background: Tremor and fatigue are the most common movement disorders found in MS patients due to pathological changes in specific myelinated tracts. Fatigue and tremor interfere with activities of daily living. To date, no research has been conducted to determine the effects of fatigue on tremor amplitude in MS patients. Objectives: Evaluate the efficacy of GA therapy in controlling the amplitude of postural tremor [PT] after fatigue-inducing task in MS patients. Methods: Each patient underwent a standard computerized tremor assessment before and after 30-40 seconds of fatigue task. The accelerometer was attached to the dorsal of the hand. The patient was instructed to keep the accelerometer attached arm out-stretched in front for five seconds of data collection at 200 samples per second. There was a five seconds of relaxation period after each repetition. In order to induce fatigue in tested arm, the patient was instructed to keep his/her arm in front out-stretched for 30-40 seconds. Three consecutive records were collected without rest period in between. The amplitude and frequency of bilateral hand PT was determined offline using standard algorithms (FFT) employing uniaxial accelerometer input for computer analysis. The distribution of ratio of post-fatigue/pre-fatigue PT amplitude data was obtained for each patient to determine the effect of fatigue on PT amplitude. Results: The amplitude of PT worsened after fatigue tasks in both GA-treated and non-GA-treated MS patients (N=96;GA=52,non-GA=44). The amplitude of PT worsened in each group after fatigue task (pre-GA=0.0107g vs post-GA=0.0132g; pre-non-GA =0.0241g vs post-non-GA=0.0725g g=9.81 m/s/s). Paired t-test statistic indicated that there was no significant difference (p=0.132) between pre- and post-fatigue GA-treated patients group indicating GA-treated MS patients did not get fatigue much during 30-40 seconds of fatigue task. However, there was a statistically significant difference (p=0.027) between pre- and post-fatigue non-GA-treated patients group indicating fatigue made PT amplitude much worse. Conclusions: GA treatment has a beneficial effect on ameliorating the post-exercise fatigue-induced PT amplitude seen after 30-40 seconds of sustained posture in MS patients.

Disclosure: BR Brooks has served as a consultant to Teva Neurosciences, Biogen, Berlex, Serono, Elian, Sanofi, Covance, Regeneron, Amgen, Avanir

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P189

OSTEOPOROSIS IN MULTIPLE SCLEROSIS PATIENTS TREATED WITH CORTICOSTEROIDS

Havrdova E, Tyblova M, Stepan J, Zikan V, Horakova D, Ticha V, Novaková I
Dpt. of Internal Medicine, Charles University, Prague, Czech Republic

Background: Corticosteroids (CS) used for treatment of acute relapses in MS may induce loss of bone as a result of inhibition of gastrointestinal absorption of Ca, reduction of tubular reabsorption of Ca with hypercalciuria, suppression of gonadal hormones secretion, and inhibition of recruitment and activity of osteoblasts. The combination of CS administration and immobility in MS may lead to higher incidence of osteoporosis.

Objectives: To determine mineral bone density (MBD) loss in patients with MS. Methods: 299 patients with MS (59 males, age 41.7 ± 12.3 yrs and 240 females, age 44.1 ± 10.7 yrs) followed in MS Center, Dpt. of Neurology, First School of Medicine, Charles University, Prague, underwent MBD density examination (neck, lumbar region, femoral trochanter). The dose of CS used in the treatment of MS, administered either orally or intravenously, was counted. Kurtzke EDSS was used to determine the ability of MS patients to move in vertical position. All fractures were documented.

Results: Osteoporosis defined as loss of bone mineral - 2.5 SD of age and sex-matched controls was found in 25% of patients, 30% of them had a history of bone fractures. Most of them were in SP MS. 38% of postmenopausal female patients with osteoporosis had a history of bone fracture. Only 2.7% patients in RR MS had osteoporosis. The average amount of CS used in the MS therapy in these patients was 27 ± 6 g. Osteopenia (+1 to -2.5 SD) was found in 45% of patients, the fractures occured in 10% of these patients. Kurtzke EDSS was lower than in patients with osteoporosis - 4.42 ± 1.97. The amount of steroids used for MS treatment did not differ from the whole group. 28% of patients had no decrease in MBD. The amount of steroids used in their treatment was 21 ± 19g, their Kurtzke EDSS did not exceed 4.0, half of them were still in remittent phase of the disease.

Conclusions: MBD loss in MS patients occurs not only due to repeated use of CS but also due to decreased mobility. Female postmenopausal patients with combination of CS administration and loss of ability to walk are in danger of bone fractures. The most effective method of bone density examination was the measurement of MBD in trochanter. Osteoporosis found in this region is predictive of bone fractures.

Disclosure: E Havrdova has nothing to disclose.

Funding: Supported by research grant IGA MZ 6780-3.

P190

BOTULINUM TOXIN IN THE TREATMENT OF DETRUSOR HYPERREFLEXIA IN MULTIPLE SCLEROSIS: CASE REPORT

Hradilek P, Krhut P, Zapletalova O, Mainer K
Neurological Clinic, University Hospital, Ostrava, Czech Republic; Urological Clinic, University Hospital, Ostrava, Czech Republic

Background: Multiple sclerosis (MS) often affects the genitourinary tract. The most common finding is the detrusor hyperreflexia and detrusor-sphincter dyssynergia. The patients suffer from urgency, frequency, urge incontinence, difficulties in emptying the bladder and urinary infections. Under these conditions the upper urinary tract could be occasionally damaged. Most extended therapeutic concept is treatment with anticholinergic drugs, but its effectiveness could be insufficient. Since few years the detrusor intramuscular injection of the botulinum toxin (BT) has been described as...
a therapeutic option in the patients with detrusor hyperreflexia following the spinal cord injury, not responding to anticholinergic therapy. We refer about use of the BT in MS patient.

Methods: M.J. 32 years old, MS patient. The voiding symptoms are present for 4 years. She suffered from urgency, frequency and urge incontinence. Intermittent catheterisation and different anticholinergic and spasmylic drugs were administered without any effect. 10/2001 BT-1.000 IU have been injected into the 40 locations of the detrusor.

Results: The patient is continent, urge-symptom-free and very satisfied 8 months after the BT application. She empties the bladder by intermittent self-catheterisation 5x per day, without any need of anticholinergic drugs. No urinary tract infections were observed, catheterised volumes are 500-600 ml. Both cystometric capacity (180ml to 577 ml) and residual volume have significantly increased (100 - 150 ml to 500 - 600 ml), intravesical pressure has significantly decreased (65 cm H2O to 15 H2O).

Conclusions: Botulinum toxin was successfully administered in our MS patient with good relief from urinary symptoms We consider this therapy useful for patients with refractory incontinence due to detrusor hyperreflexia associated with multiple sclerosis.

Disclosure: P Hradilek has nothing to disclose.

P191
SPASTICITY IN MS PATIENTS IN THE NARCOMS REGISTRY: PREVALENCE, SEVERITY AND TREATMENT PATTERNS USING ORAL AGENTS AND/OR INTRATHECAL BACLOFEN.

Rizzo M, Hadjimichael O, Buenconsejo Jr, Preiningerova J, Vollmer T* aNeurology, Yale School of Medicine, New Haven, Connecticut, USA; bNeurology, VA Conn Healthcare System, West Haven, Connecticut, USA

Background: Systematic survey of treatment of spasticity and related quality of life issues is lacking in MS patients.

Objectives: We investigated the prevalence, severity and treatment patterns of spasticity in 21,130 MS patients in the NARCOMS Registry.

Methods: The Registry collects data on demographics, MS related history, disability status, and immunologic and symptomatic therapy.

Results: MS patients with moderate to severe spasticity are more likely to be male and older in age, with longer disease duration, more frequent relapses, and more MS related disability than patients with mild or minimal spasticity. Among those with relapsing-worsening MS, 21% report moderate to severe spasticity that affects their activities several times a week, and 27% report severe or total spasticity. Overall, 44% of patients use oral anti-spasticity agents, most frequently baclofen; 17% use more than one oral agent (tizanidine, gabapentin, diazepam, clonazepam). In addition, those with moderate to severe total levels of handicap due to spasticity, 1.6% and 3.2% respectively, use intrathecal administration of baclofen (ITB), frequently in combination with an oral drug. Patients treated with ITB have more education, but are less likely to be employed (20% vs. 29%), and more likely to be disabled (65% vs. 50%) than those using oral drugs only. Their mean disability score is significantly higher than the oral drug group (Patient Determined Disease Step=6.2 vs. 4.7), and their handicap score is worse with respect to mobility, hand function, bladder and sensory symptoms. Those using ITB have an elevated mean spasticity score compared to those on oral medication only (2.9 vs. 2.5). Logistic regression analysis shows that given similar disability and spasticity levels, patients with higher income, younger age, who are treated by MS specialist neurologists, and visit physiatrists and physical therapists are more likely to be prescribed an ITB pump.

Conclusions: Spasticity is associated with higher levels of disability in MS patients. Sociodemographic factors and healthcare resources used influence treatment strategies recommended by physicians. Quality of life issues will be explored in relation to specific treatments.

Disclosure: Received research grant from Medronic
Funding: Supported by research grants from Medronic, CMSC, and Rehabilitation and Research Service, Department of Veteran Affairs.

P192
VARIABILITY IN THE QUALITIES OF CHRONIC NEUROPATHIC PAIN IN MULTIPLE SCLEROSIS ASSESSED BY THE NEUROPATHIC PAIN SCALE (NPS)

Rog DP*, Young CA†a
aNeurological Sciences, University of Liverpool, Liverpool, Merseyside, United Kingdom; †Walton Centre for Neurology and Neurosurgery, Liverpool, Merseyside, United Kingdom

Background: Central neuropathic pain occurs in between 17 and 52% of people with MS. However, little is known about the variability in the qualities of the neuropathic pain experience. The Neuropathic Pain Scale (NPS), is a 10 item numerical rating scale (each item is rated 0-10, range is therefore 0-100), which we have previously shown to be valid and reliable in assessing the qualitative and quantitative aspects of neuropathic pain in MS.

Objectives: To determine the variation in the components of chronic neuropathic pain in people with MS, using the NPS.

Methods: Thirty one patients with MS and neuropathic pain, were interviewed to determine the presence and location, using a body map, of their (most severe) neuropathic pain and then invited to complete the NPS on seven consecutive days, at the time of day when their identified pain syndrome was expected to be at its worst.

Results: Six patients were male, mean age 47 years (range 32-71), mean EDSS 5.4 (2.5-8.5), 3 had primary progressive, 9 secondary progressive and 19 relapsing-remitting MS. The sites of neuropathic pain were as follows: 21 lower limbs, 3 upper limbs, 3 hemi body, 3 neck, 1 back, 16 pains were bilateral. The average duration of pain was at least 9.9 years (range 1.5-30, SD 7.6). During the 7 day period, the mean of the 10 item NPS total varied between 47.4 and 51.8 for the group. We have previously shown that 64% of the variance in the NPS is accounted for by 3 factors: (1) “Localised”, comprising “Intense”, “Sharp”, “Unpleasant” and “Deep”, (2) “Superficial”, comprising “Sharp”, “Sensitive”, “Ichy” and “Surface” and (3) “Abnormal perception”, comprising “Hot”, “Dull”, and “Cold”. During the 7 day assessment period, the mean of factor 1 (maximum 40) varied between 23.6 and 26.4 (range 0-40, average SD 8.6), factor 2 (maximum 40) between 15.3 and 18.4 (range 0-40, average SD 9.8) and factor 3 (maximum 30) between 12.8 and 13.9 (range 0-30, average SD 0.4). The mean correlation between the 10 item NPS total was r=0.735, for factor 1, r=0.658, factor 2, r=0.7512 and factor 3, r=0.833.

Conclusions: Chronic neuropathic pain in MS can achieve relatively constant levels of severity adding to the burden of illness in these patients.

Disclosure: D Rog has nothing to disclose.

P193
BURDEN OF NEUROPATHIC PAIN IN MS

Rog DP*, Young CA†a
aNeurological Sciences, University of Liverpool, Liverpool, Merseyside, United Kingdom; †Walton Centre for Neurology and Neurosurgery, Liverpool, Merseyside, United Kingdom

Background: Central neuropathic pain occurs in between 17 and 52% of people with MS. However, little is known about the burden of neuropathic pain in MS, in terms of its qualities, duration, intensity, exacerbating factors, associated subjective sensory phenomena and response to treatment.

Objectives: To establish the burden of neuropathic pain in people with MS.

Methods: A convenience sample of 31 patients with MS and neuropathic pain, were interviewed and indicated the presence of any (neuropathic and non-neuropathic) pain syndromes on a body map. For each pain syndrome, its duration, time course, severity, qualities, response to treatment and therapy, presence of associated numbness, allodynia, hyperpathia, hyperalgesia and likely aetiology were determined.

Results: Six patients were male, mean age 47 years (range 32-71), mean EDSS 5.4 (2.5-8.5), 3 had primary progressive, 9 secondary progressive and 19 relapsing-remitting MS. A total of 113 pain syndromes (mean 3.6, range 109) were identified, 89 (79%) of which were felt to be neuropathic in origin (mean
2.8, range 1-7). Neuropathic pains were located as follows: 42 lower limbs, 27 upper limbs, 6 neck, 4 each for back and eye, and 2 each for head, trunk and hemi body pains. Thirty-nine (44%) were paroxysmal and the mean duration of neuropathic pain was 8.7 years (range 0.02-40, SD 8.5). Mean severity of neuropathic pain on a 0-10 numerical rating scale was 5.2 (range 1-10, SD 3.7). Thirty-five pains (39%) were spontaneous and 54 (61%) were evoked, mainly by fatigue in 27 (50%), changes in temperature 15 (28%), touch 12 (22%) and movement 7 (13%). Two neuropathic pains were evoked by menstruation. Seventy percent of neuropathic pains were associated with subjective numbness and 39 (66%) with a form of allodynia, 41 (66%) with hyperpathia and 21 (24%) with hyperalgesia. Only 2 neuropathic pains were completely relieved (temporarily) by analgesia, 21 were mildly and 14 moderately relieved. Other therapies including TENS and acupuncture were tried in only a small minority of patients and brought mild to moderate relief of only 3 neuropathic pains. 

Conclusions: Neuropathic pain remains a considerable burden to people with MS with respect to its long duration, multiple locations, severity, associated unpleasant sensory phenomena and resistance to analgesia and therapy.

Disclosure: D Rog has nothing to disclose.

P194

FATIGUE ASSESSMENT BY MECHANICAL AND MYOELECTRICAL OUTPUT DURING SUSTAINED MAXIMAL ISOMETRIC VOLUNTARY CONTRACTION IN MULTIPLE SCLEROSIS.

Sanjai M, Belden D, Konapacki R, Wachwik AP, Brooks BR. *Motor Performance Lab, Dept Neurology, University of Wisconsin-Madison Med Sch, Madison, Wisconsin, USA, ‡Neuril Svc, WM S Middleton Memorial VA Med Ctr, Madison, Wisconsin, USA

Background: Motor fatigue(F) measured as the decline in maximal isometric voluntary contraction [MVIC] has been reported in MS as FFI. Surface electromyography (sEMG) is used to study F by measuring changes in sEMG signal in time and frequency domain. During MVIC, sEMG amplitude decline and the median frequency (MF) shift to lower values. The decline in amplitude reflects F induced by decrease in recruitment and/or firing rate. The shift in MF reflects decline in muscle fiber conduction velocity (MFCV). As F progresses, changes in the muscle-fiber-membrane permeability interfere with the normal propagation of action potentials leading to a decrease in MFCV. 

Objectives: To simultaneously measure changes in mechanical and myoelectrical output in elbow flexors and foot dorsiflexors in MS. 

Methods: MVIC was collected from 16 normal control (NC) and 18 MS patients, using a force transducer. sEMG signal was measured by connecting a preamplifier with integral electrodes to the surface of the skin on tested muscle with a reference electrode on the back of the hand. The EMG signal is amplified (gain = 1000) and filtered (band pass = 25 to 250 Hz) before being sampled. Both signal voltages are connected to the computer via 12-bit data acquisition board for display. Digital signal processing (plotting and analyzing) was implemented using custom made software (Visual Basic). Decline in force was expressed as FFI = 100 * [1 minus (AUC 25 to 30 / AUC 2 to 7)]. Compression in the power spectrum of the sEMG signal in the last 5 seconds of the MVIC was calculated as MFS = 100 * [1 minus (MF 25 to 30 / MF 2 to 7)].

Results: MVIC was similar in MS and NC indicating comparable strength. FFI was higher in MS (p<0.05) while MFS was not different in MS and NC (p>0.05). Results were similar in both muscles.

Conclusions: Excess F in MS associated with normal MFCV may be due to pathological changes to a more fatiguable type IIa muscle fiber.

Disclosure: BR Brooks has served as a consultant to Teva Neurosciences, Biogen, Berlex, Serono, Eli Lilly, Coven, Regeneron, Amgen, Avanir Funding: Supported in part by Muscular Dystrophy Association [MDA] of America, National Institute of General Medical Sciences [NIGMS] University of Wisconsin General Clinical Research Center [M01 RR03186] and Department of Veterans Affairs.

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GLATIRAMER ACETATE TREATMENT EFFECT ON MUSCLE STRENGTH IN MULTIPLE SCLEROSIS PATIENTS. A PROSPECTIVE LONGITUDINAL MS CLINIC-BASED STUDY

Sanjai M, Belden D, Konapacki R, Brooks BR. *Motor Performance Lab, Dept Neurology, University of Wisconsin-Madison Med Sch, Madison, Wisconsin, USA, ‡Neuril Svc, WM S Middleton Memorial VA Med Ctr, Madison, Wisconsin, USA

Background: The effect of GA on maximal voluntary isometric contraction (MVIC) as a measure of strength has not been studied. MVIC measured by computerized isometric muscle strength (CIMS) testing can detect changes in a clinically important range not adequately represented by changes in EDSS.

Objectives: To study the effects of GA on MVIC in patients with MS .

Methods: CIMS testing was performed on 70 (14 M, 56 F) MS patients with relapsing- remitting MS before and after GA was added to their therapy without changing their existing drug treatment during the observation period. Over the counter medications and concurrent physical and occupational therapy were not considered to be exclusion factors. MVIC was obtained from 10 upper extremity (UE) and 10 lower extremity (LE) muscles. Patients were selected for analysis in a pre-post design when GA was their only new drug treatment and they had two test dates prior to and the two test dates following the initiation of GA.

Results: In the two testing session prior to the onset of GA (average - 247.52 days±247.52 days, p<0.01), the percent MVIC in the UE did not significantly change [-0.77%]. The percent MVIC in the LE, however, declined significantly [-5.29%] (p<0.0173). On GA treatment, (average - 286.59 d) there was a significant increase in MVIC [UE +4.00% (p=0.0073) and LE +5.7% (p=0.000143) respectively] from the second test to the third test. Following these patients for one more testing session [average - 196.79 d], MVIC decreased UE -1.60% and LE -3.41% respectively (not significant) from the third test to the fourth test.

Conclusions: These preliminary data suggest that GA may have a direct treatment effect on increasing and maintaining strength in MS patients. A prospective pre-post design with randomized delayed start of GA treatment may provide insight to mechanism of action of GA, as well as the role of other ergotropic agents in combination with GA, in the treatment of MS. 

Disclosure: Supported in part by Educational Grant from Teva Neurosciences, Muscular Dystrophy Association [MDA] of America, National Institute of General Medical Sciences [NIGMS] University of Wisconsin General Clinical Research Center [M01 RR03186] and Department of Veterans Affairs.

BR Brooks has served as a consultant to Teva Neurosciences, Biogen, Berlex, Serono, Eli Lilly, Coven, Regeneron, Amgen, Avanir Funding: Supported in part by Teva Neurosciences and Great Lakes Veterans Integrated Services Network [VISN 12], Department of Veterans Affairs.

Clinical Aspects of MS (Part 2)

P196

PREDICTIVE FACTORS TO SECONDARY PROGRESSIVE MULTIPLE SCLEROSIS POINT OUT THE POTENTIAL IMPORTANCE OF THE INFLAMMATORY PROCESS


Background: There are two hypothesis to explain progression of disability in multiple sclerosis (MS). The first is that progression of disability does not depend on the inflammatory process, the second is that recurrent inflammation causes permanent axonal damage which contributes to the irreversible deficit that occurs later in the secondary progressive phase. In relapsing remitting (RR) MS patients, the early disability due to sequelae after relapses and the
number of relapses during the first years are indicators of the inflammatory process.

Objectives: To look for whether some predictive factors of early secondary progressive (SP) MS give arguments favouring either the degenerative hypothetical or the inflammatory process.

Methods: In Brittany (France), we collected extensive data in EDMUS (European database for multiple sclerosis) for 1019 RR MS patients since 1992 to 31 December 2000. The data were analysed using Kaplan Meyer method to evaluate progression to a SP course.

Results: Among these 1019 RR MS patients, 334 (32.8%) reached a SP course in December 2000. Age at onset of MS in this SP population was 29.2. The most common symptoms at presentation was sensory deficit (35.9%), optic neuritis (33.2%) and disability at lower limbs (29.6%). The male female ratio was 0.52. The mean disease duration was 12 years (y). In the 334 SP MS population, 50% reached the SP course 9 y after the onset of the disease. The shorter time to reach the SP course for RR MS was significantly correlated for patients which had an EDSS more than 3 due to relapses in less than three y after onset (3.8 y Vs 10 y) (p<0.002), and for RR MS patients who had 2 relapses or more as opposed to 1 relapse in the first y (6.6 y Vs 11 y) (p<0.0001).

Conclusions: The unfavourable prognostic factors related to the inflammatory process found in this study favoured the hypothesis that recurrent inflammation cause permanent axonal damage which contributes to the irreversible deficit that occurs later in the disease. This study gives arguments for early treatments with active drugs against inflammatory process.

Disclosure: M Coustans has nothing to disclose.

P197

MULTIPLE SCLEROSIS ASSOCIATED WITH SYSTEMIC MASTOCYTOSIS: A SINGLE CASE REPORT

Cristina Z, Carlo F
Neurology, CIVIL Hospital, Mirano, Italy

Background: Multiple Sclerosis (MS) is a chronic, inflammatory, autoimmune demyelinating disease of central nervous system. The pathological process underlying the disease involve dysregulation of the immune system, and it is predominantly a T cell-mediated immune disorder. On the other hand, Systemic Mastocytosis (SM) is a heterogeneous rare group of stem cell disorders characterized by abnormal growth and accumulation of mast cells in more organ systems (skin, bone marrow, bone, gastrointestinal tract, liver, spleen and lymph nodes), and is considered to be a hematologic disease.

Objectives: We present a clinical definite relapsing-remitting MS (RRMS) case with associated SM.

Methods: CASE: F.L., a 40 years old man in whom MS developed in 1991 with dizziness, diplopia with right medial rectus palsy and omolateral nistagmus, sensorial disturbance of the left leg. MRI showed typical findings, CSF showed also a systemic involvement, with multifocal histological lesions in the bone marrow, spleen, lymph nodes and skeletal infiltra- tion, increasing serum total tryptase levels.

Results: Mast cells originate from pluripotent hematopoietic progenitor cells that express the CD34 antigen, while MS patients have a dysregulation of some select T-cells subpopulation. Although no effective therapy for patients with SM is known, some of them may benefit from treatment with corticosteroid or Interferon (IFN)-alpha 2b, which can exhibit inhibitory effects on factor-dependent growth of mast cells progenitor cells, and such therapies have also been used for MS patients.

Conclusions: A better knowledge of the complex immunoregulatory network implicated in both diseases could hepl us to understand if this association is the expression of two non-independent events or only a mere coincidence.

Disclosure: Z Cristina has nothing to disclose.

P198

PREDICTIVE VALUE OF THE ONE-YEAR CLINICAL RESPONSE TO BETA-INTERFERON IN RELAPSING-REMITTING MULTIPLE SCLEROSIS

Etienne R, Iliu-Florin T, Dominique P, Jean-Christophe O, Claire G, Olivier H
MS Clinic, Hopital Tenon, Paris, Ile de France, France

Background: There are no established criteria for therapeutic response to Beta-Interferon (IFN) in Multiple Sclerosis (MS). However, clinical outcomes after one year are often part of the definition of “responders” (Waubant E et al, Neurology, 2002 ; 58 : A189), or part of the inclusion criteria in add-on or rescue therapeutic trials with immunosuppressants (Smith DR et al. Neurology, 2002 ; 58 : A455).

Objectives: To evaluate the predictive value of the One-Year clinical response to IFN in Relapsing-Remitting (RR) MS patients on the further course of MS.

Methods: We selected all RR patients from our MS Clinic data base fulfilling the following criteria: first-ever prescription of IFN; follow-up by the same neurologist; duration of follow-up: one year or longer. Clinical response at one year was defined as: group 1. responders (no relapse, no increment of EDSS score); group 2. treatment failures (1 full point increment of EDSS ; 0.5 if initial EDSS 5 or higher); partial responders (unchanged EDSS at one year) were stratified according to their relapse-rate: group 3: unchanged of higher than during the year preceding treatment; group 4: lower.

Results: Preliminary results for 87 patients followed-up for 12 to 73 months by 2 (out of 4) neurologists (Avonex | *regis* | : 52 ; Betaseron | *regis* | : 12 ; Rebif | *regis* | : 22) are presented. Six patients stopped IFN before one year because of side-effects or pregnancy. In group 1 (24 pts, 30%), the EDSS of all evaluable patients was stable at 2 and 3 years, and 82 % (14/17, 2 years) and 85 % (11/13, 3 years) were still relapse-free. IFN was stopped in all group 2 patients (12 pts, 15 %); 7 of them (46 %) received rescue immunosuppressive therapy. In partial responders (45 pts, 55 %), no pre-treatment characteristics predicted outcome at 2 or 3 years. However, IFN was stopped for non-efficacy more often in group 3 than in group 4, either at 2 years (7/21 vs. 2/15) or at 3 years (5/9 vs. 0/8). There was no apparent efficacy of increasing IFN dosage.

Conclusions: A relapse-free status at one year is highly predictive of continuing efficacy at 2 and 3 years. The decision to stop IFN in still-relapsing but EDSS-stable patients might be taken after one year on treatment in some patients. Full updated results will be presented.

Disclosure: Etienne Roulet received honoraria for therapeutic trials from Schering AG, Biogen Inc. Ares-Serono and Teva

P199

EFFICACY OF MS THERAPY IS KEY DRIVER IN THERAPY CHOICE

Eyring S, Wood Cb, Sherman Sr, Simone M' a, Wood Cb, Sherman Sr, Simone M' aMarketing, Serono International SA; aMarketing, Serono, Inc., Rockland, Massachusetts, USA; aPatient Support, Serono, Inc., Rockland, Massachusetts, USA

Background: Research indicates that efficacy is the key driver for neurologists and MS patients in therapy choice. Historically, the switch rate between disease modifying drug (DMD) therapies was low (~11% over 2 years), based on a perceived lack of differentiation. Since the launch of IFN beta-1a 44mcg tiw in the US in March 2002, the percent of switches from other therapies and reasons for the switches have been evaluated.

Objectives: Identify the influence of efficacy on choosing a DMD therapy for the treatment of MS and quantify the impact of efficacy on switching from one DMD therapy to another.

Methods: Research was conducted by an independent market research agency via face-to-face interviews in early 2001 with 198 neurologists and 122 patients from 9 countries. All respondents were asked questions regarding efficacy and convenience (type/frequency of injection, product form). These data

Multiple Sclerosis
were compared against records of rationale for switching to IFN beta-1a 44mcg tiw, collected by a patient support program during its first 2 months of operation.

Results: The survey results found that 72% of neurologists and 86% of patients reported that they would choose a DMD that offered even a 10% increase in efficacy - even if the drug profile was not their initial choice with respect to frequency of injections, route of administration and formulation. These findings are borne out by records of patient rationale for switching DMD which were submitted to the IFN beta-1a 44mcg tiw patient support program at the time of initiating RxS. Of the first 350 patients who volunteered information on their past drug history, over 90% of RxSs were switches from other therapies. The specific breakdown was: 48% switched from IFN beta-1a 30mcg qw, 26% from glatiramer acetate, 18% from IFN beta-1b. 8% were naïve to therapy. Lack of efficacy was the number one reason cited for therapy switch.

Conclusions: Real-life experience confirms research that both neurologists and MS patients base their new and switch treatment decisions on efficacy. It appears that the switch rate may be increasing in the US based upon interest in optimizing MS treatment efficacy.

Disclosure: All authors are employees of Serono.

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P200

CORRELATION BETWEEN VISUAL EVOKED POTENTIALS ABNORMALITIES AND PHYSICAL HANDICAP PROGRESSION IN MULTIPLE SCLEROSIS: PROSPECTIVE FOLLOW-UP OF 65 PATIENTS

Ferriby D, Arndt C, de seze j, Stojkovic t, Hache P, Vermersch P

aNeurology, Hôpital R. Salengro, Lille, France; bOphthalmology, Hôpital R. Salengro, Lille

Background: Multiple sclerosis patients presenting with optic neuritis (ON) at the onset of the disease seem to have a lower handicap progression (Confavreux et al., 2000). However, it remains unclear if infra- or clinical ON, occurring during disease course, may influence the outcome of the disease.

Objectives: The aim of this study was to determine if visual evoked potentials (VEP) evaluation during the course of the disease can be a predictive factor of handicap progression.

Methods: We prospectively studied, during 3 years, clinical and VEP data of 65 patients (18 male, 47 female) presenting with clinically definite multiple sclerosis according to Poser et al. (1983) criteria. All had neuro-ophthalmologic and VEP assessment at entry and outcome. Mean age and disease duration at inclusion were respectively 35 years and 57 months. Mean EDSS at entry and outcome were respectively 2.4 and 3.2. 44 patients presented with uni- or bilateral ON at inclusion. 5 patients had uni- or bilateral uveitis. 28 patients presented handicap progression defined as 1-point EDSS increase or at least 3 relapses during 3 years.

Results: We did not observe any correlation between VEP data and handicap progression during the 3 years of follow-up (p=0.2). Uveitis was not significantly correlated with better prognosis.

Conclusions: Handicap progression does not seem to be associated with VEP data. Furthermore, VEP data does not seem to be a predictive factor of handicap progression.

Disclosure: D Ferriby has nothing to disclose.

P201

IMPAIRED SYMPATHETIC ACTIVITY IN PATIENTS WITH MULTIPLE SCLEROSIS AND FATIGUE


aDepartment of Neurology, University of Würzburg, Würzburg, Bavaria, Germany; bNeurological Clinic, Rehabilitation Center, Valens, St. Gallen, Switzerland

Background: Fatigue is one of the most common disabling symptoms in patients with multiple sclerosis (MS), but the mechanisms of this complex impairment remain to be determined. Dysfunction of the autonomic nervous system is also frequent in MS, but its causal association to fatigue is not clear.

Objectives: To evaluate cardiovascular autonomic function in MS patients with (MS-F) and without (MS-NF) fatigue.

Methods: 69 MS patients (52 women, 17 men) were studied by parasympathetic (heart rate response to Valsalva maneuver, deep breathing and active change of posture [HR-post]) and sympathetic function tests (blood pressure responses to active change of posture and sustained handgrip [BP-grip]) as well as by measures of heart rate variability during rest and during standing in the time- and frequency-domain (low-frequency power [LF], and high-frequency power [HF]). Results were compared to those obtained in 36 age-matched healthy volunteers (21 women, 15 men). Fatigue was assessed by Krupp’s Fatigue Severity Scale (FSS), with a score > 4.0 indicating fatigue, and by Modified Fatigue Impact Scale (MFIS).

Results: Median HR-post and BP-grip were lower in MS patients than in controls. The results of both autonomic tests were inversely related to both fatigue scores, FSS and MFIS, particularly to those measuring physical fatigue (r = -0.46, BP-grip vs. MFIS). The median LF/ HF ratio was lower in MS-F than in MS-NF patients indicating reduced sympathetic outflow in MS patients with fatigue. Worsening of symptoms with heat (“Uhthoff’s phenomenon”) was associated with elevated fatigue scores.

Conclusions: The results of this study confirm the high prevalence and pattern of abnormalities in cardiovascular reflex tests in MS patients as a whole and suggest that sympathetic nervous system activity may be reduced in MS patients with fatigue.

Disclosure: P Flachenecker has nothing to disclose.

P202

MEASURING THE OUTCOME OF INTERFERON THERAPY FROM THE PATIENTS’ PERSPECTIVE

Ford H, Johnson MH, Denton S

Department of Neurology, Leeds Teaching Hospitals Trust, Leeds, West Yorkshire, United Kingdom

Background: It is accepted that patient-perceived quality of life is important, and that self-report measures may provide reliable and valid information about this. Thus a self-report questionnaire of QoL, based upon patients’ own experiences and views, may be a valuable addition to outcome measurement for those with multiple sclerosis

Objectives: To assess the effectiveness of interferon treatment using a disease-specific patient-centred measure of quality of life and to compare this with physician-centred measures of disability.

Methods: All patients treated with interferon beta in the Leeds Multiple Sclerosis Programme had a comprehensive pre-treatment clinical assessment. This included a self-report disease-specific measure of quality of life, the Leeds Multiple Sclerosis Quality of Life scale (LMSQoL) and the Guy’s Neurological Disability Scale (GNDs). These measures were completed at three monthly intervals on treatment. The Expanded Disability Status Scale (EDSS) was assessed annually. Patients eligible for treatment had relapsing-remitting MS (RRMS) or active secondary progressive MS (SPMS).

Results: 73 patients had been started on interferon beta and observed for at least 2 years. 57 patients had RRMS and 16 had active SPMS. The mean baseline LMSQoL score was 22.8 (median 23) and this improved significantly to 26.1 (median 26) at one year. This improvement was maintained at 2 years. The baseline mean GNDs score was 12.9 (median 13) and at one year this had improved to 11.3 (median 11) and this was maintained at 2 years. The baseline mean EDSS was 4.1 (median 4.5) and there was no significant change at 1 or 2 years.

Conclusions: The EDSS, a physician-centred measure of impairment and disability, did not change significantly in this prospective clinic study. The Leeds MSQoL improved significantly in this population. The limitations of the EDSS have been reported but of particular relevance in this population is the failure of the EDSS to capture changes in fatigue and energy levels, and the insensitivity of the scale at its lower end. The ability to detect change is an essential requirement of any outcome measure. Patient-centred outcome measures may show significant effects of treatment which would be missed if physician-centred measures alone are used.

Disclosure: H Ford has nothing to disclose.
P203
COAGULATION ABNORMALITIES IN MULTIPLE SCLEROSIS AND POSSIBLE MULTIPLE SCLEROSIS

Galgani S, Cores F, Corpetti M, Pindi G, Manni M, Gasperini C
Ospedale San Camillo, Roma, Italy

Background: Misdiagnosis in MS is reported in 5-10% of cases. Diagnostic criteria for MS were recently reviewed and MRI data were integrated with clinical and paraclinical methods (McDonald 2001). In the last few years many clinical reviews showed that hypercoagulable states, especially Activated Protein C Resistance (APCR) and Hyperhomocysteinemia, could be considered as risk factors for cerebral ischemia. Nevertheless possible relationships between these conditions, CNS pathology and aspecific changes in MRI should be investigated.

Objectives: To verify the incidence of coagulation abnormalities in a cohort of patients with MS and Possible MS

Methods: A cohort of pts with MS and possible MS according to McDonald’s MS diagnostic criteria (MSDC), consecutively observed during a period of 12 months, was studied. The pts with possible MS were classified, according to MSDC, as isolated syndromes including: optic neuritis, spinal cord and brain-stem syndromes. Furthermore, we considered as possible MS two more groups of patients with “pure sensory symptoms” and “unusual symptoms”. Brain MRI was performed in all pts. Moreover, laboratory tests for C and S protein levels, APCR and homocysteine were collected. Minor Prothrombotic Coagulopathy (MPC) was defined as reduction of C and S proteins, APCR abnormality or hyperhomocysteinemia.

Results: 69 pts had MS and 51 possible MS. The age was >18<50 yrs. MPC was observed in 14/69 pts with MS and in 21/51 pts with possible MS (p=0.05). The possible MS group included: a)Isolated syndromes (15/51); b)Pure sensory symptoms (31/51); c)Unusual symptoms (5/51). MRI findings of possible MS pts were considered: A)Normal (16/51); B)Abnormal not fulfilling MSDC (35/51). MPC was found in 4/16 pts with normal MRI and in 17/35 pts with MRI changes not fulfilling MSDC (P<NS). Pure sensory syndromes group showed the highest incidence of MPC (16/34) compared to isolated syndrome group (5/20), p=0.05

Conclusions: MPC was significantly more frequent in pts with possible MS compared to pts with MS and significantly more common in pts with pure sensory symptoms compared to pts with isolated syndromes. Our preliminary data suggest that screening tests for MPC should be performed in cases clinically suggestive for MS.

Disclosure: F Corvi has nothing to disclose.

P204
THE RANGE OF INFLAMMATORY MYELOPATHIES, CLINICAL, MELINEUROLOGICAL AND IMMUNOLOGICAL FINDINGS IN 35 CONSECUTIVE CASES.

Perini P, Calabresi M, Tiberto M, Tzintzeva E, Ranzato F, Gallo P
Neurological & Psychological Sciences, University of Padova, Padova, Italy

Background: Inflammatory myopathies (IM) constitute a quite heterogeneous group of diseases characterized by inflammatory lesions in the spinal cord, whose etiopathogenesis remains often undefined.

Objectives: We analyzed clinical, MRI, cerebrospinal fluid and systemic immunological parameters in 35 consecutive patients presenting with IM, in order to define a diagnostic protocol.

Methods: Thirty-five consecutive patients (21 male, 14 female, NF=3/2; mean age at onset 44 yrs) were studied. Immunological and virological screenings consisted in the determination of ESR, PCR, RF, ANA, ENA, CIC, AT, ACTA, ACA, LAC, ACE, cryoglobulins, C3, C4, C1q, immunoelectrophoresis, lymphocyte subsets, antineural antibodies, antineutroptic antibodies, detection of viral genome by PCR. Spinal cord imaging was obtained by conventional MRI with T1, T2, DP, Gadolinium-EDTA, FLAIR sequences. Cerebrospinal fluid (CSF) examination consisted in cell count and differentiation, calculation of CSF/Serum albumin ratio and IgG indexes, demonstration of IgG oligoclonal bands by IEF, detection of antibodies to an genomic sequences of neurotrophic viruses.

Results: The onset was monosymptomatic in 25% and polysymptomatic in 75% of the cases; acute in 16, subacute in 16 e chronic-relapsing in 3 cases. A postinfectious origin of the myelitis was documented in 10 patients. In only one case an associations with an autoimmune disease (thyroiditis) was observed. In 2 patients an association with HHV6 infection was demonstrated. IgG OB were detected in one third of the CSF analyzed, but never in the post-infectious and in the chronic-relapsing cases. The diagnosis of Neuromyelitis optica was definitively achieved in three patients, while in one case IM was the presenting case of a Behcet’s disease. Postinfectious myelitis had the most extensive MRI abnormalities. Only IM having IgG OB in the CSF evolved to clinically definite multiple sclerosis.

Conclusions: While confirming the large clinical, MRI and immunological heterogeneity of IM, our study suggests that detailed immunological tests in blood and CSF, neuroimaging may lead to the definition of more appropriate diagnostic procedures.

Disclosure: P Gallo has nothing to disclose.

P205
FATIGUE AND AXONAL LOSS IN CORPUS CALLOSUM IN MULTIPLE SCLEROSIS

Centre Hospitalier de Dijon, Dijon, Bourgogne, France

Background: Recent imaging studies showed that disability correlates with axonal loss in white matter in multiple sclerosis. Magnetic resonance spectroscopy can estimate the axonal pathology with the measurements of the resonance intensity of N-Acetyl-Aspartate. Axonal damage in corpus callosum correlates with cerebral white matter lesion. Few studies showed the mechanism of the fatigue, a prominent symptom in multiple sclerosis.

Objectives: The aim of the study was to correlate the fatigue with the N-Acetyl-Aspartate in corpus callosum.

Methods: Twenty seven patients with relapsing remitting or primary progressive multiple sclerosis were include in a longitudinal study. We matched two patients groups on the basis of the disease duration. One subgroup with eleven patients have a short disease duration (<2 years) and an another subgroup with sixteen patients have a long standing disease duration (>4 years). Fatigue was estimate with a quantitative scale, the Fatigue Severity Scale. Conventional brain imaging and single voxel proton magnetic resonance of the corpus callosum were obtain at intervals of one year with concurrent fatigue evaluation.

Results: At the onset of the study we didn’t found any relation between fatigue and the chemical changes in the subgroup with short disease duration. Strong correlation was found in subgroup with long disease duration between Fatigue Severity Scale and N-Acetyl-Aspartate/Creatine (Spearman rank order correlation=0.603, p=0.05) and N-Acetyl-Aspartate/Choline (Spearman rank order correlation=0.815, p<0.05).

Conclusions: First results suggest that indices of axonal damage or loss in corpus callosum may provide a specific measure of pathological changes relevant to fatigue in patients with long disease duration. This relation is evaluate after one year.

Disclosure: C Greg has nothing to disclose.
CLOMIPHENE CITRATE CAN INCREASE RELAPSE RATE IN MULTIPLE SCLEROSIS

Moreau T1, Gere F2, greg c3, Vernay DP, Clavelou P, Giroud M4
1centre hospitalier de Dijon, Dijon, Bourgogne, France; 2Centre hospitalier universitaire de Clermont-Ferrand, Clermont-Ferrand, Auvergne, France

Background: In MS, the relapses rate varies during pregnancy with a dramatic reduction during the first and the third quarter. On the contrary, there is an increase during the three following months after delivery.

Objectives: The aim of this study was to evaluate if those variations are linked to the hormonal changes.

Methods: We report three cases of exacerbation of relapses in women with MS, treated with clomiphene.

Results: Patient 1, a 27 years old woman, was diagnosed with clinically definite MS according Poser’s criteria in 1997. She suffered two relapses in 1997 but no relapse in 1998 and 1999. She received clomiphene citrate with beta felitrapin in march 2000. The patient had presented three relapses the four following months. A MRI was performed showed new gadolinium enhancing lesions. Patient 2, a 29 year old women, was diagnosed with a definite MS in november 1999, four months after her childbirth. There were no relapses since 1999. She received clomiphene citrate in May 1999. She presented three relapses, in july, november and december 2000. Patient 3, a 32 years old woman, was diagnosed with a definite MS in January 1998. She received clomiphene and citric acid in june 1998. After this treatment, she presented monthly legs paresthesia, during more than 24 hours responding to Poser’s criteria of relapse from July to November 1998.

Conclusions: First choice treatment of chronic anovulation is an anti-oestrogen such as clomiphene or tamoxifen. Clomifen has an antioestrogenic effect on hypothalamus. As shown in PRIMS study, there is a significant decrease of relapses frequency during pregnancy. This may be secondary to physiological hyperoestrogenia wich results is an immunosuppressive effect by reducing pro inflammatory cytokines release. In post partum, there is a rapid decrease of sex hormone’s levels. This might explain the relapses rate increase by an inversion of cytokines balance in favour of the TH1 proinflammatory cytokines. The same mechanism may explain relapses reappearance in clomiphene treated women. Clomiphene antioestrogenic effect may cause a sex hormone imbalance and a release of proinflammatory cytokines.

Disclosure: c greg has nothing to disclose.

ACUTE DISSEMINATED ENCEPHALOMYELOPATHY AND PERIPHERAL NEUROPATHY ASSOCIATED WITH CHRONIC HCV INFECTION

Giannesiini C, Schelp C, Heinzlef O, Rouillet E
Tenon Hospital, Paris, France

Background: Hepatitis C virus is associated with different neurological disorders, most often peripheral neuropathies and in some case central nervous system disease; but in both cases, the mecanism of neurological involvement is a vasculitis. Only one case of acute demyelinating encephalomyelitis associated with hepatitis C virus has been reported.

Objectives: To describe a case of ADEM and peripheral neuropathy associated with HCV infection.

Methods: case report

Results: A 33 years old man developed in a few days a severe cerebellar ataxia and dysarthria, distal and asymmetrical motor deficit and altered mental state. Chronic active hepatitis C had been diagnosed seven years before but not treated. He was complaining for three month of paraesthesia in both feet and hands. On examination he had a static and kinetic cerebellar syndrome, dysarthria, nystagmus and axial hypotonia, bilateral babinski signs; ankle jerks were absent and others were weak. The patient was disoriented and had visual hallucinations. General examination was normal. Usual biological tests were normal, without inflammatory syndrome. Magnetic resonance imaging of the brain and spinal cord showed multifocal lesions, some of wich enhanced after gadolinium injection. Cerebral spinal fluid revealed an aseptic lymphocytic meningitis: 93 cells per mm3, 97% lymphocytes: 1.2 g/l protein, normal glucose. Electroencephalography showed diffuse slow activity and electromyography a diffuse axonal and demyelinating sensorimotor polyneuropathy. All investigations failed to identify any infectious agent in blood or cerebro-spinal fluid. Polymerase chain reaction for hepatitis C virus was positive in blood. Immunologic tests were negative, in particular cryoglobulinemia. A sural nerve biopsy was performed and showed recent axonal degeneration without any sign of vasculitis. The patient received high dose intravenous methylprednisone and rapidly improved.

Conclusions: This is the first case of acute disseminated encephalomyelitis associated with peripheral neuropathy during an active chronic virus C hepatitis.

Disclosure: O Heinzlef has nothing to disclose.

MITOXANDRONE FOR PROGRESSIVE TYPE OF MULTIPLE SCLEROSIS TREATMENT

Giannoulis C, Sarafianos A, Hatzidakis G, Kargadou A, Stavropoulos D, Kargeonouis KE
Multiple Sclerosis Clinic, Athens General Hospital, Neurology Department, Athens, Greece

Background: During the last decade, most of the new therapies in MS are targeted at the relapsing-remitting type of MS. However, the progressive type is a...
severe expression of the disease, and thus there is a great need for therapies against it. **Objectives:** In this study, the aim was to evaluate mitoxandron for the treatment of the progressive type of MS, in cases where other treatments failed. **Methods:** Twelve patients (7 men and 5 women), mean age 39.9 yo (26 to 50), with primary progressive (7 patients) and secondary progressive MS (5 patients), with a mean duration of the disease 13 years, were enrolled in an open study of mitoxandron. All patients were examined before with heart echo, Chest X-ray and blood tests, and were evaluated for their disability according to the EDSS scale. Seven patients were under treatment with interferon beta one two under azathoprine, two under IVIG and one patient was without treatment for 2 years. All of them had a deterioration of their clinical picture during the last few months and wanted to try a different treatment. The inclusion criteria were: age up to 50 yo, good cardiac function, white and red cells without abnormalities, progressive type of MS, failure of previous treatments, no pregnancy or breast feeding. The dose was 12mg/m² for every session. Six sessions were held (one every month) during which, all laboratory examinations were done. **Results:** After the six month period, there was an improvement of the disability, according to the EDSS scale, from 7.29 (5.5-8) to 6.7 (5-7). Side effects were reported only in two patients, myocardopathy and leucopenia. During this time only one had a moderate relapse. **Conclusions:** Mitoxandron is a promising therapeutic drug in the progressive type of MS. However, it is necessary to have a long-term follow-up of these patients to create a more certain opinion, concerning the efficacy of mitoxandron in patients with the progressive type of MS.

**Disclosure:** K Karageorgiou has nothing to disclose.
patients but it fails to evaluate a number of functions such as cognition, mood, vision and upper limb dexterity. Guy's Neurological Disability Scale (GNDS) may turn out to be a good alternative both to EDSS and MSFC and the MSFC domains.

**Objectives:** To report the results of GNDS on a sample of Brazilian MS patients, correlating it with the EDSS.

**Methods:** GNDS is a multidimensional clinical scale which provides information on 12 functional domains. The domains were graded according to their severity and their impact on patients. The total score ranges from 0 (no disability) to 60 (maximum possible disability). GNDS was individually administered to a group of relapse-free MS patients in whom the EDSS score had been shortly assigned. Additional clinical data were collected from medical record.

**Results:** Thirty-nine CDMS patients (27 women and 12 male) were selected at random from our MS Research Center. Their mean age was 42.08 years (sd=9.1), mean formal schooling was 10.23 years (sd=4.25) and the mean disease duration 8.66 years (sd=7.83). There were 23 patients with relapsing-remitting MS, 11 with secondary progressive MS and five with primary progressive MS. The median EDSS was 3.5 and the mean Ambulation Index (AI) 1.9 (sd=2.51). The GNDS mean total score was 12.9 (sd=9.79). In patients with EDSS below 3.5 the mean total score was 8.86 (sd=6.89); in patients with EDSS between 4.0 and 6.5 the mean total score was 14.67 (sd=9.08) and with EDSS 7.0 or higher the mean total score was 32.33 (sd=2.52). The most impaired domains were fatigue, mood, other disabilities, lower limb and sexual function and cognition.

**Conclusions:** In our sample GNDS total score correlated well with EDSS scores. Further studies correlating the various domains of the GNDS with function-specific scales or tests may provide better information about the value of the GNDS as a definite outcome measure tool.

*Disclosure:* M Lana-Peixoto has nothing to disclose.

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**P214**

**MULTIPLE SCLEROSIS WITH HYPER SIGNAL-FREE T2-WEIGHTED MRI**

**Lebrun C**, Bourg V, Chanalet S, Soriani M, Vermersh P, Chatel M

*Neurology, CHU Pasteur, Nice, Am, France; Radiology, CHU Pasteur, Nice, Am, France*

**Background:** The use of new MRI criteria is recommended to confirm diagnosis of multiple sclerosis. Some patients have experienced relapsing neurological symptoms but white matter lesions are not visible on brain or spinal MRI to ensure MS. Nevertheless, other paraclinical investigations (CSF sample or evoked potentials) can be in favor of dissemination of the disease in space and time according to McDonald criteria (2001).

**Objectives:** We collected prospectively in Nice MS database patients with relapsing or progressive neurological symptoms fitting with MS but hypersignal-free brain and spinal T2-weighted MRI.

**Methods:** Fifteen cases have been gathered and studied for demographic characteristics, blood tests, CSF analysis, Visual Evoked Potentials (VEP), brain and spinal MRI with 6 month-interval, 5-yr MRI follow up and brain spectroscopy of normal appearing white matter (NAWM).

**Results:** The demographic features and characteristics of MS patients were: age: 16-49 years; mean time of neurological evolution: 6.86 years; progressive disease: 2/15; relapsing-remitting disease: 13/15 (61 declared relapses); abnormal CSF: 9/15 (oligoclonal bands: 2); abnormal VEP: 7/15, N acetyl aspartate decrease in NAWM: 5/15. Ten patients had CDMS according to Poser criteria, 11 were MS according to Mc Donald criteria, 3 were corresponding to Devic syndrome according to Wingerchuck criteria.

**Conclusions:** Absence of MRI hypersignal in FLAIR or T2-weighted sequences in patients with objective neurological relapsing polyssymptomatic symptoms is confusing if we considered them as potential MS patients. Many hypothesis can be envisioned: MRI sensitivity, pre-MS state with clinical good prognosis, expression of axonal lesions. Until now, we considered those patients as authentic relapsing or progressive MS. Given the above-mentioned difficulties, indications for immunomodulatory treatment should be carefully evaluated.

*Disclosure: C Lebrun has nothing to disclose.*

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**P213**

**ACUTE DEAFNESS REVEALING MULTIPLE SCLEROSIS**

**Lebrun C**, de seze P, Bourg V, Soriani M, Vermersh P, Chatel M

*Neurology, CHU Pasteur, Nice, Am, France; CHU Lille, Lille, N, France*

**Background:** Hearing loss is an uncommon presenting symptom (1%) declaring MS, while symptoms of brain-stem demyelination occur in more than 20% of cases during either one of the first relapses of the disease.

**Objectives:** To determine the frequency and clinical evolution of patients revealing MS by acute deafness.

**Methods:** Documented MS patients who has revealed MS with acute deafness were selected out of the files of 950 patients in Northern (Lille=L) and Southern (Nice=N) French MS databases. Every patients had documented MS according to Mc Donald criteria, 11 were MS according to Poser criteria, 11 were MS according to Mc Donald criteria, 3 were corresponding to Devic syndrome according to Wingerchuck criteria.

**Results:** Twelve patients (1.2%) were identified: 10 females, 2 males, mean age 32.5 years, median age N=32 (24-54) ; L=33 (22-48), unilateral deafness (11/12). MS diagnosis was suspected after this first symptom for 3 of them who presented subacute signs of myelitis or brain stem involvement simultaneously. All patients had brain T2 hypersignals corresponding to MS and CSF examination with for 6/12 positive oligoclonal bands. 4/6 had increased IgG index. In all cases, hearing loss subjectively recovered completely after intravenous methylprednisolone administration but BAEPs remained abnormal. The time before a second relapse was about 27.25 months (L median time 27.5 : 9-54 ; N median time 27 : 12-48).

**Conclusions:** Although unilateral or bilateral hearing loss may occur in MS, it has rarely been reported as an initial solitary manifestation. After a mean follow-up of 7 years, all patients remain in relapsing-remitting form with mean EDSS score of 2.5. Clinical prognosis appears to be good despite MRI Barkhof criteria are fulfilled at presentation. Patients profiles in North and South of France are identical.

*Disclosure: C Lebrun has nothing to disclose.*

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**P215**

**PROGNOSIS IN RELAPSING-REMITTING MULTIPLE SCLEROSIS. A HIERARCHICAL MODEL.**

**Martinez-Yélamos S**, Casado V, Carmona O, Martinez-Yélamos A, Ramón JM, Arbizu T

*Multiple Sclerosis Unit, C.S.U.Bellvitge. Universitat de Barcelona, L’Hospitalet de Llobregat, Barcelona, Spain; Neurology, Hospital de Viladecans, Viladecans, Barcelona, Spain; Public Health Department, C.S.U. Bellvitge.Universitat de Barcelona, L’Hospitalet de Llobregat, Barcelona, Spain*

**Background:** Some clinical variables may be related with the long-term outcome in patients with MS.

**Objectives:** To analyze the prognostic variables related to three different endpoints in patients with MS: a) incomplete recovery after the first relapse b) time to EDMUS Impairment Scale (EIS) 6, and c) time to the onset of secondary progressive phase in a series of patients with RRMS.

**Methods:** Clinically-based series of 452 patients with RRMS at onset. Standardized follow-up was performed by means the EDMUS. No patients were treated with disease modifying agents. Variables were included in a hierarchical way, based on the sequence in which clinical information is generally revealed. Statistics: Logistic regression and Cox regression for survival models.

**Results:** Variables with independent prognostic relevance for incomplete recovery after the first relapse were: Sex (men): ROR=1.90 (1.20-3.00); age at onset (10 yrs.): ROR=1.37 (1.08-1.75), lower extremity dysfunction: ROR=4.11 (2.24-5.64); sphincter disturbance: ROR=4.51 (2.06-0.89). The time to reach a severe residual disability (EIS 6) were related to: Incomplete recovery after the first relapse: ROR=2.51 (1.34-4.72), first interattack interval (1 yr.): ROR=0.86 (0.78-
0.94), and time to first examination at the MS'unit (1 yr.): ROR=0.92 (0.88-0.97). Parameters associated with early conversion to secondary MS were: Age at onset (10 yrs.): ROR=1.40 (1.06-1.86); incomplete recovery after the first relapse: ROR=1.56 (1.72-2.09) and first interattack interval (1 yr.): ROR=0.92 (0.86-0.98).

Conclusions: In patients with RMS, an incomplete recovery after the first relapse and a short first interattack interval are related with an earlier onset of SPMS and with a shorter time to reach a severe residual disability.

Disclosure: S. Martinez-Yelamos has nothing to disclose.

P216

CLINICAL ISOLATED MYELITIS: EARLY PREDICTION OF MULTIPLE SCLEROSIS BASED ON MAGNETIC RESONANCE IMAGING AND CEREBROSPINAL FLUID FINDINGS

Meluzínová E, Bojar M, Houzovická E, Gloslová L, Belsan T

*Neurology, Charles University, 2nd Medical School, Motol Hospital, Prague, Czech Republic; †Radiology, Charles University, 2nd Medical School, Motol Hospital, Prague, Czech Republic

Background: The term myelitis is used to describe inflammatory involvement of the spinal cord. Etiology of this disorder can be variable and it may occur as the first attack of multiple sclerosis (MS). Whether the patient presenting with myelitis as the first symptom develops in later course MS is often difficult to predict.

Objectives: The aim of this study is to assess if MS patients presenting in onset with myelitis can be distinguished on the basis of MRI findings from patients with myelitis of another aetiology.

Methods: 34 patients presenting with clinical isolated myelitis were included in this study. MRI of the spinal cord and MRI of the brain and CSF analysis were performed at the onset of the disease in all the patients. After 6 months to 6 years of follow-up diagnosis of MS or myelitis of another cause was concluded.

Results: Spinal cord MRI was abnormal in all the patients, brain MRI was abnormal in 13 patients, CSF finding was abnormal in 29 patients: pleiocytosis and/or higher protein level were found in 22 patients, positive oligoclonal bands (OB) in 23 patients. Six patients were diagnosed as having MS on follow-up. Retrospectively 5 of them had abnormal brain MRI, all of them had more than one lesion on spinal MRI and all of them were OB positive when examined for myelitis.

Conclusions: In patients with clinical isolated myelitis the association of abnormal brain MRI with multifocal spinal cord lesions and with positive OB is highly predictive for later progression to MS. Isolated spinal cord lesion affecting multiple thoracic segments seems to have better prognosis and low probability for progression to MS.

Disclosure: E Meluzínová has nothing to disclose.

Funding: Supported by research program No.111300003.

P217

COLLABORATIVE STUDY ON CHILDHOOD ONSET MULTIPLE SCLEROSIS IN FRANCE (KIDMUS): ABOUT A COHORT OF 495 PATIENTS

Mikaeloff Y*, Tardieu MP, Catherine L, Edan G, Vallee L, Ponsot G, Confavreux C

*Pediatric neurology, Saint-Vincent de Paul Hospital, Paris, France; †Pediatric neurology, Kremlin-Bicêtre Hospital, Paris, France; ‡Neurology, Pitie-Salpetriere Hospital, Paris, France; §Neurology, CHU, Rennes, France; ¶Pediatric neurology, CHU, Lille, France; ‡Neurology, CHU, Lyon, France

Background: The term myelitis is used to describe inflammatory involvement of the spinal cord. Etiology of this disorder can be variable and it may occur as the first attack of multiple sclerosis (MS). Whether the patient presenting with myelitis as the first symptom develops in later course MS is often difficult to predict.

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Conclusions: In patients with clinical isolated myelitis the association of abnormal brain MRI with multifocal spinal cord lesions and with positive OB is highly predictive for later progression to MS. Isolated spinal cord lesion affecting multiple thoracic segments seems to have better prognosis and low probability for progression to MS.

Disclosure: E Meluzínová has nothing to disclose.

Funding: Supported by research program No.111300003.

P218

PATTERNS OF MS TREATMENT WITH DISEASE MODIFYING THERAPIES BEFORE ENTRY INTO AN OPEN-LABEL CLINICAL TRIAL OF REBIF® INJECTIONS

Mikol D, Burns TP, Bennett S, Lopez-Bresnahan M

*Department of Neurology, University of Michigan, Ann Arbor, Michigan, USA; †Data Management, Serono, Inc., Rockland, Massachusetts, USA; ‡Clinical Development, Serono, Inc., Rockland, Massachusetts, USA

Background: Disease modifying drugs (DMDs) approved for MS in the US include interferons, Rebif® and Avonex® (interferon beta-1a) and Betaseron® (interferon beta-1b), glatiramer acetate (Copaxone®), and mitoxantrone (Novantrone®). No definitive algorithm exists for use of DMDs, and patients may be treated with one or all of these during their course.

Objectives: To assess previous use of DMDs in MS patients entering a study of Rebif® injections.

Methods: 1870 patients with RRMS were randomized, in a multi-center, parallel group, open-label, 12-week study comparing the tolerability of Rebif® injections (44 mcg TIW) with and without RebifTM Mini, an auto-injection device. Injection site reactions are assessed on a weekly basis. Previous DMD treatment was recorded at the time of entry. The study is ongoing.

Results: Preliminary results are available for 991 patients. Previous DMD Treatment: Of 991 patients 277(28.0%) had no previous DMD treatment; 714(72%) had one or more DMDs; 499(50.4%) had one DMD; 165(16.6%) two DMDs; 44(4.4%) three DMDs; and 6(0.6%) four DMDs. Previous Treatment with Specific DMDs: At study start Rebif® was not commercially available in the US. Of 991 patients 516(52.1%) had previous treatment with Avonex®; 223(22.5%) with Betaseron®, 176(17.8%) with Copaxone®, and 15(1.5%) with Novantrone. 48(4.8%) had received other therapies not otherwise specified. Therapy Switches Before Study Entry: Of 991 patients, 600(60.5%) discontinued one or more MS treatments no more than 30 days before entering the Rebif® study. Of the 600 patients 402(600(67.0%) discontinued Avonex®, 101(600(16.8%) Betaseron®, 97(600(16.1%) Copaxone®, 8(600(1.3%) Novantrone®, and 18(600(3.0%) discontinued other therapies not otherwise specified.

Conclusions: Currently, no definitive algorithm exists for use of DMDs in MS. These preliminary results show that most patients entering a study in RRMS have been treated with at least one, and up to four, DMDs. As more data regard-
ing effective doses of DMDs, and especially of interferons, become available and widely accepted, a rational pattern of use of these therapies will emerge.

Disclosure: I have received honoraria from Berlex, Biogen, Immunex, Serono and Teva (speaker’s bureau for Berlex, Immunex and Serono).
Funding: Supported by Serono, Inc.

P219

FATIGUE IN MULTIPLE SCLEROSIS. A LONGITUDINAL STUDY

Clinical Neuroimmunology Unit, University Hospital Vall d’Hebron, Barcelona, Catalonia, Spain

Background: Fatigue is one of the most troubling symptoms in patients with MS. Longitudinal studies of fatigue are scarce.

Objectives: The aim of the study was to assess the changes on fatigue over time in a cohort of consecutive MS patients and to correlate them with changes in disability and mood status.

Methods: The Modified Fatigue Impact Scale (MFIS), the Fatigue Severity Scale (FSS), the Beck depression inventory (BDI) and EDSS were applied to 144 consecutive MS patients. After a period of at least one year, all patients were re-assessed. Patients suffering a relapse at the time of evaluation were excluded.

Results: Fatigue (FSS*5) was observed in 58% of MS patients at baseline and in 59% after a mean follow-up period of 17 months. Most patients (83%) with fatigue at baseline remained fatigued after follow-up. Sixty-one percent of patients without fatigue at baseline remained without fatigue after follow-up. Although EDSS score was significantly higher (3.1 vs 2.6, p=0.0001) over time in the total cohort, no significant differences were observed in BDI, FSS and MFIS scores over this period of time. Changes in fatigue (MFIS score) correlated significantly with changes in BDI (r= 0.48, p<0.0001). However, no correlations were seen between disability changes and fatigue score changes.

Conclusions: Patients with MS and fatigue remain fatigued after short-term follow-up. Changes in mood status, though not in disability score, may influence changes in fatigue over time.

Disclosure: X Montalban has nothing to disclose.

P220

RELAPSING MMLELITIS: A DEMYELINATING SYNDROME DISTINCT FROM MULTIPLE SCLEROSIS

Nicholas R, Fletcher N, Boggild M
The Walton Centre for Neurology and Neurosurgery, Liverpool, Merseyside, United Kingdom

Background: Relapsing myelitis (RM) is a rare demyelinating syndrome that consists of recurrent episodes of spinal cord symptoms and signs. Unlike MS, which is characterised clinically by recurrent neurological symptoms and signs disseminated in time and place, in RM the deficits appear to relate to a single spinal cord lesion.

Objectives: To establish the clinical features, imaging and immunological characteristics of RM and ascertain its response to immunological therapy.

Methods: Retrospective review of the case notes of patients with a diagnosis of RM who are currently under review at the Walton Centre.

Results: 8 patients (5 women, 3 men) were identified with RM in whom the mean age of onset was 45 years (range 37-57). There were on average 3.5 relapses/patient (range 2-7 relapses) during a mean follow-up of 4.5 years (range 2-9 years), no patients showed evidence of progression between relapses. MR imaging of the brain was normal in 5 and in the remaining 3 patients abnormalities atypical for demyelination were seen. Spinal cord imaging revealed a single inflammatory lesion in 7 and in the remaining patient imaging was normal on two occasions. CSF analysis showed oligoclonal bands in 4 and increased lymphocytes and/or elevated CSF protein in 3. Acute relapses responded to intravenous or oral steroids. In 5 patients long-term immunosuppression was initiated and has been continued for a mean of 2 years (range 4 months - 4 years) this consisted of azathioprine +/- prednisolone in 4 and cyclosporin +/- prednisolone in 1 patient. On immunosuppression the relapse rate fell from 1.1 relapses/year to 0.2 relapses/year. 1 patient had two relapses whilst on immunosuppression whilst no relapses were seen in the remaining 4.

Conclusions: This is the largest group of RM patients presented. During the period of follow-up no patient with RM has developed MS as defined by the MacDonald criteria. This entity appears to represent a distinct demyelinating syndrome. Follow-up to date would suggest there is a response to long term immunosuppression in this group

Disclosure: RS Nicholas has nothing to disclose. NA Fletcher has nothing to disclose. MD Boggild has received grant support for staff from Serono and is on an advisory panel for Teva and Biogen

P221

HAEMATOLOGICAL ABNORMALITIES RELATED TO INTERFERON BETAL-1A THERAPY

University of Toronto, Toronto, Ontario, Canada; 5Serono, Inc., Rockland, Massachusetts, USA

Background: Interferon (IFN) therapy is associated with haematological abnormalities.

Objectives: To analyse the haematological effects of IFN therapy in MS patients.

Methods: Safety data were pooled from 6 controlled studies for patients on IFN beta-1a 22-132mcg weekly in single or divided doses and placebo. Data were analysed after 6 months (1995 IFN-treated and 824 placebo patients) and 2 years (789 IFN-treated and 389 placebo patients) for haematological laboratory abnormalities reported as adverse events (AEs). The overall safety population of 3996 patients was analysed for AE dropouts and serious haematological AEs (SAEs).

Results: In the pooled safety population, 6-month data revealed leucopenia in 1.8% of placebo patients, 0.3-2.0% of once weekly IFN patients (22, 30, and 44mcg) and 7.3-10.2% of three times weekly (twi) IFN patients (22 and 44mcg). Lymphopenia was reported as an AE in 3.8%, 11.1% and 11.8% of patients on placebo, 22mcg twi and 44mcg twi, respectively. Neutropenia rates were 1.3%, 6.0% and 7.2%. Over 2 years, lymphopenia/leucopenia were reported as AE in 27%/20% of 44mcg twi patients, 19%/11% of 22mcg twi patients and 13%/4% receiving placebo. On patients 22 and 44mcg twi with WBC abnormalities, 12.5% and 18%, respectively, were graded severe. Mild thrombocytopenia (<75,000 platelets) was seen in 7.2% and 2.5% of patients on 44 and 22mcg twi vs. 1% on placebo. Anaemia was reported in 6% and 2% of patients on 44 and 22mcg twi vs. 3% on placebo.

Data demonstrate that hematological abnormalities diminish over time and are not associated with increased risk of infectious events. Although the proportion of patients on 44 mcg twi with any grade lymphocyte abnormality is up to 43% over two years, only 23% are abnormal at the end of year 2 demonstrating tachyphylaxis for lymphopenia. Of those developing lymphopenia, 61% did so within 3 months of starting therapy. Cytopenias were asymptomatic and reversible with dose adjustments. Only 0.3% (12/3996) of patients stopped therapy due to cytopenia. Of the 8 WBC SAEs reported, 7 were lymphopenia.

Conclusions: Cytopenia, predominantly affecting the WBC lineage is common with IFN therapy and is dose related. Events are asymptomatic, not associated with increased infections, and rarely lead to discontinuation of therapy.

Disclosure: P O’Connor has served as consultant to Serono; F O’Brien, J Alsop, P Chang, Y Grumser, and J Abdalla are employees of Serono

Funding: Supported by Serono, Inc.
P222

OPTIC NEURITIS: CORRELATION OF CLINICAL, VISUAL FIELD AND NEUROIMAGING FINDINGS AND THEIR PROGNOSTIC ROLE


*2nd University Department of Neurology, Aristotle University, Thessaloniki, Greece; 1Department of Ophthalmology, Aristotle University, Thessaloniki, -, Greece; 1Department of Radiology, Aristotle University, Thessaloniki, -, Greece

Background: Optic neuritis (ON) can be defined as visual loss caused by primary demyelination of the optic nerve. It is widely believed that monosymptomatic ON (MON) is usually a first manifestation of multiple sclerosis (MS). The proportion of patients with MON who will ultimately develop MS, is still a matter of debate.

Objectives: To analyze the findings of automated perimeter and neuroimaging in patients with ON and define possible prognostic factors that can predict the evolution of ON or its progression to MS.

Methods: Twenty patients with ON (3 men-17 women, age 17-52 years) were studied retrospectively. Findings of the examination of visual fields with automated perimeter and of brain, spinal cord (cervical and thoracic), optic nerves and paranasal sinuses with magnetic resonance imaging (MRI) during the acute phase of the disease were evaluated. Optic nerves were examined by MRI for abnormal enhancement and signal, atrophy or swelling and/or enlargement of its subarachnoid spaces. The ON was a manifestation of MS with relapsing-remitting course in 8 of the 20 patients (all women); the remaining 12 had MON (3 men-9 women). Other pathological causes of ON were excluded during a 2-year follow-up.

Results: Out of 12 patients with MON, 7 had brain and/or spinal cord demyelinating lesions revealed by MRI, and pathological visual fields (3 bilateral); 5 of them later developed MS and all had positive brain and spinal cord MRI. Out of 8 patients with MS, 7 had pathological visual fields and 3 had positive MRI of the optic nerves. In 4 patients we observed paranasal sinuses, in two of which MON was associated with enhancement of the optic nerve.

Conclusions: Asymptomatic regions in the optic nerves, brain and spinal cord revealed by MRI increase the likelihood for the progression of ON to MS. Correspondingly, the type of optic nerve MRI findings (atrophy or swelling) correlate with the severity of initial visual loss and could be predictive of visual recovery.

Disclosure: M Paschalidou has nothing to disclose.

P223

ASSOCIATION OF UVEITIS AND MULTIPLE SCLEROSIS COULD INFLUENCE THE CLINICAL COURSE OF MULTIPLE SCLEROSIS

Patrick V, Jean-yves G, Pierre L, Didier F, Tanya S, Albert V, Jérôme D

Neurology, University of Lille, Lille, France

Background: Uveitis is more common in patients with multiple sclerosis (MS) than in the general population.

Objectives: The aim of the study was to compare the clinical data of MS patients with or without uveitis.

Methods: MS patients were included according to the McDonald criteria. In the subgroup of patients with uveitis (MS-U), only patients with clinical symptoms of uveitis were included and classified according to the international uveitis study group. Other inflammatory diseases (sarcoidosis, Behcet disease) were excluded in all patients. To avoid biases, for each MS-U patients, we included two MS patients consecutively seen at the same outpatient clinic.

Results: Fifteen MS-U patients and 30 MS patients were compared. Types of uveitis were panuveitis, pars planitis and anterior uveitis in 9, 4 and 2 cases respectively. In 9 cases, neurological signs occurred before onset of uveitis. Mean ages at onset of neurological symptoms were 29 years in both groups. Mean disease durations were 11.6 and 10.3 years in the MS-U group and in the MS group respectively. In the MS-U group, the evolution was relapsing-remitting (RR) in 14/15 cases and secondary progressive (SP) in one case. In the MS group, the evolution was RR in 16/32, SP in 11/32 and primary progressive in 5 cases (p < 0.01). The mean EDSS scores were 2.6 and 5 (p < 0.002) and the mean relapse rates during the first two years of MS were 1.8 and 2.6 (p < 0.05) in the MS-U and MS groups respectively.

Conclusions: MS patients with uveitis seem to have a better prognosis than MS patients without uveitis. Specific immune targets or dysregulation could explain different clinical courses in MS

Disclosure: V Patrick has nothing to disclose.

P224

THE EFFECT OF CORTICOSTEROID ON CONDUCTION IN THE VISUAL PATHWAYS: A SERIAL STUDY USING VISUAL PSYCHOPHYSICS.

Pye EM, Weatherby SP, Kesson D, Foster DH, Hawkins CP

*Dept. of Neurology, North Staffs Royal Infirmary, Stoke-on-Trent, Staffordshire, United Kingdom; 1Dept. of Optometry & Neuroscience, UMIST, Manchester, Gtr Manchester, United Kingdom

Background: Acute relapses of MS are characterised by episodes of neurological dysfunction secondary to CNS inflammation, oedema and demyelination. IV corticosteroid is the mainstay of treatment, hastens resolution of acute inflammatory change and promotes more rapid recovery. Subclinical involvement of the visual pathways in MS is common. Visual psychophysics provides an established measure of sensory deficit, which may be selective for different functional pathways. A course of corticosteroid might be expected to improve signal conduction in affected pathways, leading to measurable improvements in vision.

Objectives: To further elucidate the nature of the sensory deficit and effect of corticosteroid on conduction in the visual pathways during an acute MS relapse we undertook a serial study using visual psychophysics.

Methods: 5 patients (10 eyes) experiencing an acute, non-visual MS relapse having a three-day course of IV corticosteroid, had visual psychophysics performed at baseline, after the third dose and again one week later. Contrast thresholds were measured at three spatial frequencies (0.25, 1.0 and 4.0 cyc/deg) with counter-phase (chromatic-modulated) and in-phase (luminance-modulated) red-green gratings.

Results: At low spatial frequency (0.25 cyc/deg) throughout the study, chromatic thresholds were significantly lower than corresponding achromatic thresholds (p < 0.0003). At low and medium spatial frequencies a non-significant improvement in both chromatic and achromatic thresholds from baseline was seen following the third dose and was sustained at one week. At high spatial frequency (4.0 cyc/deg) improvements in chromatic and achromatic thresholds from baseline were not seen until one week post-corticosteroid.

Conclusions: MS patients have subtle, subclinical deficits in vision that are not significantly improved by a course of corticosteroid. We suggest this is because chronic visual deficit may result from permanent structural injury e.g. glossos and axonal loss. A degree of acute inflammatory change may, however, still be present as shown by the trend towards improvement in contrast thresholds. This trend favoured neither chromatic nor achromatic pathways.

Disclosure: E Pye has nothing to disclose.

P225

A COMPARISON OF BILATERAL SIMULTANEOUS AND BILATERAL SEQUENTIAL OPTIC NEURITIS USING VISUAL PSYCHOPHYSICS.

Pye EM, Weatherby SP, Kesson D, Foster DH, Hawkins CP

*Dept. of Neurology, North Staffs Royal Infirmary, Stoke-on-Trent, Staffordshire, United Kingdom; 1Dept. of Optometry & Neuroscience, UMIST, Manchester, Gtr Manchester, United Kingdom

Background: Bilateral optic neuritis (BON) affects the eyes either simultaneously (BsimON) or sequentially (BseqON). Pathogenesis may differ. Visual

Disclosure: V Patrick has nothing to disclose.
psychophysics provides an established measure of sensory deficit, which may be interpreted in terms of large and small fibre pathways.

**Objectives:** We wished to investigate the nature of the visual deficits in BON using visual psychophysics and determine whether these differed in the two clinically distinct forms of BON.

**Methods:** Visual psychophysics was performed on 4 patients (7 eyes) with BismON, i.e. both eyes affected within one week and 4 patients (8 eyes) with BsegON, i.e. second eye affected more than 3 months after the first (range 3-72), and 5 controls (10 eyes). Mean time from optic neuritis to psychophysical testing was 41 months for BismON and 31 months for BsegON. Contrast thresholds were measured at 3 spatial frequencies (0.25, 1.0 and 4.0 c/deg) with counter-phase and in-phase red and green gratings.

**Results:** Visual acuity recovered to at least 6/9 in 5/7 BismON eyes and 7/8 BsegON eyes but all eyes had thresholds higher than controls at all spatial frequencies. Mean thresholds were 7 times greater than controls in BismON eyes (p<0.0001), although not significantly different from controls in BsegON eyes (p=0.04). BismON eyes appear to have a preferential parvocellular deficit at 0.25 c/deg (p<0.05).

**Conclusions:** Despite good recovery of visual acuity in most eyes, visual psychophysical thresholds were much higher in BismON eyes than BsegON eyes. The small fibre parvocellular pathway seemed preferentially affected.

Disclosure: E Pye has nothing to disclose.

**P226**

**PHYSICAL ACTIVITY AND FATIGUE IN MULTIPLE SCLEROSIS PATIENTS INITIATING INTERFERON-BETA-1A THERAPY.**

**Riskind P, Brown V, Kevin K**

Neurology, UMMHC, Worcester, Massachusetts, USA

**Background:** One limitation in understanding MS fatigue is the lack of any objective measures, other than standard questionnaires.

**Objectives:** We postulated that assessment of physical activity might be a useful objective means of assessing changes in MS fatigue. Since initiation of interferon therapy may be associated with transiently increased fatigue, we studied activity and energy in patients initiating interferon beta-1a therapy (30 mcg IM once weekly).

**Methods:** Twenty-five interferon-naive patients with RRMS were monitored with wrist actigraphic devices for 2 weeks pre-therapy and 6-months post treatment. Subjective energy (VAS) was documented daily by each patient.

**Results:** Post-injection day energy levels were lowest during the first week of treatment, but increased in a linear manner, reaching baseline at 5 weeks. In contrast, activity on the days pre- or post-injection did not significantly change over the initial 10 weeks of therapy. Daily energy and activity was significantly but minimally correlated (r=+0.11, p <0.001). Median weekly energy and activity scores in individual patients over 10 week epochs were more closely correlated than daily scores being either positive or negative (r=−0.809 to +0.836). Activity and energy decreased during flares (both p<0.001). Activity, but not energy scores were also significantly lower during flares than the week before flares (p<0.001). Mean energy scores throughout the study correlated with entry assessments of global fatigue (Schwartz J, Jandorf L, Krupp L 1993) (r=−0.62, p<0.001) but not with entry BDI or other fatigue subscales. Mean activity during the study did not correlate with entry BDI or fatigue scores.

**Conclusions:** These results suggest that subjective energy does not substantially limit or modulate daily physical activity in MS patients initiating interferon beta-1a therapy. However, activity is a more objective and dynamic measurement than energy assessment and may be of value as an independent outcome measure in MS patients.

Disclosure: Dr. Riskind has received honoraria and grant support from Biogen and is on the Biogen Speakers Bureau. Funding: Supported by Biogen.

**P227**

This abstract was also presented at the platform.

**COURSE AND PROGNOSIS IN EARLY ONSET MULTIPLE SCLEROSIS.**


*Department of Neurological and Psychiatric Sciences, Clinical of Neurology, Bari, Italy; †Department of Clinical Pharmacology and Epidemiology, Istituto di Ricerche Farmacologiche Mario Negri- Consorzio Mario Negri Sud, S. Maria in Baro, Chieti, Italy

**Background:** Older age at onset of multiple sclerosis (MS) is frequently associated to a poor prognosis; by contrast the role of an early onset MS (EOMS) with regard to long-term outcome is not well defined.

**Objectives:** To assess the prognostic role of clinical and demographic factors in MS patients categorized by age at onset.

**Methods:** We analyzed a hospital-based historical cohort of 793 MS patients. Eighty-three patients were classified as EOMS (<16 yrs) and 710 as adult onset MS (>16 yrs) (AOMS). Patients were observed for a mean period of 5 yrs. Univariate and multivariate analyses of predictors for rapid progression (secondary progressive-SP- course) and disability (EDSS 4) were performed using a stepwise COX regression model with time dependent covariates.

**Results:** Female/ratio did not differ in EOMS and AOMS, however a female predominance occurred in puberal period. Despite a longer disease duration in EOMS (p=0.0006), the EDSS evaluated at last examination was lower (p<0.0001) than in AOMS. The probability to reach growth disability and SP course was lower in EOMS than in AOMS (p<0.001). Median times to reach EDSS 4 and SP were longer in EOMS than in AOMS (p<0.001), but the age at both end points was significantly lower in EOMS (p<0.002). In both MS groups an irreversible disability was associated with SP course (p<0.001) and sphincteric system at onset (p=0.041, p=0.007); in AOMS other factors were an older age at onset (p=0.001), a pyramidal system at onset (p=0.002) and high relapse frequency in the first two yrs (p=0.001). The risk of entering SP course was influenced by high relapse frequency in the first two yrs (p=0.016) in EOMS and by a higher age at onset (p<0.001) and a short first inter-attack interval (1 year) (p=0.022) in AOMS.

**Conclusions:** A slower rate of progression disease characterized EOMS, suggesting a more plasticity to recover than AOMS. Nevertheless early clinical manifestation cannot be considered as a positive prognostic factor.

Disclosure: I Simone has nothing to disclose.

**P228**

**MS PHENOTYPE : AN AGE-DRIVEN DISEASE ?**


Neurologie A, Hôpital Neurologique, Lyon Cedex 03, France

**Background:** Age at onset of multiple sclerosis (MS) has been shown to be a good predictor of the time spent to reach different levels of disability. However, it can be questioned if disability could be mostly age-driven.

**Objectives:** The aim of this study was to determine whether ages at which MS patients reach DSS 4, DSS 6 and DSS 7 were different according to their age at onset of the disease.

**Methods:** The Lyon MS database was closed in April 1997 for the purpose of epidemiological studies. It contained then data of 1844 definite or probable MS patients, with a mean follow-up of 10 years. Eighty five percent had a relapsing-remitting onset and 15 % a progressive one. Patients were stratified according to their age at MS onset in five classes : less than 20 years, 20 to 29, 30 to 39, 40 to 49, 50 years or more. For each patient, we determined the age at entry into irreversible levels of disability, as measured by the Kurtzke Disability Status Scale. Three key levels of disability have been chosen : DSS 4, DSS 6 and DSS 7. When a patient was lost to follow-up or had not reached the defined level of disability, censoring was done at the age of the last visit. We performed survival analyses by the Kaplan-Meier method to determine the median age at DSS 4, DSS 6 and DSS 7, for each initial course and class of age at onset, and compared the results by the log-rank test.

**Results:** A total of 1026 patients (56 percent), 595 (32 percent), and 380 (21 percent) reached DSS 4, DSS 6 and DSS 7 respectively. In relapsing-remitting...
onset patients, the median age at DSS 4, 6 and 7 were 44.8, 55.3 and 62.8 respectively. In progressive onset ones, they were 42.1, 53.0 and 63.1 respectively. These results were significantly different for DSS 4 and 5 (p = 0.001 and 0.002), but not for DSS 7 (0.24). However, even when statistically different, it can be underlined that the differences were not significant at the clinical level.

Conclusions: Analyses for the role of age at MS onset are still in progress. Results will be available in September 2002.

Disclosure: S Vukasic has nothing to disclose.

P229

NEUROGENIC SYNCOPE IN MULTIPLE SCLEROSIS PATIENTS

Zapletalova O, Dolezil D, Hradilek P, Stipal R

Background: Heart disorders with reduction of cerebral blood flow raising neurological symptoms are widely known. The same attention should be devoted to the opposite situation, when brain pathology could influence heart function.

Objectives: Various central nervous system (CNS) disorders could result in important cardiac dysfunction. Electrocardiographic changes in brain ischemia and subarachnoid haemorrhage or in brain tumours are referred. CNS lesions could cause autonomous failure in degenerative diseases like Shy Drager syndrome, systemic atrophy or in high cervical spinal injuries. We suggest, that CNS lesion in multiple sclerosis (MS) could also influence circle arising from insular cortex through hypothalamus, limbic structures, oblongata to intermediolateral funicules of spinal cord. These changes could present as abnormal reflex, which could result in syncope. Syncope is defined as transient loss of consciousness and postural tone, which is caused by global reduction of cerebral blood flow. There are two types of syncope: 1) cardio- genetic 2) neurogenic. The second one could have trigger zone in peripheral reflex circle or could be result of activation of central part of autonomous nervous program.

Methods: We present case report of MS woman with syncopes. During repeated head up tilt table test 25- second asystoly occurred with need of resuscitation. Malignant form of neurocardiogenic syncope was disclosed and cardio stimulator was implanted.

Results: We identified 11 patients with and without the ε4 allele. Clinical and demographical characteristics of the two groups were comparable. Both groups showed similar significant (p=0.003) decreases in central brain NAA levels (normalized to creatine [Cr]) with respect to NC (NAA/Cr in ε4 patients = 2.83±0.18, NAA/Cr in patients without ε4 allele = 2.77±0.14, NAA/Cr in NC = 3.05±0.25). In contrast, patients carrying the ε4 allele showed a significantly lower normalized brain volume (NBV) values than NC (NBV in ε4 patients = 1560±49 cc, NBV in NC = 1617±35 cc, p<0.03). Values of NBV were not significantly different between patients without the ε4 allele and NC (NBV in patients without ε4 allele = 1600±61 cc, NBV in NC = 1617±35 cc, p=0.5).

Conclusions: Decreases in NAA/Cr, similar in the two patient groups, suggest that a relevant axonal damage is present in patients in early disease stages independently of their ApoE genotype. In contrast, significant brain volume loss was found only in patients carrying the ε4 allele, confirming that this genotype can influence more severe tissue destruction in MS.

Disclosure: M Amato has nothing to disclose.

Funding: Supported by MURST (Rome) and PAR grant of the University of Siena.

P231

THE RELATION BETWEEN BRAIN MRI LESIONS AND DEPRESSIVE SYMPTOMS IN MULTIPLE SCLEROSIS

Beneoová V, Niedermayerová P, Mechl M

Background: This study investigates the relation between involvement of the specific areas of the brain and the depression in multiple sclerosis (MS) patients.

Objectives: Magnetic resonance (MRI) regional lesions between the depressed and nondepressed groups of MS patients were compared.

Methods: We studied 20 (10 depressed and 10 nondepressed) patients, 14 females and 6 males, average age (37.3±7.2 SD) with definite relapsing-remitting MS, treated with interferons. EDSS score ranged from 1.0 to 4.0 (mean EDSS 2.5 in the whole group, 2.8 in depressed group and 2.2 in nondepressed group). All patients underwent 1.5 Tesla magnetic resonance examination, including T1 and T2 weighted images. MRI data were analyzed by measuring the regional frontal, temporal, corpus callosum and total number and area (in mm2) of lesions and the side of lesions. The emotional state of the patients was assessed with the following psychological tests: Hamilton Depression Rating Scale, Zung Self-Rating Depression Scale and Montgomery Depression Scale.

Results: There were 10 patients with the diagnosis of depression (three of them with severe depression, two of them with moderate depression and five of them with light depression). Besides, there were ten nondepressed patients. The data from MRI scans showed that all patients had several demyelinating lesions. There were detect statistically significant differences in the regional frontal lesions area between the two groups (p = 0.02) but not in temporal and corpus callosum location. The data from MRI scans showed that the diagnosis of major depression correlated with frontal lesion load.

Conclusions: Our findings showed that depressive symptoms were associated with specific brain lesions.

Disclosure: V Beneoová has nothing to disclose.
P232
DISABILITY AND AXONAL LOSS IN EARLY RELAPSING AND REMITTING MULTIPLE SCLEROSIS. ASSESSMENT WITH MSFC AND 1H-MRS
Casanova B1, Martinez C1, Valero C1, Celda B1, Mati-Bonmati L2, Pascual A1, Coret P3
1Neurology, Hospital La Fe, València, Valencian Country, Spain; 2Neurology, Hospital Clinic, València, Valencian Country, Spain; 3Chemistry, Universitat de València, València, Valencian Country, Spain; 4Imagin, Clínica Quirón, València, Valencian Country, Spain

Background: The NAA/Cr ratio in the normal-appearing white matter (NAWM) is a measure of the axonal loss. The MSFC is a more objective and sensitive measure of disability in MS patients because it integrates three aspects: the arms function, the legs function and the cognitive function.

Objectives: To study the presence of axonal loss, measuring the NAA/Cr ratio in the NAWM in early RRMS and its correlations with the MSFC.

Methods: We studied 23 RRMS patients, 69% females and 31% males, mean age 29.7 years old, mean evolution time 2.6 years (1-5) and mean EDSS 1.5 (0-3.5). The Z-score of our series was compared with the Z-score for a normalized population according the guidelines published by Fischer et al. The third Z-score obtained after two previous exams performed one year and six months previously was used for comparisons. MRS: a two-dimensional phase encoded turbo spectroscopic image (2DTSI) was performed in a 1.5 T superconductive unit. A slice of 60x50x20 mm (volume of interest -VOI-) was located parallel to the transversal plane which contains the anterior and posterior commissure plane, at the pons and the cerebellar peduncles. The FID’s from voxels were Fourier transformed and absolute metabolites were determined using the jMRUI software.

Results: The correlation between our MSFC Z-score and the normalized Z-score was 0.931 (Spermann rank). Linear regression analysis between the NAA/Cr ratio and the Z-score was significant (r2=0.194, p<0.035 -ANOVA-).

As we expected no correlation was reached between Z-score and EDSS (-0.35 p=0.09). No correlation between EDSS and the NAA/Cr ratio, r2=-0.225, p=0.302, and with any of the compounds of the Z-score was reached.

Conclusions: The MSFC is a reproducible and sensitive scale to measure the disability in MS, and it correlates with axonal loss in the NAWM in early RRMS patients. Realization of proton MRS at this location is easy, not expensive and reliable in clinical practice, and gives us an objective measure of the pathological state of patients “in vivo” that correlates with his disability.

Disclosure: B Casanova has nothing to disclose.
Funding: Ares-Serono laboratories.

P233
EARLY CORTICAL ATROPHY IN RELAPSING REMMITTING AND PRIMARY PROGRESSIVE MULTIPLE SCLEROSIS AND ITS RELATIONSHIP TO OTHER MR AND CLINICAL MEASURES
De Stefano N1, Matthews PM1, Filippi M1, Iannucci G1, De Luca M2, Bartolozzi ML3, Guidi L4, Ghezzi A5, Montanari E6, Federico A1, Smith S7
1Institute of Neurological Sciences, University of Siena, Siena, Italy; 2Neurosciences, S. Raffaele Hospital, Milan, Italy; 3Clinical Neurology, University of Oxford, Oxford, UK; 4United Kingdom; 5Neurology Unit, Empoli Hospital, Empoli, Italy; 6MS Center, Gallarate Hospital, Gallarate, Italy; 7MS Center, Fidenza Hospital, Fidenza, Italy

Background: Axonal transection and apoptotic loss of neurons due to cortical demyelination have been demonstrated in MS brains. Thus, cortical pathology may contribute to neurological impairment in MS.

Objectives: To assess cortical atrophy in patients with the relapsing and progressive forms of MS at different disease stages and evaluate its relationship with clinical disability.

Methods: We obtained conventional MRI examinations in 90 patients with definite MS who had either the relapsing remitting (RR, n= 65) or the primary progressive (PP, n= 25) form of the disease. Conventional T1-weighted MR images were used to obtain cortical brain volumes normalized for head size using a method for computing measurements of brain volume. Proton density and T2-weighted MR images were used to assess total brain lesion load.

Results: Normalized cortical volumes (NCV) were significantly lower in both RR and PP MS patients than in age matched normal controls (p<0.001 for both), but similar in the two patient groups (p>0.5). Significant NCV decreases were seen in both RR and PP even when MS patients were grouped for early disease duration (<5 years, p<0.005) and low brain lesion accumulation (<5 cc, p<0.005). Patients with minimal or mild disability (EDSS <4) showed significant (p<0.0001) NCV decreases in the RR MS group only. In addition, measures of NCV showed a close relationship with those of T2-weighted lesion volume only in RR MS patients, whereas NCV correlated with EDSS better in PP than in RR MS patients.

Conclusions: These data confirm decreases in cortical volumes in MS patients and suggest that cortical grey matter pathology may occur early in the course of the disease. The significant negative correlation between clinical disability and NCV suggest that, especially in PP MS patients, grey matter pathology may contribute significantly to neurological impairment.

Disclosure: N De Stefano has nothing to disclose.
Funding: Stichting Vrienden MS Research.

P234
1H-MAGNETIC RESONANCE SPECTROSCOPY IN CORTICAL GREY MATTER, HIPPOCAMPUS AND THALAMUS OF HEALTHY SUBJECTS AT 1.5 T - A REPRODUCIBILITY STUDY.
Geurts J1, Barkhof F2, Casteljins JA1, Polman CH1, Pouwels PP1
1Radiology, MR Center for MS Research, VU Medical Center, Amsterdam, Noord-Holland, Netherlands; 2Neurology, MR Center for MS Research, VU Medical Center, Amsterdam, Noord-Holland, Netherlands; 3Clinical Physics and Informatics, VU Medical Center, Amsterdam, Noord-Holland, Netherlands

Background: Proton Magnetic Resonance Spectroscopy (1H-MRS) is widely used to study metabolite concentrations of the brain in vivo. Although some reproducibility studies have been performed, little is known about the reproducibility in structures such as the thalamus and hippocampus.

Objectives: To investigate within what ranges of error metabolite concentrations of healthy subjects, as measured with 1H-MRS, are reproducible.

Methods: Using single voxel 1H-MRS (STEAM, TR/TE 6000/20 ms, 64 averages, Siemens Vision 1.5T), 13 healthy subjects were measured, mean age 28 years. In total, 10 spectra were obtained from parietal cortex (12 ml), 10 from hippocampus (6 ml) and 7 from thalamus (7 ml). After a time lapse varying from 1 day to 2 months, subjects were re-examined and spectra were obtained from the same brain regions. Metabolite concentrations were calculated using LCModel. For each metabolite, variation between the two measurements was expressed statistically by the coefficient of variation (CoV). In addition, metabolite concentrations were compared between brain regions using an unpaired Student’s t-test.

Results: Significant regional differences were observed for most of the metabolite concentrations. For the major metabolites tNAA,Cho,Cr, these differences were observed from 8-20% in all regions, with highest values in the hippocampus. For Glu and Gin the CoV was as low as 15 and 35% in cortex, but up to 60 and 75% in hippocampus. Mean concentration of tNAA was 7.4 ± 0.6 mM in the cortical grey matter (CoV: 10%), 6.7 ± 0.9 mM in the hippocampus (CoV: 17%) and 8.3 ± 0.8 mM in the thalamus (CoV: 8%). No systematic bias in the measurements was found.

Conclusions: Clear regional differences exist both in metabolite concentrations and in reproducibility in cortex, hippocampus and thalamus of healthy human subjects. This has to be considered when studying these brain regions in follow-up studies of MS patients.

Disclosure: J Geurts has nothing to disclose.
Funding: Stichting Vrienden MS Research.
P235

INCREASED GLUTAMATE/GLUTAMINE LEVELS MEASURED BY 1H-MRS IN MULTIPLE SCLEROSIS BRAIN.


*Multiple Sclerosis Institute, Philadelphia, Pennsylvania, USA; 2Neurology, Temple University School of Medicine, Philadelphia, Pennsylvania, USA; 3Diagnostic Imaging, Temple University School of Medicine, Philadelphia, Pennsylvania, USA; 4Department of Occupational Therapy, Temple University College of Allied Health Professions, Philadelphia, Pennsylvania, USA

Background: Glutamate excitotoxicity may play a role in the pathogenesis of MS by producing oligodendroglial loss, axonal dystrophy; factors which may contribute to disease progression. Elevated CSF and increased tissue glutamate levels have been found in MS. In active lesions, macrophages are the major producers of glutamate and are associated with oligodendroglial loss and axonal dystrophy. AMPA receptor antagonists have afforded neuroprotection without reduced inflammation in EAE, suggesting that glutamate antagonists may be a viable therapeutic option in MS in addition to anti-inflammatory agents.

Objectives: This study evaluated glutamate/glutamine levels in MS brain using 1H-MRS. Areas of acute inflammation; of chronic axonal loss and of normal appearing white matter (NAWM) were examined and compared with normal white matter.

Methods: Sixty one MS patients (RRMS=36, SPMS=24, PPMS=1) and 10 normal controls were studied. Eighty five lesions and 10 normal areas were examined using single voxel 1H-MRS. All studies were performed with a GE 1.5T/UX MRI using TE=35msec, TR=1150msec. A point-resolved spectroscopy (PRESS) sequence was used. NAA, choline, creatine, myo-inositol and alpha (3.56ppm) and beta/gamma (2.05-2.45ppm) glutamate/glutamine peaks were resolved. Gadolinium-enhancing, T2+/T1-, T2+/T1+ lesions as well as NAWM were compared with normal control white matter.

Results: Both alpha and beta/gamma glutamate/glutamine peaks were elevated in all MS areas examined. Levels were elevated in RRMS, SPMS and PPMS. Much greater elevations were seen in gadolinium-enhancing lesions when compared with the other MS lesions and NAWM. Increased glutamate levels were particularly associated with acute T1 hypointense areas.

Conclusions: Diffusely elevated levels of glutamate/glutamine are present in both acute and chronic MS lesions as well as NAWM as measured by 1H-MRS. Particularly elevated levels are associated with acute inflammation. This confirms and extends the histopathological evidence for glutamate toxicity and suggests that glutamate antagonism should be further investigated in MS.

Disclosure: J Greenstein has nothing to disclose.

P236

GLOBAL MAGNETIC RESONANCE SPECTROSCOPY METABOLIC VARIATIONS AS INDICATORS OF DISEASE ACTIVITY IN RELAPSING REMITTING MULTIPLE SCLEROSIS

Inglese M, Rusinek H, Babb JS, Grossman RI, Gonien O

Radiology, New York University School of Medicine, New York City, New York, USA

Background: The widespread, diffuse activity of Multiple Sclerosis (MS), even during its clinically stable phase, potentially leads to continual accumulation of neuronal and axonal damage. Although not visible on conventional MRI, this activity also involves the normal-appearing white matter (NAWM) and plays an important role in the evolution of the disease from the initial relapsing-remitting (RR) to the later, more debilitating, secondary progressive phase.

Objectives: To ascertain the presence and the extent of disease activity in the NAWM of the brain of patients with relapsing-remitting (RR) MS using proton magnetic resonance spectroscopy (1H-MRS)

Methods: The absolute metabolic concentrations of N-acetyl-aspartate (NAA), Creatine (Cr) and Choline (Cho) were obtained with 3-dimensional 1H-MRS at 1.5 Tesla from a large, 0.5L, brain volume of eleven RR MS patients (median EDSS: 2.5; range: 0.0-6.0) and nine age and sex matched healthy controls.

Results: The NAA level in the NAWM of the patients was significantly lower than the controls’ while their Cr and Cho were significantly higher (p<0.04, 0.001 and <0.0001, respectively). The Cho level was the only metric able to differentiate RR MS patients from the controls at 100% specificity and sensitivity exceeding 90%.

Conclusions: The NAA, Cho and Cr deviations from controls’ “normal” indicate that even the clinically silent phase of the disease is characterized by abnormal elevated metabolic activity. Specifically, the elevated Cho and Cr probably reflect microscopic inflammation/demyelination and remyelination processes. Therefore, they are potential prognostic indicators of disease activity.

Disclosure: M Inglese has nothing to disclose.

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P237

PROTON MAGNETIC RESONANCE SPECTROSCOPY EVIDENCE FOR EARLY GRAY MATTER INVOLVEMENT IN RELAPSING REMITTING MS


*Radiology, New York University School of Medicine, New York City, New York, USA; 2Neuroimaging Research Unit, Scientific Institute and University, San Raffaele Hospital, Milan, Italy

Background: MS has traditionally been viewed as an inflammatory/demyelinating white matter (WM) disease of the CNS. However, progressive clinical cognitive deficits have always suggested gray-matter (GM) involvement in addition to sub-cortical dementia from WM pathology. Indeed, recent pathology and MRI studies have shown lesions in the GM as well.

Objectives: To ascertain the extent of gray-matter (GM) involvement in MS, from the concentration of N-acetylaspartate (NAA), a metabolite found almost exclusively in neuronal cells, over the whole brain of MS patients versus matched controls.

Methods: The whole-brain-NAA (WBNAa) concentration: The ratio of this metabolite’s amount, obtained with non-localizing proton MR spectroscopy, to the parenchymal volume from high resolution MRI, was measured in 71 relapsing-remitting (RR) MS patients (51 women, 20 men, 25-55 years old) and 41 controls (27 women, 14 men, 23-55 years old).

Results: The average WBNAa difference between the patients and the controls was -2.9 mM (-22%, p<0.0001) range: +1.2 to -7.8 mM (+8% to -63%).

Conclusions: Since WM and GM constitute ~30% and 60% of the brain volume, respectively, and the NAA concentration in the former is 2/3 the latter’s, then losses greater than 32% cannot be explained in terms of WM pathology alone and must include widespread GM deficits. Therefore, the concept of MS as a WM disease might need to be reexamined as a diffuse “whole brain disorder.”

Disclosure: M Inglese has nothing to disclose.

Funding: Supported by Shering.
P238

BRAIN VOLUME CHANGES AFTER SUPPRESSION OF MRI-VISIBLE INFLAMMATION IN PATIENTS WITH SECONDARY PROGRESSIVE MS TREATED WITH AUTOLOGOUS STEM CELL TRANSPLANTATION
Department of Neuroscience Scientific Institute and University Ospedale San Raffaele, Neuroimaging Research Unit, Milan, Italy; Department of Neurological Sciences and Vision, University of Genova, Genova, Italy; Neuroscience, University of Cagliari, Cagliari, Italy; Neuroscience, University of Pisa, Pisa, Italy; Neurological and Psychiatric Sciences, University of Florence, Florence, Italy; Oncology and Neuroscience, University of Chieti, Chieti, Italy; Bone Marrow Transplantation Unit, Careggi Hospital, Florence, IT, Italy; Neuroradiology and University of Siena, Siena, Italy.

Background: Magnetic resonance (MR)-based brain volumetric measures have the potential to quantify the destructive pathological aspects of MS, thus providing reliable surrogate markers of disease progression. A recent, multicenter, phase II/I, trial of patients with secondary progressive (SPMS) has shown that autologous hematopoietic stem cell transplantation (AH SCT) has a dramatic and sustained effect on disease activity, as measured by counting the number of enhancing lesions seen after the injection of a triple dose (TD) of gadolinium (Gd).

Objectives: To investigate whether brain atrophy continues to occur even in the unique condition of complete and sustained suppression of MRI-visible inflammation.

Methods: Ten patients with severely-disabling and rapidly evolving SPMS from 5 centers participating into this trial underwent brain post-contrast T1-weighted scans one month after the treatment and 12 and 24 months later. Normalized brain volume (NBV) was measured using a fully automated technique called SIENA.

Results: The mean percentage change of NBV from baseline to month 12 was -1.8% (range: -5.2% to 1.0%; 7 patients) and from month 12 to month 24 was -1.9% (range: -1.3% to -2.5%; 5 patients).

Conclusions: This study shows that continuous and marked brain tissue loss can occur even in the absence of activity on TD enhanced MRI, thus suggesting that, at least in SPMS, “neurodegeneration” occurs also in absence of overt inflammatory changes.

Disclosure: M Inglese has nothing to disclose.
Funding: Partially supported by Nycomed.

P239

COGNITIVE AND EMOTIONAL STATUS IN A POPULATION OF PATIENTS PRESENTED WITH CLINICALLY ISOLATED SYNDROMES SUGGESTIVE OF MULTIPLE SCLEROSIS
Neurology, CHU Timone, Marseille, France; Laboratoire de Neurophysiologie et de Neuropsychologie, Faculté de Médecine

Background: Cognitive and emotional dysfunctions are frequent findings in multiple sclerosis (MS) that can occur even at the early stage of the disease.

Objectives: To investigate the potential cognitive and emotional dysfunctions in a population of patients presented with clinically isolated syndromes suggestive of MS (CIS/MS).

Methods: We compared the performances of forty-five patients presenting with CIS/MS and twenty-four sex-age-socioeconomic matched healthy volunteers for an extensive neuropsychological battery (memory: Selective Reminding Test, Delayed Recall of SRT, 10/36 Spatial Recall Test, Delayed Recall of 10/36 Spatial Recall Test, WMS-R subtests; Grober & Buschke test; attention and executive functions: PASAT (2,3s) and verbal fluency, WAIS-R subtest, WMS-R subtests, Stroop test, Trailmaking test (A and B), Wisconsin Card Sorting Test; language: Boston naming test; visuo-spatial functions: WAIS-R subtest, Symbol Digit Modalities Test). Emotional status was assessed with the Toronto Alexithymia Scale and emotional visual task.

Results: No significant difference was found between CISSMS and controls for emotional tasks, language, memory, executive functions and visuospatial abilities. However, patients with CISSMS had a significant impairment of PASAT (2s and 3s) than did control volunteers (p<0.05 for each condition).

Conclusions: These findings suggest the potential value of PASAT as surrogate marker of cognitive dysfunction at the very early stage of MS.

Disclosure: P Jean has nothing to disclose.
Funding: Supported by grants from ARSEP, CNRS and Fondation Duranton de Magny.

P240

NEURAL SUBSTRATES UNDERLYING PERFORMANCE OF A CONTROLLED MOTOR TASK IN PATIENTS WITH MULTIPLE SCLEROSIS.
NIH, NINDS/NIH, Bethesda, Maryland, USA; Human Cortical Physiology Section, NINDS/NIH, Bethesda, Maryland, USA; Clinical Brain Disorders Branch, NIMH/NIH, Bethesda, Maryland, USA; Laboratory of Radiology Research, NIH, Bethesda, Maryland, USA; Department of Neurology, University of Giessen, Giessen, Germany.

Background: In animal models, nonprimary motor regions contribute to recovery of motor function after cortical lesions, possibly through unmasking of direct corticomotoneuronal connections originated in area 6.

Objectives: We hypothesized that neural substrates underlying motor performance in MS patients include nonprimary motor regions, particularly premotor cortex, to a larger extent than in healthy controls.

Methods: We studied BOLD fMRI activation patterns associated with carefully controlled performance of the same hand motor task (voluntary thumb flexion movements) in nine patients with multiple sclerosis and nine healthy controls (HC). Disease stages, duration and EDSS scores were variable, but all patients were capable of performing independent thumb movements with the right hand (dominant). Thumb movement kinematics and EMG activity were monitored during scanning.

Results: Thumb movement kinematic measures (magnitude of first peak acceleration, movement direction and frequency) were comparable in patients and controls indicating task consistency across groups in the absence of EMG mirror activity. Performance of thumb movement was associated with more activation in contralateral premotor (area 6) and association (BA 46) cortices in MS patients relative to controls.

Conclusions: These results are consistent with the idea that activity in nonprimary motor areas, particularly premotor cortex, may play a compensatory role in recovery of motor function in multiple sclerosis.

Disclosure: N Kadom has nothing to disclose.

P241

INTERCUADRATE NUCLEUS RATIO (ICR) IN MS PATIENTS AS A LINEAR MEASURE OF BRAIN ATROPHY.
Khan O, Zvartau-Hind M, Caon C, Ching W, Lisak R, Tsielis A Neurology, Wayne State University, Detroit, Michigan, USA.

Background: Brain atrophy is being investigated as an outcome measure in MS therapeutic trials. It has been suggested that measurement of ICD and ICR is a potentially reliable linear measure of brain atrophy in MS patients. Measurement of ICD does not require software application and is independent of image acquisition techniques. We performed ICR measurement to confirm

Multiple Sclerosis
reproducibility of this technique and to examine potential association with disease variables in our MS patient population.

Objectives: To study the reliability of ICR as a linear measure of brain atrophy and to examine the relationship between ICR and disability in MS patients with different disease types.

Methods: 190 MRI scans of MS patients seen in our clinic were analyzed for the measurement of ICD (defined as the distance between the medial border of caudate nuclei identified on the most caudal axial slice) and ICR (defined as ICD divided by the inter-inner table of the skull distance at the same level). Correlation between ICR and multiple disease variables including EDSS, disease duration, sex, and age, were examined. Comparison of ICR in RRMS with SPMS patients was also performed.

Results: Of 190 patients analyzed, 167 patients had RRMS and 23 had SPMS. Mean EDSS was 3.2 (SD 1.7), mean age was 40.2 years (SD 9.3), mean disease duration was 8.5 years (SD 7.2). Mean ICR was 0.11 (SD 0.04). 142 patients were female. Male patients had significantly higher ICR (Means 0.12 mm and 0.1 mm, respectively; p<0.001). There was a significant correlation between ICR and EDSS (Rho=−0.67; p<0.0001). There also was a significant although weaker correlation between ICR and disease duration (r=−0.32; p<0.001). There was a significant difference between the mean ICR of RR and SP MS patients, 3.9 mm vs. 7.1 mm, respectively (p<0.0001).

Conclusions: ICR appears to be a reliable and reproducible linear measure of brain atrophy. ICR appears to correlate with disability and disease duration in MS patients. Furthermore, patients with SPMS have a larger ICD (ICR) than patients with RRMS indicating greater brain atrophy. ICD and ICR measurement can be performed in an outpatient setting and may be a useful clinical tool in the management of MS patients. Further longitudinal studies are necessary to validate this technique.

Disclosure: O Khan has nothing to disclose.

P242
CEREBRAL ATROPHY IN SECONDARY PROGRESSIVE MULTIPLE SCLEROSIS: EFFECT OF INTRAVENOUS IMMUNOGLOBULINS
∗Division of Clinical Neurology, University of Nottingham; †European Charcot Foundation, Nijmegen, Netherlands

Background: Several studies have demonstrated the importance of measuring brain volume as a surrogate marker of disease progression in MS. The recently completed European study of IVIG at the dose of 1g/kg/body weight monthly in patients with secondary progressive MS has provided an opportunity to assess the effect of IVIG treatment on cerebral atrophy using 3-dimensional (3-D) MRI.

Objectives: To assess the effect of IVIG on brain atrophy in secondary progressive MS.

Methods: A subgroup of 43 patients from 5 centres underwent and completed T1-weighted magnetisation prepared rapid acquisition gradient echo (MPRAGE) images at 12-month intervals from month 0 to month 24. Expanded Disability Status Scale (EDSS) scores were acquired at 3-month intervals. The volumes of supratentorial and infratentorial structures and the lateral ventricles were estimated using modern design stereology and point counting on 3-D MPRAGE images.

Results: The placebo (n=22) and treated (n=21) groups were well matched for the baseline demographics and the volumes of supratentorial (902+/-53 vs 911+/-45 ml), infratentorial (138+/-13.5 vs 134+/-14.2 ml) brain and lateral ventricles (27.1+/-17.4 vs 23.4+/-10.8 ml). There was a significant enlargement of the lateral ventricles at month 24 in both placebo and IVIG treated groups, with a mean increase of 9.8 and 7.4%, respectively. In parallel, there was a smaller reduction of supratentorial brain volume in IVIG than placebo treated patients (-0.5 vs -2.1%) over 24 months, but this difference was not significant (p=0.25). However, the placebo group showed a significantly greater infratentorial brain volume reduction at month 12 (-1.8 vs +2.1%, p=0.0022) and month 24 (-3.3 vs +0.8%, p=0.026) compared with the IVIG treated group. In the total cohort, the percentage change of lateral ventricles was correlated with the change in EDSS over 24 months (r=-0.58, p<0.0001).

Conclusions: Brain volume reduction was smaller in the IVIG treated group with a statistically significant difference regarding changes in infratentorial brain volume over 24 months. The mechanism(s) responsible for the impact of IVIG on brain atrophy are yet to be elucidated.

Disclosure: Professors’ Hommes, Blumhardt, and Fazekas are members of the steering committee for the ESIMS trial.

Funding: This trial was supported by Bayer pharmaceutical Ltd.

P243
CORRELATES OF MAGNETIZATION TRANSFER IMAGING METRICS IN PRIMARY PROGRESSIVE MULTIPLE SCLEROSIS
*Clinical Neuroimmunology Unit, University Hospital Vall d’Hebron, Barcelona, Catalonia, Spain; †Magnetic Resonance Unit (IDH), University Hospital Vall d, Barcelona, Spain

Background: Weak correlations between clinical and MRI parameters in PPMS have been reported.

Objectives: The purpose of this study was to establish correlations between normal appearing grey (NAGM) and white matter (NAWM) magnetization transfer (MT) metrics and clinical measures in a cohort of PPMS patients.

Methods: Proton density, T2- and T1-weighted and MT images of the brain were obtained from 38 PPMS patients. MT histograms from NAWM and NAGM were calculated. Clinical assessment was performed by means of the EDSS and the MSFC. The Symbol Digit Modalities Test (SDMT) was also administered.

Results: No correlations were demonstrated between brain MTR measures (mean, mode) and the MSFC or the PASAT. The SDMT showed better correlation with NAGM (r=0.435; p=0.006) than with NAWM (r=0.347; p=0.03) MTR mean values.

Conclusions: This study suggests that the alterations observed in the NAGM of patients with PPMS might play a role in cognitive impairment when measured with the SDMT.

Disclosure: X Montalban has nothing to disclose.

P244
DISSEMINATION IN SPACE IN BRAINSTEM SYNDROMES
*Clinical Neuroimmunology Unit, University Hospital Vall d’Hebron, Barcelona, Catalonia, Spain; †Magnetic Resonance Unit - IDH, University Hospital Vall d’Hebron, Barcelona, Catalonia, Spain; ‡Institute of Neurology, National Hospital for Neurology and Neurorehabilitation, London, United Kingdom

Background: Barkhof criteria have been adopted in the new multiple sclerosis (MS) diagnostic criteria to demonstrate dissemination in space (DIS) because of their high specificity for predicting conversion to MS. One of the Barkhof criteria is the presence of infratentorial lesions which, in clinically isolated syndromes (CIS) of the brainstem (CISB), does not demonstrate DIS. This would suggest that Barkhof criteria would have less specificity in CISB as compared to other CIS.

Objectives: To determine the specificity of Barkhof criteria in a cohort of CISB and to compare it with other CIS; and to assess the performance of each one of its four parameters.

Methods: 153 patients with CIS (51 CISB, 46 myelitis, 56 optic neuritis) were longitudinally studied. Barkhof criteria (cut-off: 3 out of 4) were applied. To
find out the performance of each one of the four parameters of Barkhof criteria we analysed fulfilling of four subsets of modified Barkhof criteria using only three parameters at a time and setting positivity in 2 out of 3.

Results: A specificity of 61% in CISB versus 73% in other CIS was found. The modified subsets of Barkhof criteria that retain the infratentorial lesion assessing parameter have lower specificities in the CISB group (52%, 52% and 42%) than the subset not including it (61%). In other CIS all four subsets of Barkhof criteria performed similarly (66%, 70%, 68% and 66%).

Conclusions: When applied to CISB, Barkhof criteria are not as specific as in other CIS, and should be differently applied. We hypothesize that another criterion demonstrating DIS in CISB might be more specific.

Disclosure: X Montalban has nothing to disclose.

P245

COMPUTER-ASSISTED VOLUMETRIC ANALYSIS OF GADOLINIUM ENHANCEMENT IN MULTIPLE SCLEROSIS

Preiningerova P1, Ding Z2, Caminitracci C3, Sun HF, Vollmer T4, Anderson A5

1Neurology, Yale University, New Haven, Connecticut, USA; 2Diagnostic Radiology, Yale School of Medicine, New Haven, Connecticut, USA

Background: Quantification of gadolinium enhancement (gd) in provides an important outcome measure in clinical trials in multiple sclerosis (MS). Automated quantification of Gd enhancement on brain MRI is technically challenging due to several reasons including a range of signal intensities within or between lesions or among scans, and misclassification of vessels as enhancing lesions.

Objectives: The goal was to develop and evaluate a computer-assisted method of quantitative analysis of Gadolinium enhancing lesions on brain MRI in MS.

Methods: Contiguous 3mm, axial scans of T1 pre and post gd, FLAIR, and proton density weighted images were acquired on a 1.5 Tesla GE scanner. The locations of enhancing lesions were first identified by an expert reader, and the three dimensional extent of gd+ lesions was determined with a computer-assisted algorithm using a combination of seed-growing and local thresholding techniques. The number and volume of Gd+ lesions were then automatically calculated. An interface is provided for simultaneous display of the three series of MR images, and identification of corresponding pixels, which facilitate correct and efficient determination of lesions by the reader.

Results: A total of 18 monthly scans of two consented subjects who participate in a clinical trial was included in the validation study. An expert reader can register the location of lesions and determine the volume in 2-8 min/per scan depending on the number of lesions. Variability in the method is introduced at the level of expert reader when localizing lesions and at the level of volumetric analysis. Intrarater variability of a trained operator performing the volumetric quantification based on the expert determined locations of lesions is 5.6%. Variability introduced in localizing lesions by the expert reader is much larger. Our data show that such variability is as large as 37.8% for subjects with small lesion load.

Conclusions: Volumetric analysis with expert identified lesions is highly reproducible. Variability in this method is largely introduced by different thresholds an expert reader applies when identifying lesions. We are making an effort to develop a well defined protocol to assist in localization of lesions so as to reduce this source of variability.

Disclosure: J Preiningerova has nothing to disclose.

Funding: Supported by the Nancy Davis Foundation.

P246

COMPARISON OF CEREBRAL PERFUSION IN MULTIPLE SCLEROSIS USING A NOVEL TECHNIQUE

Rashid W1, Parkes L2, Ingle G3, Chard D3, Symms M4, Miller D5

1NMR unit, Institute of Neurology, Queen Square, London, United Kingdom; 2FC Donders Centre for Neuroimaging, University of Nijmegen, Nijmegen, Netherlands

Background: Cerebral perfusion in multiple sclerosis (MS) has never been estimated using Continuous Arterial Spin Labelling (CASL), an MRI technique using endogenously labelled arterial water. It is potentially a more accurate methodology, as it allows quantitative measurements and is less susceptible to contrast loss in blood brain barrier disruption.

Objectives: To compare perfusion changes between MS and its subgroups against controls using CASL.

Methods: 33 controls and 59 MS subjects (12 primary progressive (PP), 21 relapsing remitting (RR) of which 11 were on beta-interferon (RRi) and 10 not (RRn), 13 benign and 13 secondary progressive (SP)) were compared. Segmentation based on the differing T1 relaxation times of white (WM) and grey matter (GM) produce averaged perfusion values for these areas. Also, voxel based morphometry (VBM), using SPM99, was applied to compare perfusion maps between groups.

Results: Multiple linear regression accounting for age and gender effects, show a significant increase in whole white matter perfusion (WMP) in RR (p=0.02) and SP (p=0.04) groups using T1 relaxation map values. In RRi there is no significant increase in WMP compared with controls, whereas in RRn there is a significant increase (p=0.03). However, there is no significant difference in WMP between the two RR subgroups. No significance is seen in whole GM perfusion. From VBM, significant decreases (p=0.03) are seen comparing all MS subjects to controls in bilateral thalami, cingulate gyri and in regions of both frontal and parietal lobes with both WM and GM affected. In RRn, significant increase (p=0.01) is seen in a WM area in the left frontal lobe, no increase is seen in RRi. SP shows significant increase around the left superior temporal gyrus (p=0.01) and decrease mainly at the right superior frontal gyrus (p=0.01). In PP, significant decreases (p=0.04) are seen in areas of the left frontal lobe.

Conclusions: This is the first study to show such a widespread pattern of perfusion change in both GM and WM. The increase in whole WMP may reflect inflammatory activity particularly in RR and SP patients. This suggests CASL has potential in monitoring disease activity.

Disclosure: W Rashid has nothing to disclose.

Funding: Supported by MS Society of Great Britain and Northern Ireland.

P247

FUNCTIONAL CORTICAL CHANGES IN PATIENTS WITH MULTIPLE SCLEROSIS AND NON-SPECIFIC CONVENTIONAL MAGNETIC RESONANCE IMAGING SCANS OF THE BRAIN

Rocca MA1, Pagani E2, Ghezzi A3, Falini A4, Zaffaroni M5, Colombo B6, Scotti G7, Comi G8, Filippi M9

1Neuroimaging Research Unit, Department of Neuroscience, Scientific Institute and University HSR, Milan, Italy; 2Department of Neurology, Ospedale di Gallarate, Gallarate, Italy; 3Department of Neuroradiology, Scientific Institute and University HSR, Milan, Italy; 4Department of Neurology, Scientific Institute and University HSR, Milan, Italy

Background: Cortical adaptive changes have been shown in patients with MS and different clinical courses.

Objectives: To evaluate, using fMRI, the movement-related brain patterns of cortical activations in patients with clinically definite MS (CDMS) and non-specific conventional MRI abnormalities of the brain. To quantify tissue damage of the normal appearing white (NAWM) and gray (NAGM) matter of these patients, using diffusion tensor imaging (DTI).

Methods: We investigated 12 right-handed patients with CDMS and 12 sex- and age-matched controls. Patients were included if they had three or fewer lesions on brain T2-weighted scans. In each subject we acquired: 1) fmRI (flexion-extension of the last four fingers of the right and left hands, respectively). 2) dual-echo sequence and 3) pulsed-gradient spin-echo sequence. FMRI data were analyzed using SPM99. Lesions were measured using a local thresholding segmentation technique. Mean diffusivity (MD) histograms of NAWM and NAGM were produced.

Results: In CDMS patients, the mean T2-weighted lesion load was 0.3 ml. MS patients had lower MD histogram peak height (p=0.0001) and higher peak position (p=0.03) of the NAGM than healthy subjects. When performing the simple motor task with the dominant hand, MS patients had more significant activations in both frontal and parietal lobes with both WM and GM affected.

Conclusions: There is a significant increase (p<0.0001) of the NAGM than healthy subjects. When performing the simple motor task with the dominant hand, MS patients had more significant activations in both frontal and parietal lobes with both WM and GM affected.

Disclosure: W Rashid has nothing to disclose.

Funding: Supported by MS Society of Great Britain and Northern Ireland.
On the contrary, controls showed more significant activations of the medial part of the contralateral parieto-occipital fissure and the ipsilateral primary sensorimotor cortex (SMC) than patients with MS. In patients with MS, the relative activation of the ipsilateral SMA was correlated with the peak height (r=-0.88, p<0.001) and position (r=0.87, p<0.001) of the GM mean diffusivity histogram.

Conclusions: Cortical reorganization occurs over a rather distributed sensorimotor network in MS patients with non-specific abnormalities on conventional brain MRI scans.

Disclosure: M Rocca has nothing to disclose.

P248
THE ROLE OF SPINAL CORD DAMAGE ON BRAIN CORTICAL PLASTICITY: A FUNCTIONAL MAGNETIC RESONANCE IMAGING STUDY

Background: Functional cortical changes have been demonstrated in patients with MS and diffuse white matter abnormalities of the brain and the spinal cord. The contribution of spinal cord damage, taken in isolation, on brain adaptive changes, has not been elucidated yet.

Objectives: To assess, using fMRI, the brain pattern of movement-associated cortical activations in patients with a previous remitting episode of acute myelitis of possible demyelinating origin. To investigate whether the extent of cortical functional reorganization is associated with the extent of cervical cord pathology measured using magnetization transfer imaging (MTI).

Methods: We studied 12 right-handed patients in a chronic phase after an isolated myelitis and 12 healthy controls. In each subject, we obtained: a) fMRI (repetitive flexion-extension of the last four fingers of the right hand), b) brain dual-echo scans, c) cervical cord fast-STIR and MT-MRI scans. FMRI data were analyzed using SPM99.

Results: No brain abnormalities were detected in the two groups studied. In comparison with healthy subjects, patients with myelitis had lower average cord MTR (r=-0.91, p<0.001) and cord MTR histogram peak location (r=0.001). They also had more significant activation of the ipsilateral primary somato-motor cortex (SMC), supplementary motor area and middle frontal gyrus (MFG). Relative activation of the ipsilateral MFG was correlated with average cord MTR (r=-0.91, p=0.001) and cord MTR peak location (r=-0.001).

Conclusions: This study demonstrates that cervical cord damage can elicit functional cortical changes that could have an adaptive role in limiting the clinical consequences of cord structural pathology.

Disclosure: M Rocca has nothing to disclose.

P249
SHORT-TERM CORRELATIONS BETWEEN CLINICAL AND MRI FINDINGS IN RELAPSING-REMITTING MULTIPLE SCLEROSIS
Filippi M, Rovaris M, Rocca MA, Ladkani D, Shifroni G, Wolinsky JS, Comi G

Background: The relationship between clinical and MRI findings in MS patients has not been fully elucidated yet.

Objectives: We investigated the short-term correlations between clinical and MRI-measured disease activity in a large sample of patients with RRMS by analysing the dataset from the European/Canadian glatiramer acetate (GA) trial.

Methods: The trial was a nine-month, double-blind, placebo-controlled study, where 239 RRMS patients were randomized to receive either 20 mg GA (n=119) or placebo (n=120). Brain dual echo, pre- and post-gadolinium (Gd) T1-weighted MRI scans were obtained at baseline and every month. Gd-enhancing and new T2-hyperintense lesions were counted and total T2-hyperintense and T1-hypointense lesion volumes (LV) measured.

Results: Significant univariate correlations were found between number of relapses during the study period and number of Gd-enhancing lesions at baseline (r=0.25) and during the follow-up (r=0.30) in the study population. When the two study arms were considered in isolation, the degree of the correlations was higher in the placebo than in the GA group. A multivariable model showed that the independent factors more strongly correlated with the frequency of relapses during the study period were the number of relapses during the two years before study entry and the number of on-trial Gd-enhancing lesions, both in the study population as a whole and in the placebo group. In the whole patient sample, both T2 and T1 LV at baseline were significantly correlated with baseline EDSS. Changes of MRI lesion volumes and EDSS were significantly correlated in the whole patient sample and in the GA group. Both T2 and T1 LV at baseline were significant predictors of the amount of MRI activity during the study period. T2 and T1 LV at baseline were significantly correlated with the lesion volume changes during the subsequent nine months.

Conclusions: In RRMS, MRI-measured inflammatory activity is only modestly, but significantly correlated with the occurrence of clinical attacks over a short-term period.

Disclosure: Drs Filippi, Wolinsky and Comi, as members of the Clinical Steering Committee and MRI Steering Committee, were reimbursed for their specific services on a contractual basis by Teva Pharmaceutical, Ltd. Funding: Supported by Teva Pharmaceutical, Ltd.

P250
PROTON MAGNETIC RESONANCE SPECTROSCOPY IN FAMILIAR AND SPORADIC MS
Sieg-Zajdel M, Selmaj K

Background: There are some indication that familial and sporadic multiple sclerosis (MS) patients might differ in clinical and MRI features of the disease. These differences might be explained by the heterogeneity of white matter pathology in both form of MS.

Objectives: The aim of this study was to compare abnormalities of normal-appearing white matter (NAWM) between familial and sporadic MS patients using water suppressed proton magnetic resonance spectroscopy (H-MRS).

Methods: Fifteen familial MS (male/female ratio 0.6, mean age=35.0, mean disease duration 5.9, mean EDSS=2.3) and fifteen sporadic MS patients (male/female ratio=0.87, mean age=34.6, mean disease duration=6.63, mean EDSS=3.63) were included in the study. Additionally, thirteen age and sex matched healthy individuals were selected as control. H-MRS was performed using a point-resolved slice-selective (PRESS) sequence (TE 135 msec, TR 3000 msec, 256 averages). Volume of interest (VOI)(8ml, 20x20x20 mm3) was located in the left centrum semiovale. Using post processing software peak area ratio of N-acetyl-aspartate/choline-containing compounds (NAA/Cho), N-acetyl-aspartate/Creatine (NAA/Cr) and phosphocreatine (Cho/Cr) were estimated.

Results: In a combined MS patient group NAA/Cho and NAA/Cr were significantly lower than in control group (NAA/Cho 1.88 vs 2.35 p<0.05; NAA/Cr 1.90 vs 2.28 p<0.05). In familial MS NAA/Cho was significantly decreased compared with control group (NAA/Cho 1.86 vs 2.35 p<0.05). Similarly in sporadic MS group NAA/Cho and NAA/Cr were significantly decreased compared with control group (NAA/Cho 1.90 vs 2.35 p<0.05; NAA/Cr 1.87 vs 2.28 p<0.05). A comparison of the familial and sporadic MS group showed that NAA/Cho was slightly lower and NAA/Cr and Cho/Cr were slightly higher in
familial patients than in sporadic MS patients (NAA/Cho 1.86 vs 1.90; NAA/Cr 1.94 vs 1.87; Cho/Cr 1.03 vs 1.01).

Conclusions: The results of our study suggest that there are some subtle differences in white matter metabolic concentration between familial and sporadic MS detected by H-MRS.

Disclosure: M Siger-Zajdel has nothing to disclose.

P251
A COMPARISON OF DIFFERENT QUANTITATIVE MRI TECHNIQUES TO MEASURE WHOLE BRAIN ATROPHY IN RELAPSING-REMITTING MULTIPLE SCLEROSIS

Zivadinov R1, Bakshi R2, Grop A3, Sharma P4, Bratina A5, Kuwata JMP6, Nasuelli D7, Tjoa CW1, Zorzon M1
1Department of Neurology, University of Trieste, Trieste, Italy; 2Buffalo Neuroimaging Analysis Center, University of Buffalo, Buffalo, New York, USA

Background: A variety of techniques for measuring brain atrophy have been applied to patients with multiple sclerosis (MS).

Objectives: The goal of this study was to compare the correlations between clinical and MRI characteristics among three different normalized measures of whole brain atrophy obtained by semiautomated and automated segmentation processes.

Methods: We studied 34 patients with relapsing-remitting MS (median disease duration 8.5 years, median age 36.5 years, median EDSS 1.5). T2- and T1-leesion loads (LL) were obtained using a reproducible semiautomated technique. Whole brain atrophy was measured on conventional spin-echo T1-weighted axial sequences by three different software programs developed to measure brain parenchymal fraction (BPF). The Buffalo and Trieste software programs are semiautomated methods, whereas SIENAX is automated. The scan-rescan inter- and intra-observer variability (COV) was estimated for all three methods and ranged from 0.32 to 0.44 (inter-observer) and 0.33 to 0.44 (intra-observer), the lowest for the SIENAX method.

Results: Mean T2- and T1-LL were 23.1 ml (SD 19.7) and 2.7 ml (SD 3.9), respectively. Mean BPF was 0.830 (SD 0.04) with Buffalo, 0.824 (SD 0.04) with Trieste and 0.826 (SD 0.04) with SIENAX method (p = NS). Buffalo and Trieste BPF demonstrated remarkably stronger correlation with EDSS (r = 0.37, p = 0.034 and -0.36, p = 0.039, respectively) compared to SIENAX BPF (r = -0.16, p = 0.219). All three BPFs demonstrated significant correlations with T2- and T1-LL (R = -0.35 to -0.49, p = 0.04 to 0.006), although the magnitude was slightly higher for Buffalo and Trieste BPF. In stepwise multiple regression analysis only the Buffalo and Trieste BPF predicted EDSS (R = 0.48, p = 0.004 and R = 0.50, p = 0.004).

Conclusions: The Buffalo and Trieste normalized semiautomated brain atrophy methods showed significantly stronger correlations with physical disability than the fully automated SIENAX method. This study suggests the utility of computer assisted semiautomated methods in determining whole brain atrophy in MS.

Disclosure: R Zivadinov has nothing to disclose.

P253
SHORT-TERM BRAIN ATROPHY CHANGES IN RELAPSING-REMITTING MULTIPLE SCLEROSIS

Zivadinov R1, Bagnato F1, Nasuelli D2, Bastianello S2, Bratina A3, Finamore L1, Locatelli L1, Catalan M3, Clemenzi A1, Grop A1, Millefiorini E4, Zorzon M1
1Department of Neurology, University of Trieste, Trieste, Italy; 2Department of Neurological Sciences, University of Rome La Sapienza, Rome, RO, Italy; 3European Biomedical Foundation, Rome, RO, Italy

Background: Several studies have demonstrated that patients with multiple sclerosis (MS) can develop brain atrophy (BA) within a time frame of six to twelve months. Recently, it has been reported that BA can progress even in a shorter time period of three months.

Objectives: To demonstrate whether monthly brain atrophy changes can predict physical disability changes in a short time period.

Methods: We studied 30 patients with relapsing-remitting (RR) MS (median disease duration 4.9 years, median age 34.4 years and median EDSS 1.4). Patients were assessed at baseline and monthly for a period of three months with clinical, EDSS and MRI examinations. Calculations of T2 lesion load (LL), T1 Gad-enhancing LL, T1-LL and brain parenchymal fraction (BPF) have been performed. No immunomodulatory drug was administered six months before the study entry. Three patients were treated with intravenous methylprednisolone for a relapse during the study period.

Results: EDSS remained stable during the study period (p = 0.980) and the patients experienced a mean rate of 0.3 (SD 0.5) exacerbations. Mean and median BPF did not change significantly at any timepoint of the study, although BPF slightly decreased from baseline scan to scan 3 (-0.05%, p = 0.111). Mean and median T2-, T1- and T1 Gad-enhancing-LLs were not significantly different at any timepoint of the study. Longitudinally, a correlation between changes in T2-LL and T1-LL was detected (r = 0.49, p = 0.008). BPF did not correlate cross-sectionally and longitudinally with any MRI measure. Changes in BPF were not correlated with changes in physical disability at any timepoint of the study.

Conclusions: We did not observe significant brain atrophy progression in our group of patients during a three months period. We failed to demonstrate any negative function and MRI measures in neurodegenerative disorders. Therefore, there is still a controversy regarding the optimal regional brain parenchymal atrophy measurement technique.

Objectives: The aim of this study was to develop a reproducible regional brain parenchymal atrophy method of measurement.

Methods: We studied 27 patients with relapsing-remitting (RR)- MS (median disease duration 9 years). T1- and T2- lesion load (LL) of frontal lobes were measured using a reproducible semiautomated technique. The neuropsychological performances and the disability of the patients have been assessed The regional brain parenchymal volume (RBPV) of frontal lobes was obtained using a computerized interactive program, which incorporates semiautomated and automated segmentation processes. A normalized measure, the regional brain parenchymal fraction (RBPF) was calculated as the ratio of RBPV to the total volume of the parenchyma and the cerebrospinal fluid (CSF) in the frontal lobes. A digital 3D version of Harvard Medical School Atlas was used as a reference for the segmentation of the frontal lobes after the coregistration. The boundaries have been outlined on each anatomic slice using a 1 mm computer displayed atlas image protocol based on standard neuroanatomic landmarks. The mean CV for RBPF of the frontal lobes was 1.6% for intra-observer reproducibility and 2.1% for inter-observer reproducibility.

Results: RBPF demonstrated remarkably stronger correlation with neuropsychological functioning, disability and quantitative MRI lesion measures compared to RBPV: r = -0.54, p = <0.0001 vs. r = -0.36, p = 0.04 for Paced Auditory Serial Addition Test, r = -0.28, p = 0.01 vs. r = -0.17, p = N.S. for EDSS, r = -0.31, p = 0.04 vs. r = -0.21, p = N.S. for T1-LL. There was no significant correlation between T2-LL of the frontal lobes and both regional parenchymal atrophy measures.

Conclusions: These data suggest that RBPF is a sensitive and reproducible method for measuring regional brain parenchymal atrophy.

Disclosure: R Zivadinov has nothing to disclose.
Disclosure: R Zivadinov has nothing to disclose.

P254

MRI MEASURES IN RELAPSING-REMITTING MULTIPLE SCLEROSIS. A SHORT-TERM OBSERVATION STUDY

Nasuelli Da, Bagnato Fb, Zivadinov Ra, Bratina Aa, Bastianello Sb, Finamore Lb, Locatelli Lb, Grop Aa, Di Pofi Ba, Millefiorini Eb, Zorzino Ma

aDepartment of Neurology, University of Trieste, Trieste, Italy; bDepartment of Neurology, CHU Lille, Lille, North, France; cCNRS 1309, Institut Biologie de Lille, Lille, Italy; dEuropean Biomedical Foundation, Rome, Italy

Background: Several conventional and non-conventional MRI measures have been used to monitor disease activity in phase II and III clinical trials of patients with multiple sclerosis (MS). Gd-enhancement is a good predictor of the occurrence of relapses, but not a strong predictor of the development of cumulative impairment or disability, indicating that different pathogenetic mechanisms are operative in the occurrence of relapses and in the development of long-term disability in MS.

Objectives: To investigate which MRI measure change is the best predictor of physical disability accumulation in a short time period.

Methods: Thirty patients with relapsing-remitting (RR) MS (median disease duration 4.9 years, median age 34.4 years and median EDSS 1.4) were included in the study. Patients were assessed at baseline and monthly for a period of three months with clinical, EDSS and MRI examination. The following monthly changes of MRI measures have been calculated: number of brain lesions in T2-, FLAIR- and T1 Gd enhanced-weighted images, T2-lesion load (LL), T1 Gd enhancing-LL, T1-LL and brain parenchymal fraction (BPF). No immunomodulatory drug was administered six months before the study entry. Three patients were treated with intravenous methylprednisolone for a relapse during the study period.

Results: The only MRI measure that significantly increased from baseline scan to scan 3 was the number of FLAIR lesions (r 4.7%, p= 0.04). In stepwise multiple regression analysis, EDSS changes during the three months period were predicted only by the changes in number of T1 Gd-enhancing lesions (R= 0.30, p= 0.032), whereas the number of new exacerbations were predicted only by the changes in T1 Gd enhancing-LL (R = 0.54, p= 0.0008).

Conclusions: Although different quantitative non-conventional MRI measures have been recently proposed to monitor disease activity in phase II and III of clinical trials, Gd-enhancement remains the best monitoring tool for determining short-term disease activity changes.

Disclosure: R Zivadinov has nothing to disclose.

P255

NEW CANDIDATES IN MULTIPLE SCLEROSIS IDENTIFIED BY AN ANALYSIS OF IGG REPERTOIRE COUPLED WITH A PROTEOMIC APPROACH

Almeras Lb, Lefranc Dd, Drobecq Hd, de Seze Jd, Dubucquoi Sd, Vermersch Pb, Prin La

aImmunology, Medicine school, Lille, North, France; bNeurology, CHU Lille, Lille, North, France; cCNRS 1309, Institut Biologie de Lille, Lille, North, France

Background: Today, none of the myelin-associated antigen (Ag) targets definitively discriminates the immune response observed in MS patients and in healthy subjects. However, it has recently been shown that the analysis of global immune Ab profiles can distinguish between normal individuals and patients suffering from various immune diseases.

Objectives: To compare the global autoantibody response to central nervous system (CNS) Ags in sera from 82 MS patients and 27 healthy subjects, and to process these data against antigenic targets.

Methods: The analysis of the immune profiles was performed by Western blotting. Data were submitted to linear discriminant analysis, and a proteomic approach was realized to characterize the relevant antigens.

Results: Our analysis could discriminate IgG antibody responses towards CNS Ags between healthy subjects and MS patients (sensitivity: 96.3%; specificity: 95.1%). Moreover, this approach separated the three clinical forms of MS with a high concordance rate with the clinical data (Kappa value = 77.8%). Seventeen auto-antigens were pointed out for the discrimination of profiles between healthy subjects and MS patients and 26 Ags were exhibited for separation of the three clinical forms of MS. A proteomic approach allowed us to identify some of these auto-antigens.

Conclusions: Our study suggests, that the serum Ab repertoire discriminate between MS and normal subjects and model the pathological processes associated with the various forms of MS. The antigen identification related to this specific immuno-pathological recognition could be used to develop a biological diagnostic test for MS.

Disclosure: Almeras L received a grant fellowship from CHRU of Lille.

Funding: Supported by Biogen.

P256

IMMUNOPATHOGENIC AND CLINICAL RELEVANCE OF ANTIBODIES AGAINST MOG IN MULTIPLE SCLEROSIS


Neurology, University of Innsbruck

Background: Recently, 4 different neuropathological subtypes were defined in MS, one among them characterized by antibody mediated demyelination. A potential target antigen for autoantibodies might be the CNS specific MOG.

Objectives: Recently we demonstrated that in 130 MS patients a subgroup mounted a persistent autoantibody response to the extracellular IgG domain of MOG in CSF and serum. We were further interested whether these antibodies exert biological features commonly seen in other autoimmune diseases. If these antibodies are pathogenic, we anticipated that we should demonstrate clinical effects regarding disease activity and treatment influences.

Methods: 261 MS patients were analysed for immunoglobulin subclasses of anti-MOG antibody subclasses revealed anti-MOG IgM antibodies as dominant. IgG isotype analysis exhibited a predominance of anti-MOG IgG1 and IgG3 corresponding to the prevailing IgG isotypes in other autoimmune diseases. In addition, we found that anti-MOG positive MS sera able to fix complement correlate with anti-MOG IgM titers and that some anti-MOG positive MS sera effectively activate the terminal complement pathway. Clinical relevance is suggested by a) the prognostic value of anti-MOG antibodies in patients suffering from a first demyelinating event and b) successful treatment with plasma exchange in selected MS patients suggestive of anti-body-mediated demyelination.

Conclusions: Our studies may contribute for an immunopathogenic subtyping of MS and thus stratifying MS patients within a differentialtherapeutic concept in the future.

Disclosure: T Berger has nothing to disclose.
Disclosure: V Daskalovska has nothing to disclose.

Conclusions: There was a statistically significant higher HHV-6 DNA prevalence between beta-interferon treated and untreated patients. Further analyses disclose a higher reactivity in CSF of MS patients than controls for some of the possible target antigens. Interestingly, different patterns of immune reactivity were observed among individual MS patients, suggesting heterogeneity in the intrathecal immune response.

Disclosures: S Cepok has nothing to disclose.

Disclosure: S Cepok has nothing to disclose.

P259

SEQUENTIAL STUDY OF SERUM SEX HORMONES AND TH1/TH2 CYTOKINE BALANCE DURING AND AFTER RELAPSE OF MULTIPLE SCLEROSIS


*Immunology, General University Hospital Gregorio Marañón, Madrid, Spain; †Biochemistry, General University Hospital Gregorio Marañón, Madrid, Spain; ‡Neurology, General University Hospital Gregorio Marañón, Madrid, Spain

Background: MS and other autoimmune diseases have a clear female preference, suggesting that sex hormones have a role on susceptibility to these diseases. Sex hormones have proved trophic/repair actions and are capable of cytokine synthesis modulation.

Methods: To overcome the oscillations in sex hormones during menstrual cycle, we studied 7 men (mean age, 32.3 years) with relapsing-remitting MS, and 7 age-matched healthy men controls. No patient had taken immunosuppressive nor hormonal drugs at least 3-mo prior to the study. Serum cortisol, testosterone, estradiol (E2), and progesterone (P) were assayed using a chemiluminescent immunosassay, and TNF-a and IL-10 by specific ELISAs at MS relapse and 2-mo later in parallel.

Results: All hormones measured and IL-10 were significantly higher in the MS group than in controls at relapse (cortisol p<.001, P p<.005, E2 p<.005 and testosterone p<.05, respectively), and all of them but progesterone at 2-mo later. P levels were systematically increased over the reference range for males (0.1 to 0.3 ng/ml) in all 7 MS patients (mean, 1.8±0.4). Cortisol levels fell to 38% after relapse (p<0.04), P decreased 21% and E2 even increased in 14%. Serum IL-10 decreased significantly from relapse to 2-mo follow-up (p=0.01). No correlation was observed among hormone levels and cytokines during relapse. A strong inverse correlation between P levels and P/E2 ratio and TNF-a/IL-10 ratio after relapse was noted (p<0.01, r=0.95, and p<0.02, r=0.82, respectively).

Conclusions: We show an activation of the hypothalamic-pituitary-adrenal and gonadal axis during relapse not correlated to any cytokine pattern. In the follow-up study, maintained higher levels of sex hormones than controls were observed, and P and P/E2 ratio strongly correlated with a Th2 cytokine pattern, suggesting trophic/repair functions, or/and an anti-inflammatory role.

Disclosure: C de Andrés has nothing to disclose.

P258

PREVALENCE OF HERPESVIRUS DNA IN MS PATIENTS

Daskalovska VA, Daskalovski ZV, Petkova-Boskovska T

Multiple Sclerosis, Clinic of Neurology, Skopje, Macedonia

Background: Multiple sclerosis (MS) is a chronic inflammatory and demyelinating disease of the central nervous system (CNS) with an as yet unknown aetiology. Studies on immune cells in the cerebrospinal fluid (CSF) and CNS lesions of MS patients have demonstrated oligoclonal expansion of B cells and hypermutations of the B-cell receptor genes compatible with extensive antigen maturation. Similarly, oligoclonal intrathecal immunoglobulin G (IgG) synthesis is observed in most MS patients. Both findings are consistent with an ongoing humoral immune response in the CNS of MS patients to as yet unknown target antigens.

Objectives: To investigate the antigen specificity of the local humoral antibody response in MS patients.

Methods: We applied a novel protein array technology. The arrays, comprising 35,000 inserts from a human foetal brain library, were probed with CSF and serum of 15 MS patients and 5 controls. Proteins with high immunoreactivity in CSF of MS patients were used for further ELISA and immunoblot analyses.

Results: Immune responses to 25 proteins were identified in MS patients, which were not observed in controls. Further analyses disclosed a higher reactivity in the CSF of MS patients than controls for some of the possible target antigens. Interestingly, different patterns of immune reactivity were observed among individual MS patients, suggesting heterogeneity in the intrathecal immune response.

Conclusions: The protein array technology provides a new tool to decrypt the antigen specificity of the intrathecal immune response in MS patients. The identified candidate antigens will be investigated for specificity of oligoclonal bands and for encephalitogenicity in experimental animal models to support the role of these antigens in the pathogenesis of MS.

Disclosure: V Daskalovska has nothing to disclose.

P260

CHEMOKINE RECEPTOR EXPRESSION ON T CELLS IS RELATED TO NEW LESION DEVELOPMENT IN MULTIPLE SCLEROSIS

Eikelenboom MP, Killestein J, Izeboud T, Kalkers NF, van Lier RA, Barkhof F, Uitdehaag BM, Polman CH

*Neurology, VU Medical Centre, Amsterdam, Noord Holland, Netherlands; †Radiology, VU Medical Centre, Amsterdam, Noord Holland, Netherlands; ‡Immunology, CEH, Amsterdam, Noord Holland, Netherlands

Background: In multiple sclerosis (MS) the expression of specific chemokines and their receptors is thought to play an important role in the mechanism by which antigen driven T cells can migrate to sites of inflammation, but as yet data to support this concept are limited. Therefore studies of chemokine expression in well documented patient groups are important to obtain further insight in its role in MS.

Objectives: To investigate the expression of chemokine receptors CCR5 and CXCR3 on T cells in patients with different sub forms of MS and to correlate this expression to changes in lesion load on MRI.

Methods: In 124 MS patients, the expression of chemokine receptors CCR5 and CXCR3 on T cells was measured by flowcytometry and correlated to clinical characteristics. In a subpopulation of 69 patients that was
followed longitudinally for about 3 years this expression was correlated to annualised changes in brain MRI hyperintense T2 and hypointense T1-weighted lesion load.

**Results:** CCR5 expression on CD8 positive lymphocytes is increased in patients with progressive disease compared to patients with relapsing remitting (RR) MS. The annualised change in T2 lesion load correlated with CXC/CR3 expression on CD8 positive cells (p=0.35; p=0.01), with CCR5 expression on CD8 positive cells in RR patients (p=0.60; p=0.01), and with both CXC/CR3 CD4 positive (p=0.56; p=0.01) and CD8 positive cells (p=0.68; p=0.001) in secondary progressive patients. No correlations were found for T1 lesion load and the chemokine receptors.

**Conclusions:** CCR5 and CXC/CR3 expression on CD4 positive and CD8 positive lymphocytes has significant impact on annualised change in T2 lesion load in MS, but no impact on T1 lesion load. Our observations suggest that interaction of specific chemokines and their receptors, probably by regulating the trafficking of inflammatory cells into the CNS, is one of the crucial mechanisms involved in new lesion development in MS, reflecting demyelination, giosis and inflammation.

**Disclosure:** M Eikelenboom has nothing to disclose.

**P261**

**SEVERE URTICARIA AS REACTION TO INTERFERON-β-1A ADMINISTRATION**

Fazio MC, Mazzero L, Buccafusca M, Dattola V, Scalfari A, Ferlazzo E, Girlanda P, Messina C

*Neurosciences, Psychiatry and Anaesthesiology, University of Messina, Messina, Italy; † O.U. Allergology and Clinical Immunology, University of Messina, Messina, Italy*

**Background:** Dermatological side effects after Interferon-β (IFN-β) administration in the site of injection are quite common in Multiple Sclerosis (MS) patients. Up to now, an urticarial IgE-mediated reaction to Interferon-β-1b has been reported in a MS patient with multiple drugs allergies and asthma while no urticarial reaction to IFN-β-1a has been described.

**Objectives:** Case report: a 30 years old woman affected by Relapsing Remitting MS had received IFN-β-1a for 6 months. The patient exhibited severe widespread urticaria and pruritus, that ameliorated after corticosteroids and antihistamines. The IFN-β-1a administration was discontinued. The patient was not atopic and she didn’t take any other drug.

**Methods:** The patient resulted negative to tests for inhalant and food allergens. Total IgE levels were within normal ranges, routine laboratory investigations, autoantibodies and serum complements levels were also normal. Skin prick test and intradermal test were performed (histamine as positive control, physiologic saline as negative control) and, after giving informed consent, IFN-β-1a and its diluent were tested.

**Results:** The diluent was negative but the IFN-β-1a resulted positive at 1: 100 I.D. dilution. The patient underwent, successively, a challenge test to Glutamater acetate as alternative drug with negative results and therefore started such therapy. She has been undergoing this treatment for nine months without developing, up to now, any adverse drug reaction.

**Conclusions:** We suppose that in our not atopic patient it has been an IgE-mediated reaction to IFN-β-1a administration with urticaria as clinical manifestation.

**Disclosure:** M Fazio has nothing to disclose.
subsets, and only after 6 months in the CD3+, CD3+CD8+ and CD45RO+ subsets. Interestingly, mitogen-induced apoptosis was also significantly reduced in the CD19+CD5+ subset after 6 months of treatment.

Conclusions: These data show that after 6 months of therapy, IFN beta-1b exerts a profound reduction of both spontaneous and mitogen-induced apoptosis in several specific T-cell subpopulations, already evident at one month for some subsets. With regard to B cells, we only found a reduction of induced apoptosis at 6 months.

Disclosure: A Garcia-Merino has nothing to disclose.
Funding: Supported by Shering Espana, SA.

P264

SPONTANEOUS EX-VIVO AND MITOGEN-INDUCED APOPTOSIS ARE INCREASED IN SEVERAL LYMPHOCYTE SUBSETS OF PATIENTS WITH RELAPSING-REMITTING AND SECONDARY-PROGRESSIVE MS

*Neuroimmunology Unit, Clínica Puerta de Hierro, Universidad Autonoma, Madrid, Spain; **Universidad de Alcala, Alcala de Henares, Madrid, Spain

Background: Dysregulation of lymphocyte apoptosis has been reported in MS, but there are no studies regarding the incidence of apoptosis in specific lymphocyte subpopulations of these patients. The currently used apoptotic index (AI) measures the presence of apoptosis in a phenotypically defined cell population.

Objectives: To quantify spontaneous and induced apoptosis in lymphocytes of MS patients with regard to a control population of healthy individuals

Methods: Peripheral blood mononuclear cells from 46 interferon-naive patients with relapsing-remitting and secondary progressive MS and 6 healthy controls were separated and characterized in a FACscalibur analyzer using monoclonal antibodies. The AI was calculated for T-cells expressing CD3, CD4, CD8 and CD45RO/RA antigens and for B-cells expressing CD19/CD5 antigens. These AI were determined after 24 hour culture. All studies were performed in two different conditions: spontaneously, and after phytohemagglutinin induction. Comparisons between MS patients and healthy controls were carried out using the non parametric Wilcoxon test.

Results: A significant increase in spontaneous ex vivo apoptosis was found in peripheral blood lymphocytes from MS patients (p<0.05). This increase occurred in T lymphocytes (p<0.05) but not in B lymphocytes, and was observed in the CD4+ subpopulation for the CD4+CD45RO+ subset (p<0.05), as well as in the CD8+ subpopulation for the CD45RO+ and CD45RA+ subsets (p<0.05). Similar increases in AI were found in mitogen induced apoptosis of several populations: CD3+ (p<0.05), CD4+CD45RA+ (p<0.05), CD8+CD45RA+ (p<0.05) and CD8+CD45RO+ (p<0.01). There was a marked increase of induced apoptosis in the CD19+/CD5+ subset (p<0.01)

Conclusions: We have found an impressive increase of apoptosis in spontaneous and mitogen induced apoptosis within several T-cell subpopulations of MS patients. For B cells, apoptosis was increased only after mitogen induction.

Disclosure: A Garcia-Merino has nothing to disclose.
Funding: Supported by Shering Espana, SA.

P265

INTERFERON BETA-1A REGULATES G PROTEIN COUPLED RECEPTORS IN MONONUCLEAR CELLS FROM HEALTHY DONORS AND MULTIPLE SCLEROSIS PATIENTS

Giorrelli M, Livrea P, Defazio G, Ricchiuti F, Mola A, Di Monte E, Trojano M
Dept. of Neurologic and Psychiatric Sciences, University of Bari, Bari, Italy

Background: Beta-adrenergic and chemokines systems play a relevant part in the modulation of immune responses in Multiple Sclerosis (MS). Both beta-adrenergic and chemokines receptors belong to the large family of the G protein coupled receptors (GPCRs) which are regulated by G protein coupled receptor kinases (GRKs), β-arrestins, and regulators of G protein signaling (RGS). Lymphocytes activation modify responses to GPCRs agonists through regulation of the expression of GRKs, β-arrestins and RGS. Interferon beta is known to counteract effects of mitogens on lymphocytes in vitro and in vivo in MS patients.

Objectives: To verify whether interferon beta-1a can regulate the activity of GPCRs through the tuning of GRKs, β-arrestins and RGS in mononuclear cells (MNL).

Methods: MNL from healthy donors were stimulated in vitro up to 72 h with none, phitoemmaglutin (PHA) 5µg/ml, interferon beta-1a (Rebif) 1500 U/ml, or both. Differently, freshly ex-vivo isolated (0,3,6 months) MNL were used for all studies on Multiple Sclerosis patients (10) who underwent to interferon beta-1a (Rebif 22 µg/3 times a week) treatment. Expression of GRKs and β-arrestins was assessed either by Western Blotting and semi-quantitative RT-PCR. Receptor activity was tested by measuring cAMP accumulation upon cells exposure to the β-adrenergic agonist isoproterenol.

Results: In in-vitro experiments on MNL from healthy donors, PHA-induced activation downregulated (10 folds) β-adrenergic receptors through the overexpression of GRK 2 (4 folds), GRK 3 (5 folds), and RGS 16 (12 folds) and the down-regulation (6 folds) of β-arrestin 1. Interferon beta-1a (1500 U/ml) counteracted all the mitogen-induced modifications. In Multiple Sclerosis patients, GRK2 levels increased (2 folds) at the third month, whereas turned to basal levels at the sixth month of interferon beta-1a treatment. No modifications were found when analysing GRK3 and β-arrestin 1.

Conclusions: Beta-adrenergic, chemokines, PAF and substance P receptors are GPCRs involved in the control of MNL functions. Modulation of signalling through GPCRs suggests a novel antiinflammatory property of Interferon beta therapy in MS patients.

Disclosure: M Giorrelli has nothing to disclose.
Funding: Supported by Serono.

P266

ORAL TERBUTALINE DIFFERENTIALLY AFFECTS CYTOKINE (IL-10, IL-12, TNF, IFNG) RELEASE IN MULTIPLE SCLEROSIS PATIENTS AND CONTROLS

Gold SM*, Heesen C*, Sundermann J*, Tessmer W*, Schulz K*
*Neurology, University Hospital Eppendorf, Hamburg, Germany; *Medical Psychology, University Hospital Eppendorf, Hamburg, Germany

Background: Increasing evidence supports the notion of autonomic dysfunction in multiple sclerosis (MS). Recently, an increased density of beta-adrenergic receptors (beta-AR) has been demonstrated in MS. Beta-receptor agonists were found to have therapeutic effects in EAE.

Objectives: To investigate the effects of oral terbutaline administration on inflammatory and anti-inflammatory cytokines in MS patients and healthy controls. Associations of cytokine response with measures of disease activity and disability were examined.

Methods: Terbutaline (5mg) was administered orally to 10 healthy controls and 48 MS patients classified according to disease course and disability. Blood samples were taken before and 2 hours after administration. PHA-stimulated cytokine production (IL-10, IFNg, TNFaalpha, IL-12) was analyzed in whole blood culture.

Results: While all subjects showed increases in heart rate and blood pressure, no significant difference was observed between patients and controls. IL-10 and IL-12 were significantly induced in controls but not in MS patients (p=0.03 and p=0.001 respectively). These differences were seen in patients with or without ongoing immunomodulatory therapy. Furthermore, we found some indication that the reported neuroimmunological dysregulation in MS may be associated with the extent of handicap and disability.

Conclusions: We conclude that a single dose terbutaline administration induces TH-2 (IL-10) as well as TH-1 (IL-12) cytokine production in healthy controls but not in MS patients. Our findings might reflect a disturbed autonomic control of the immune system in MS.

Disclosure: S Gold has nothing to disclose.
Funding: Supported by Gemeinschaftszentrum HertieForschung (GHS 2/447/9).
P267
CROSS-REACTIVE ANTIBODIES AGAINST MYELIN BASIC PROTEIN, ACINETOBACTER SP. AND PSEUDOMONAS AERUGINOSA IN MULTIPLE SCLEROSIS
*Health and Life Sciences, Kings College London, SE1 9NN, United Kingdom; 
Neuroinflammation, Imperial College School of Medicine, London, W6 8RF, United Kingdom

Background: Cross-reactivity or molecular mimicry may be one of the aetiological mechanisms involved in multiple sclerosis. The microbes Acinetobacter and Pseudomonas have been shown to carry amino acid sequence similarities to brain components, including myelin basic protein. Antibodies to both Acinetobacter and Pseudomonas (Hughes et al. Clin. Diag. Lab. Imm. 2001;8: 1181) and specific peptide mimicry sequences in these bacteria (Wilson et al. Proceedings of Acinetobacter 2000; abstract 1: 1) have been previously demonstrated in multiple sclerosis patients.

Objectives: The aim of this study was to determine cross-reactivity between bacterial peptides and brain antigens.

Methods: Antisera to Acinetobacter (SRFAWGE), Pseudomonas (TRHAWGD) and myelin basic protein (SRFSWGAE) peptides were produced in Biozzi AB/H mice and analysed using ELISA.

Results: Peptide antisera raised against myelin basic protein was inhibited by preincubation with 100μg/ml of both the Acinetobacter and Pseudomonas peptides. Prior to incubation, antisera to myelin basic protein had a mean antibody binding activity of 0.752 ± 0.005 at a dilution of 1: 25 and reacted at a dilution of up to 1: 6400. After incubation with Acinetobacter and Pseudomonas peptides the binding activity was reduced to 0.38 ± 0.005 and 0.34 ± 0.012 respectively at a dilution of 1: 25 and reacted at dilutions up to 1: 800. Pre-incubation with an irrelevant peptide did not affect binding activity.

Conclusions: The results obtained demonstrate cross reactivity between similar amino acid sequences in Acinetobacter, Pseudomonas and myelin basic protein. Further work needs to be carried out in order to assess the role, if any, of antibodies to these peptide sequences in the aetio-pathogenesis of multiple sclerosis.

Disclosure: All authors have nothing to disclose.

Funding: Supported by American Friends of Kings College.

P268
THE PLASMA LEVELS OF ALPHA-2-MACROGLOBULIN AND THE TRANSFORMED FORM ARE SIGNIFICANTLY DIFFERENT BETWEEN PATIENTS WITH MULTIPLE SCLEROSIS AND CONTROLS

Jensen PH, Datta P, Jorgensen S, Oturai AB, Sorensen PS
MS Research Unit, Neuroscience Centre, Copenhagen University Hospital, Rigshospitalet, Copenhagen

Background: Alpha-2-macroglobulin (a2M) is a major protease inhibitor and cytokine-binding protein in the human body fluids. Its involvement in the immune system may be of importance for the regulation of immune responses.

Objectives: Determinations of the concentrations of a2M and the transformed form were performed in plasma samples from MS-patients and controls for the analysis of a possible increased protease activity and an possible a2M-dependent regulation of the immune system.

Methods: Measurements of a2M and the transformed form in plasma samples from 60 MS-patients and 132 control individuals were performed by use of single radial immunodifussion, and by ELISA using an a2M transformation specific monoclonal antibody.

Results: Measurements of the a2M concentration in plasma from MS-patients and controls demonstrated a significantly lower concentration in the patients. When the concentration of the transformed a2M was measured, there was a significant difference between the MS-patients and controls, but in this case the concentrations were highest in the patients. The significant difference between the patients and controls was still valid, when the fractions of transformed a2M to total a2M was compared.

Conclusions: The significant differences in the a2M concentrations may suggest that a2M is of importance for the regulation of proteases and cytokines in the immune system in MS-patients.

Disclosure: P. Jensen has nothing to disclose.

Funding: Supported by The Multiple Sclerosis Society of Denmark.

P269
LOW MOLECULAR WEIGHT GLYCOCONJUGATES IN BRAIN HAVE IMMUNOREGULATORY ACTIVITY

Lindsey JW*, Waxham MN*, Stephens NE*, Weiser S*
*Neurology, University of Texas-Houston, Houston, Texas, USA; 
Neurobiology and Anatomy, University of Texas-Houston, Houston, Texas, USA

Background: Our hypothesis is that normal brain tissue contains immunoregulatory signals which prevent autoimmune damage. A defect in the presentation or recognition of these signals could contribute to the pathogenesis of multiple sclerosis. In previous work we demonstrated that homogenized brain tissue has profound immunoregulatory activity in culture. The activity was in the insoluble fraction and became soluble with NaOH treatment.

Objectives: Our objective was to further characterize the immunosuppressive activity found in brain tissue.

Methods: Brain tissue from naive mice was homogenized, solubilized using NaOH or detergent, and fractionated by gel filtration chromatography. Fractions were tested for activity in suppressing antigen driven proliferation of lymph node cells in culture. Fractions were visualized on polyacrylamide gels stained with Coomassie to demonstrate proteins and Alcian to demonstrate glycoconjugates.

Results: After solubilization with NaOH and separation on a gel filtration column, maximum activity was in a high molecular weight fraction containing many different proteins and low molecular weight material which contained glycoconjugates. These components were presumably part of a macromolecular complex. When this active fraction was treated with the detergent CHAPS and refractonated on the gel filtration column, activity was in fractions containing few proteins and the majority of the glycoconjugates. Alternative methods of solubilization were tried, and activity always correlated closely with the presence of glycoconjugates. Thin layer chromatography demonstrated that these glycoconjugates contained glycolipids.

Conclusions: A low molecular weight glycoconjugate appears to be a major immunoreulatory component of normal brain tissue. These may be either glycolipids or small glycopeptides. Further work is needed to identify the active molecule and determine the relevance of these findings for immune regulation in vivo.

Disclosure: J Lindsey has nothing to disclose.

Funding: Supported by the National Multiple Sclerosis Society and the Clayton Foundation for Research.

P270
LEVELS OF Sapo-1/Fas in the Serum of Multiple Sclerosis Patients Before and After Steroid Treatment

Mitosek-Szewczyk K, Bartosik-Psujek H, Belniak E, Dobosz B, Stelmasiak Z
Department of Neurology, School of Medicine in Lublin, Poland

Background: Soluble APO-1 (sAPO-1) may prevent apoptosis of lymphocytes induced by activation of the APO-1/Fas receptor.

Objectives: Investigation if abnormal lymphocyte apoptosis occurs in multiple sclerosis (MS) during relapse of illness and to study the impact of steroid therapy on apoptosis.

Methods: Determination of the sAPO-1/Fas levels in the serum of 24 multiple sclerosis patients in relapse phase and after steroid treatment and in the serum of 9 controls by the test ELISA method.

Results: In controls mean sAPO-1 level was 22.51 pg/ml. During the relapse mean sAPO-1 level was 23.43 pg/ml and after steroid treatment mean level elevated to 32.66 pg/ml. No differences were detected in mean serum sAPO-1 lev-
els between multiple sclerosis patients and controls, however high sAPO-1 level was stated in the serum of MS patients after steroid treatment as compared to sAPO-1 level before treatment and the difference was statistically significant (p = 0.004).

Conclusions: Results of this preliminary study suggests that resistance of peripheral blood lymphocytes to apoptosis mediated by sAPO-1 is not likely to be a major factor in the development of autoreactive cells in MS, but increased sAPO-1 level after the use of steroids may protect the cells against apoptosis.

Disclosure: K Młosek-Szczyczyk has nothing to disclose.

P271
UPREGULATED SURVIVIN EXPRESSION IN ACTIVATED T LYMPHOCYTES CORRELATES WITH DISEASE ACTIVITY IN MULTIPLE SCLEROSIS
Noori M, Sharief MK
Dept of Neuroimmunology, GKT School of Medicine, Guys Hospital, London, United Kingdom

Background: Programmed cell death (apoptosis) is critical for the normal development and homeostasis of the immune system. There is emerging evidence that failure of apoptosis to eliminate potentially pathogenic, autoreactive T lymphocytes may be involved in the pathogenesis of MS. This failure is related to multiple abnormalities of apoptosis-regulatory molecules that involve survivin, a recently described cell cycle-regulated anti-apoptosis protein.

Objectives: To investigate the relationship between survivin expression in peripheral T lymphocytes and clinical features of MS.

Methods: Using combined dot-blotted immunohistochemistry and Western blotting, we analyzed the expression of survivin and other apoptosis regulatory proteins in mitogen stimulated T lymphocytes from patients with MS and relevant controls.

Results: We detected a significant over-expression of survivin in mitogen stimulated T lymphocytes from patients with active MS compared to corresponding expression in patients with stable MS or those with inflammatory and non-inflammatory neurologic disorders. This overexpression of survivin in patients with active MS correlated with cellular resistance to apoptosis and with features of disease activity, such as disease duration and the number of enhanced lesions on cranial magnetic resonance imaging. There was no correlation between cellular survivin levels and the expression of other apoptosis-inhibitory proteins, such as Bcl-2 and FLIP.

Conclusions: Findings presented here indicate that cellular overexpression of the novel anti-apoptosis protein survivin is a feature of clinically active multiple sclerosis.

Disclosure: MK Sharief has received honoraria from Seesno International.

P272
DYSGREGULATION OF PROGRAMMED CELL DEATH ACTIVATION IN MULTIPLE SCLEROSIS PATIENTS
Saresella M, Clerici M, Trabattoni D, Speciale L, Piacentini L, Caputo D, Ferrante P
Dept of Neuroimmunology, GKT School of Medicine, Guys Hospital, London, United Kingdom

Background: Multiple sclerosis (MS) is a chronic neurologic disease characterized by multifocal inflammation and damage involving the myelin sheath. An immunopathologic mechanism, mainly mediated by the activation of cell mediated immunity (CMI) and secondary to molecular mimicry with cross-reacting and yet undefined epitopes, is linked to destruction of the myelin sheath. Apoptosis of autoreactive T lymphocytes is hypothesized to be a major mechanism of tolerance, and in particular, programmed cell death (PCD) of antigen-specific immune cells in MS could modulate immune-mediated destruction of the myelin sheath.

Objectives: In this study we analyzed markers of susceptibility to PCD in 28 MS patients with either acute (AMS; N=14) or stable (SMS; N=14) disease, and in 6 other patients undergoing IFNβ/IFNα therapy.

Methods: Thus, we performed cytofluorometric analysis of AAD-permeability and we evaluated the percentages of FAS and bcl2 expressing CD8+, CD4+, and CD4+ cells. PBMC (5x10^5/ml) were cultured for 24 h in the presence of: a) medium alone; b) PMA (25ng/ml) and ionomycin (1k&16154g/ml); or c) anti-CD28 (2k&16154g/ml) + MBP (10k&16154g/ml).

Results: Results showed that: 1) the percentage of MBP-stimulated AAD-permeable and Fas-expressing CD8+ and CD4+ cells (apoptotic cells) is significantly augmented in SMS compared to treated patients, and 2) the percentage of PMA+IONO-stimulated AAD-permeable and Fas-expressing CD8+ and CD4+ cells is significantly increased in AMS and SMS compared to IFNβ/IFNα treated MS patients.

Conclusions: These observations suggest that PCD of myelin-specific lymphocytes could reduce immune-mediated destruction of the myelin sheath in SMS patients, and that augmented PCD could be one of the beneficial mechanisms associated with IFN therapy.

Disclosure: m saresella has nothing to disclose.

P273
THE EXPRESSION OF CD5 ON PERIPHERAL B LYMPHOCYTES CORRELATES WITH DISEASE ACTIVITY IN PATIENTS WITH MULTIPLE SCLEROSIS
Seidi O, Sharief MK
Dept of Neuroimmunology, GKT School of Medicine, Guys Hospital, London, United Kingdom

Background: Although activated T lymphocytes play a key role in the pathogenesis of MS, there is growing evidence that implicates B lymphocytes and their products in this disease. A subpopulation of B lymphocytes expressing the CD5 antigen are involved in several autoimmune disorders through the release of autoantibodies.

Objectives: To examine the relationship between CD5 expression on peripheral B cells and MS disease activity.

Methods: In this study, we used three-color flow cytometry to examine the expression of CD5 antigen on B lymphocytes from patients with relapsing remitting MS, and correlated this expression with features of disease activity and circulating levels of autoantibodies against myelin basic protein.

Results: CD5 expression on B lymphocytes was significantly higher in patients with active MS when compared to patients with clinically stable MS or those with inflammatory or non-inflammatory neurologic disorders. CD5+ B lymphocytes from patients with active MS correlated significantly with the number of gadolinium-enhancing MRI lesions, and inversely with disease duration. The expression of CD5 on B lymphocytes in MS patients also correlated with circulating levels antibodies against myelin basic protein.

Conclusions: Results presented here indicate that clinically active MS is associated with an expanded population of peripheral CD5+ B lymphocytes.

Disclosure: MK Sharief received honoraria from Serono International.

P274
GLUCOCORTICOID SENSITIVITY IN PATIENTS WITH MULTIPLE SCLEROSIS
Wineen LE, Muris D, Dijkstra CA, Polman C, van den Berg T, Uitdehaag B
aNeurology, VU Medical Centre, Amsterdam, The Netherlands; bMolecular Cell Biology, VU Medical Centre, Amsterdam, The Netherlands; cMolecular Cell Biology, VU Medical Centre, Amsterdam, The Netherlands

Background: Glucocorticoids (GC) are frequently used in treatment of Multiple Sclerosis (MS). Both endogenous production and treatment with GC may suppress inflammatory activity in the central nervous system. In earlier studies increased hypothalamic-pituitary-adrenal (HPA) axis activity leading to higher endogenous cortisol levels has been described in MS patients probably secondary to inflammation. This may lead to a decreased GC sensitivity and an increase in disease activity.

Objectives: To study the GC sensitivity in patients with MS in comparison to healthy subjects and to evaluate differences between subgroups of MS patients.

Methods: GC sensitivity was determined by an in vitro dexamethasone (DEX) suppression of LPS stimulated tumor necrosis factor-alpha (TNF-α) production and expressed as IC50. Blood samples from 130 MS patients and 49 healthy controls (HC) were collected between 9-11:30 am in endotoxin free,
Neuropsychology

P275
SCREENING FOR EARLY COGNITIVE IMPAIRMENT IN MULTIPLE SCLEROSIS PATIENTS USING THE CLOCK DRAWING TEST.

BARAK Y, ACHIRON A
Psychogeriatrics, AABARANEL Hospital, Bat-Yam, NA, Israel

Background: The clock drawing test (CDT) is a complex task assessing integrative functions, abstract thinking and visuospatial organization.

Objectives: In the present study we evaluated the CDT as a possible screening instrument for early cognitive impairment in MS patients.

Methods: 107 MS patients completed the CDT as well as a battery assessing integrative functions, abstract thinking and visuospatial organization.

Objectives: In the present study we evaluated the CDT as a possible screening instrument for early cognitive impairment in MS patients.

Results: Mean CDT score was 2.6±1.4. In 53% of patients the CDT was normal while in 11.2% dementia was apparent. The CDT score did not correlate with the total EDSS. Significant correlations were obtained with mental functional system score of the EDSS (r=0.78; p<0.0001), visual learning and recall, sustained attention and concentration.

Conclusions: Mean CDT score was 2.6±1.4. In 53% of patients the CDT was normal while in 11.2% dementia was apparent. The CDT score did not correlate with the total EDSS. Significant correlations were obtained with mental functional system score of the EDSS (r=0.78; p<0.0001), visual learning and recall, sustained attention and concentration.

Disclosure: Y BARAK has nothing to disclose.

P276
QUANTITATIVE MRI AND NEUROPSYCHOLOGICAL ASSESSMENT IN 48 RR-MS PATIENTS.

Brescia Morra V, Lanzillo R, Brunetti A, Salvatore E, Schiavone V, Quarantelli M, Coppola G, Oreife G

Background: Cognitive dysfunctions have already been reported in MS. Prevalence may vary between 30 and 70%. Previous reports showed correlations between neuropsychological and MRI data. Brain atrophy has been demonstrated in MS, mainly involving the White Matter (WM). Non conventional and automated segmentation MRI techniques have been produced in order to quantify the extension of abnormal WM and the degree of brain atrophy.

Objectives: The aims of our study were (1) to evaluate the prevalence and the profile of cognitive impairment, (2) to quantify the lesion load and the brain atrophy and (3) to analyse possible correlations between cognitive and neuroradiological data in a group of RR-MS patients.

Methods: A group of 48 RR-MS patients, with a mean age of 35.4 ± 7.9 years (21-50) underwent to EDSS evaluation, conventional MRI with an automated unsupervised segmentation post-processing in order to quantify lesion load and absolute and fractional WM, grey matter (GW) and CSF volumes, neuropsychological evaluation by means of Mini Mental State Evaluation, Story Recall Test, Controlled Oral Word Association Test, Delayed Memory Test (from the Wechsler Memory Scale), Standard Raven Progressive Matrices and Ideomotor Apraxia Test. Fifty-five normal subjects were the MRI control group. The statistical analysis was performed by Pearson Coefficient and Mann-Whitney U test.

Results: We found at least one abnormal neuropsychological test in 29 patients (60.4%), in accordance to the literature. The mean lesion load was 23.0 ± 25.5 ml. GM percentage was lower in MS than in controls (49% vs. 51%, p<0.01), despite of the older age of the control group. WM percentage was lower in MS than in controls (36% vs. 38%, p<0.01). We found a significant inverse correlation between the lesion load and the GM volume (r= -0.37, p<0.01). Ideomotor Apraxia Test showed a slightly significant correlation with lower GM volume (p=0.05).

Conclusions: As a main and unexpected data, we found a significant reduction of GM volume, in accordance to the hypothesis of retrograde axonal degeneration in MS. Moreover, we confirmed the high prevalence of cognitive dysfunction in MS. GM atrophy seems to correlate with some of the cognitive functions investigated.

Disclosure: V Brescia Morra has nothing to disclose.

P277
COGNITIVE PERFORMANCES ARE ALTERED IN EARLY MULTIPLE SCLEROSIS

EA 2966 (Neurobiology of Myelin Disorders Laboratory), University Victor Segalen, Bordeaux, Bordeaux Cedex, France; INSERM U 330, University Victor Segalen, Bordeaux, Aquitaine, France; Federation des Neurosciences Cliniques, CHU de Bordeaux, Bordeaux, Aquitaine, France

Background: 40% to 60% of patients with established MS exhibited cognitive deficits. Very few studies describe cognitive functions at early stages of multiple sclerosis.

Objectives: To determine the cognitive skills of MS patients at the time of the diagnosis.

Methods: 69 MS patients (58 RRMS, 7 T2MS, and 4 PPMS) were recruited by the AQUISEP network less than six months after the time of diagnosis. After written informed consent, they underwent a comprehensive neuropsychological examination. Patients were individually matched with healthy controls, who undertook the same cognitive tasks. The neuropsychological assessment comprised Rao Brief Repeatable Battery (Selective Reminding Test (SRT), 10/36 Spatial Recall Test, Symbol Digit Modalities Test (SDMT), Paced Auditory Serial Addition Task (PASAT), Word List Generation Task), the Boston Naming test, the Ruff Figural Fluency test and the Similarities WAIS-R subtest. A computed inhibition battery including a reaction time test, Stroop tests and the negative priming test was also administered.

Results: The RR patients performed significantly worse than the controls in SDMT (complex attention), SRT (verbal immediate and delayed recall memory), 10/36 spatial recall test (spatial delayed recall memory), Similarities WAIS-R subtest, Ruff test part 1 (non verbal fluency), PASAT 2s (sustained

Disclosure: L Winsen has nothing to disclose.
attention). On the contrary, patients did not performed worse than controls in PASAT 3s. 18 patients (26,1%) scored &lt;861502; 2 DS below the control mean on SDMT, 14 (20,3%) on WAIS-R subtest, 11 (15,9%) on 10/36 spatial recall test, 13 (18,8%) on delayed recall SRT, 4 (5,9%) on PASAT2s. 15 (21,7%), 13 (18,8%) and 13 (18,8%) scored &gt; 2 DS below the control mean on long-term storage, consistent long term retrieval and delayed recall SRT respectively. No patients scored below 2 DS on Ruff test. Other results as well as correlations with other clinical data and quality of life scores will be presented at the congress.

Conclusions: Significant cognitive dysfunction is observed in multiple sclerosis at the time of the diagnosis

Disclosure: B Brochet has nothing to disclose.
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P278
PACED AUDITORY SERIAL ADDITION TEST AND MAGNETIC RESONANCE IMAGING FINDINGS IN MULTIPLE SCLEROSIS PATIENTS. PRELIMINARY STUDY.

Callegaro D, Fuso S, Otaduy M, Costa M, Lacerda M, Bachesci L, Leite C, Bueno O
*Neurology, Medical School of the University of São Paulo, São Paulo, Brazil; bRadiology, Medical School of the University of São Paulo, São Paulo, Brazil; cPsychobiology, Medical School of São Paulo EPM, São Paulo, Brazil

Background: The Paced Auditory Serial Addition Test (PASAT) represents an efficient tool for evaluating information processing speed in MS patients. However the literature on the correlation between PASAT performance and lesion burden on brain MRI has been contradictory. A recent study suggested that by using the Mean Dyad Score (MD) on the PASAT the correlation between the test and disease burden found on brain MRI could be improved.

Objectives: To correlate a series of MRI findings in MS patients to the PASAT results using the MD score system.

Methods: Eight MS patients, two male and six female, (mean age = 35 ± 8 years, mean disease duration = 8 ± 4 years, mean EDSS = 3.5 ± 2.0 and years of education = 12 ± 3) were chosen for the combined neuropsychological and MRI study. The PASAT was applied to all patients on the same day of the MRI exam. Performance on the PASAT was quantified using the MD score. The MRI exam, performed on a 1.5 T scanner, included acquisition of axial T2, T1, PD and diffusion weighted images. The total area of lesions on PD weighted images was quantified by two radiologists. Firstly a treshold was applied to the image and after manual selection of the lesions the corresponding area was calculated automatically by the software Scion (NIH, USA). Diffusion weighted images were post-processed on a workstation in order to generate Aparent Diffusion Coefficient (ADC) maps and histograms. Mean ADC, ADC peak position and peak height were calculated from the diffusion histograms.

Results: The mean MD score was 8.75 ± 11.06 (range 0 to 31.75). The mean total area of lesions was 1706 ± 1955 mm² (range 142 to 5564 mm²). Mean ADC was 992 ± 45 x 10⁻⁶ mm²/s, mean ADC peak height was 449 ± 7.4. The MD score presented a tendency to decrease with total lesion area, but this was statistically insignificant. The correlation between MD and ADC parameters was stronger but still statistically insignificant.

Conclusions: No significant correlation was found between MD scores on the PASAT test and MRI parameters quantifying MS disease burden. A larger number of patients need to be studied in order to confirm these findings.

Disclosure: D Callegaro has nothing to disclose.

P279
COGNITIVE SCREENING OF MS PATIENTS RECRUITED FOR A CLINICAL TRIAL.

Christodoulou C, Krupp LB, Melville P, Schei W, Morgan T, McIree C
Neurology, SUNY Stony Brook, Stony Brook, New York, USA

Background: Cognitive dysfunction affects approximately half of MS patients. Effective screening tools are needed to identify patients who might benefit from treatments to enhance cognition.

Objectives: To describe baseline characteristics of MS patients screened for mild cognitive dysfunction enrolled in a 6-month clinical trial of an acetylcholinesterase inhibitor (ACHEI).

Methods: 64 participants without major depression met the following cognitive screening criteria: scores at least 0.5 standard deviations (SD) below published age and gender based normative data on the Rey Auditory Verbal Learning Test (RAVLT), and Mini Mental State Examination score > 25 (to exclude severely impaired persons unable to comply with protocol). Subjects completed a modified version of the Brief Repeatable Battery (BRB). Their performance was compared to published data from demographically similar healthy persons as well as another large MS sample.

Results: Mean age was 44.5 (SD=8.6), education 14.6 (SD=2.3), EDSS 3.7 (SD=1.8), and 69% were female. MS subtypes were RR (56%), SP (42%), and PP (2%). Approximately 90% were on disease modifying agents, and 55% were employed. Mean RAVLT performance was 1.6 SD below norms. Not surprisingly, the most impairment on a BRB task was on the Selective Reminding Test (SRT), another verbal learning/memory task, with scores 2 SD below healthy controls (Boringa, 2001; Camp, 1999) and 1.3 SD below a large RR MS sample (Weinstein, 1999). Performance on other BRB tasks ranged from 0.8 to 1.7 SD below healthy norms and from 0.3 to 0.7 SD below the other MS sample. More than 90% of the sample scored 1.5 SD or more below each healthy sample on at least 1 of 4 BRB tests, and 67% compared to Weinstein’s MS sample. 33 subjects underwent baseline MR. Overall BRB scores correlated strongly with central cerebral atrophy (r = -.70, p < .000) and the ratio of n-acetyl-laspartate over creatine (indicative of axonal injury and/or loss) (r = -.64, p < .000).

Conclusions: The screening criteria selected persons with substantial cognitive impairment. Self-selection may have contributed to the level of impairment, since participants had enough concern about their cognition to participate in a 6-month clinical trial.

Disclosure: C Christodoulou has nothing to disclose.
Funding: Supported by NMSS RG042-A-2, NIDRR H133G990058, NIH HD38107-01, NIH GCRC 5M01 RR1071002.

P280
COGNITIVE FUNCTIONS IN MULTIPLE SCLEROSIS PATIENTS TREATED WITH INTERFERON-BETA: A CONTROLLED TWO YEAR FOLLOW-UP STUDY.

Dann M, Viti B, Splendiani G, Giambartolomei S, Arabi S, Provincioli L, Cervolvo G
Neurorehabilitation Clinic General Hospital “Umberto I”, Ancona, Italy

Background: Prevalence of cognitive decline in MS pts is ranging from 43% to 72%. Few Authors have investigated if IFN treatment affects cognitive functions in MS.

Objectives: To monitor cognitive functions in MS pts undergoing IFN-beta treatment for at least 2 yrs, and compare them with untreated subjects.

Methods: 52 pts with definite MS were studied. Clinical data were: M/F: 20/32; median EDSS: 2 (0-7); mean age: 38.6 yrs (16-63); mean disease duration: 7 yrs (1-33); mean education: 11.3 yrs (4-18). 27 pts received IFN-beta; 25 did not assume any drug. Cognitive functions were assessed using Digit Symbol, Buschke-Fuld Test, Wisconsin Card Sorting Test (WCST), Verbal Fluency FAS Test and Kohs’cube Test, tested at baseline (TO) and 2 years later
Background: Cognitive deficits are frequent in multiple sclerosis (MS) with a prevalence between 43 to 60%. However, there is no data concerning active MS, a particular subgroup of MS.

Objectives: To evaluate the frequency of cognitive dysfunction in active MS compared to progressive forms of the disease and healthy control subjects.

Methods: A battery of 23 tests assessing general cognitive efficiency, memory, planning abilities, visuo-spatial processing and executive function was administered to a battery of 23 tests assessing general cognitive efficiency, memory, planning abilities, visuo-spatial processing and executive function was administered to each participant. Nineteen patients with active MS, defined as an increase in EDSS score higher than 1.5 during the last year in patients with relapsing remitting MS, participated in the study. Their performance was compared to that of 41 patients with progressive MS (14 primary progressive and 27 secondary progressive) and 15 healthy control subjects. The subjects performance at each test was transformed as a z-score in order to operationalize their deviation from the control group. A score higher than 1.5 was considered as a significant decline.

Results: We did not observe any difference of EDSS score between the 3 subgroups (mean EDSS±SE = 5.9±0.8). Disease duration was longer in the secondary progressive group. Age at onset was higher in the primary progressive MS group. After a mean disease duration of 6.5 years, all the patients of the active MS subgroup had a z-score higher than 1.5 in one subtest or more. The z-score was significantly impaired at 7 among the 23 tests mainly on visuo-spatial processing and executive function tests. Although cognitive impairment was more important in secondary progressive MS, active MS patients were not different compared with primary progressive MS.

Conclusions: Cognitive deficits are frequent in active MS and involve visuo-spatial and executive dysfunction. It should be interesting to evaluate the effect of intensive immunosuppressive drugs (mitoxantrone) in this subgroup of MS patients.

Disclosure: J de seze has nothing to disclose.

P282

EMOTIONAL ADJUSTMENT IN PATIENTS WITH MULTIPLE SCLEROSIS

Pires-Barata S, Henriques J
Neurology, Hospital Espirito Santo, EVORA, Portugal

Background: Psychopathological disorders are described in the literature in MS patients. It is difficult to make a retrospective evaluation of the psychopathology of the patient, but an evaluation after the diagnosis can help finding a trait that might improve the psychological treatment and counselling of these patients.

Objectives: We pretended to screen for possible psychological traits in patients with multiple sclerosis.

Methods: We interviewed 6 patients with MS (3 women and 3 men), at least 6 months after the diagnosis. Age varies between 24 to 41 years. We used the SCL-90 scale as a screening to search for psychopathology signs. This scale is validated for our country population and evaluates emotional adjustment items, such as somatization, obsessive/compulsive, interpersonal relations, depression, anxiety, hostility, phobic anxiety, paranoid ideation, psychosocial, eating disorders, sleep disorders, thoughts of death and guilty feelings. We performed a 30 minutes interview to each patient.

Results: We observed after performing the scale that the most prevalent items were obsessive/compulsive behaviour and sleep disorders (independently of either it was on the awake, in falling a sleep or a restless sleep) that were present in all patients. Eating disorders (either eating too much or loss of appetite) were also present in 5 patients.

Conclusions: These results suggested that the obsessive/compulsive behaviour and sleep disorders are common in MS. Further research is needed to verified the association between obsessive/compulsive and sleep disorders in MS patients. Inclusion of a group control, enlarging the sample and using specific scales for the psychopathology disorders that we found, will reinforce this preliminary results.

Disclosure: Nothing to disclose.

P283

SUCCESSFUL ADULT DEVELOPMENT AS A FRAMEWORK FOR NEUROPSYCHOLOGICAL COUNSELING IN MULTIPLE SCLEROSIS

Lana-Peixoto MA, Haase VG, Lacerda SS, Lima EP
CIEM MINAS UFMG Center for Investigation of Multiple Sclerosis, Brazilian Committee for Treatment and Research in Multiple Sclerosis, Belo Horizonte, MG, Brazil

Background: The chronic and progressively incapacitating nature of MS, poses a major challenge to rehabilitative efforts. Approaches based solely on the pursuit of functional restitution have a high risk of failure and additional distress. Compensatory approaches have consistently shown effective in the empowerment of individuals both to cope with daily life in the short run and to plan their life courses in the long run, thus regaining control and autonomy as well as improving their sense of well-being. Compensation requires the establishment of a positive agenda.

Objectives: To establish a parallel between the processes of cognitive impairment and psychosocial adaptation to MS and normal adult development and aging.

Methods: A selective literature review, a conceptual analysis and a comparison between the patterns of psychosocial adaptation and cognitive impairment in MS and normal aging individuals were conducted.

Results: In MS and normal aging individuals deficits are observed preferentially in the domains of mechanic intelligence, such as episodic memory and executive functions. In both situations most severely affected cognitive performance relates to efficient and speeded information processing. On the other hand, cognitive functions related to the construct of pragmatic intelligence, such as verbal expression and comprehension, insight, social abilities and social cognition or wisdom, remain relatively preserved.

Conclusions: Focusing on the profile of cognitive impairment and assets in the broader context of adult development may help health professionals to establish an effective framework for neuropsychological rehabilitation in MS. Neuropsychological counseling in MS may benefit from incorporating a model of successful adult development which foresees the selection of domains in which there is a prospect for development, the optimization of investment and resources in the selected domains, as well as compensation for domains with declining performance.

Disclosure: M Lana-Peixoto has nothing to disclose.
P284
DEVELOPMENT OF THE BRAZILIAN VERSION OF THE MULTIPLE SCLEROSIS FUNCTIONAL COMPOSITE MEASURE: RESULTS FROM A PILOT STUDY.
Lana-Peixoto MA, Haase VG, Lacerda S, Lima EP
CIEM MINAS UFMG Center for Investigation of Multiple Sclerosis, Brazilian Committee for Treatment and Research in Multiple Sclerosis, Belo Horizonte, MG, Brazil

Background: The Multiple Sclerosis Functional Composite (MSFC) is an outcome measure in MS which assesses cognitive function (PASAT) in addition to motor function of the legs (T25) and arms/hands (9-HPT). The MSFC was designed to achieve better sensitivity and sounder psychometric foundations than traditional measures of disability.

Objectives: To report initial efforts to develop a Brazilian version of the MSFC, herein named MSFC-BCTRIMS.

Methods: We conducted a cross-sectional correlation analysis of the MSFC comparing performance by 15 MS patients and 15 demographically matched controls. The mean age of patients in the MS Group was 39.3 years (sd=10.4) and 38.6 (sd=11.8) in the Control Group. The mean formal schooling was 9.5 years (sd=3.6) for MS individuals and 11.9 (sd=4.6) for control participants. Women comprised 60% of participants in the MS Group and 67% in the Control Group. Patients had a mean disease duration of 8.21 years (sd=8.08), mean EDSS scores of 3.31 (sd=2.33) and mean Ambulatory Index (AI) scores of 2.08 (sd=2.33). Eighty seven percent of MS participants had relapsing-remitting disease. Z-scores were calculated using values from the international database employed to build the MSFC.

Results: A series of Mann-Whitney tests showed significant between-group differences both for the MSFC total scores (U=37.0, p<0.001) and the partial scores (T25: U=51.0, p<0.001; 9HPT: U=46.0, p<0.07; PASAT: U=50.0, p<0.003). Correlations between EDSS and AI scores were significant (rho=0.685, p<0.001), as well as between MSFC and EDSS (0.801, p<0.001), but not between the MSFC and the AI (rho=0.908, p<0.076). Significant correlations were also observed between the EDSS and all MSFC partial scores, but not between the MSFC and the AI.

Conclusions: Results of this pilot study suggest that the MSFC-BCTRIMS version is an appropriate disability measure for administration in Brazilian MS patients. Further studies are required to validate this version for clinical and research use in Brazil.

Disclosure: M Lana-Peixoto has nothing to disclose.

P285
BECK DEPRESSION INVENTORY AND GENERAL HEALTH QUESTIONNAIRE: RESULTS OF A COMPARISON BETWEEN MULTIPLE SCLEROSIS PATIENTS AND CONTROLS
Lana-Peixoto MA, Haase VG, Lacerda SS, Lima EP
CIEM MINAS UFMG Center for Investigation of Multiple Sclerosis, Brazilian Committee for Treatment and Research in Multiple Sclerosis, Belo Horizonte, MG, Brazil

Background: Major depression is highly prevalent in MS. Although many depression scales have been developed there is still a need for a reliable self-reported diagnostic measure. Beck Depression Inventory (BDI) and General Health Questionnaire (GHQ), which had been previously validated for the general Brazilian population. Specific MS self-report measures used were the Cognitive and Physical Fatigue Scales (CPFS) and the Multiple Sclerosis Self-Efficacy Scale (MSSE).

Objectives: To investigate the psychometric properties of some self-reported measures of psychosocial functioning adapted for use in Brazil.

Methods: The sample comprised 34 MS patients with a mean age of 42.6 years (sd=9.06) and mean formal schooling of 11.06 years (sd=4.39). Females comprised 76.5% of the cases. Twenty patients had relapsing-remitting MS, 9 secondary progressive MS and 2 primary progressive MS. The mean duration of the disease was 9.18 years (sd=5.0); the mean AI scores 2.47 (sd=2.00) and the mean EDSS score 3.00 (sd=2.47). Measures assessed included General Health Questionnaire (GHQ) and Beck Depression Inventory (BDI), which had been previously validated for the general Brazilian population. Specific MS self-report measures used were the Cognitive and Physical Fatigue Scales (CPFS) and the Multiple Sclerosis Self-Efficacy Scale (MSSE), which have been specifically translated and adapted for the present investigation.

Results: Estimated Cronbach Alpha values were 0.8612 for the BDI, 0.9584 for GHQ, 0.9520 for overall MSSE, 0.9050 for function MSSE, 0.9272 for control MSSE, 0.9302 for overall fatigue, 0.8634 for cognitive fatigue, and 0.8946 for physical fatigue. Disease duration did not correlate with any self-report measure. Functional MSSE correlated with AI (r=0.559, p<0.002) and EDSS (r=0.729, p<0.02) scores. Neither fatigue scores, BDI nor GHQ correlated with impairment measures. Both GHQ and BDI total scores correlated moderately and significantly with all fatigue and self-efficacy scores (r between 0.406 and 0.616, p between p<0.001 and p<0.02).

Conclusions: The assessed self-reported instruments presently in use in Brazilian MS patients exhibit reliable psychometric characteristics and can be employed confidently.

Disclosure: M Lana-Peixoto has nothing to disclose.

P287
PSYCHOSIS IN MULTIPLE SCLEROSIS
Niedermayerová Š, Benečová Y, Mechl M
1Department of Neurology, University Hospital, Brno, Czech Republic; 2Department of Radiology, University Hospital, Brno, Czech Republic

Background: The presence of psychiatric symptoms as a part of the picture of multiple sclerosis (MS) has long been recognized. The commonest symptom is participants were also also evaluated by the Cognitive and Physical Fatigue Scales (CPFS) and the Multiple Sclerosis Self-Efficacy Scale (MSSE).

Results: Eight patients (23.53%) had BDI scores above 20 and death cognition scores above the 90th on the GHQ. Both BDI, GHQ and GHQ-subscales discriminated between the MS and Control groups (t between 2.038 and 2.895, p<0.046 to p<0.003). In the MS Group, correlations between BDI, GHQ and disease markers (disease duration, AI and EDSS) were not significant. Intercorrelations between BDI, GHQ and GHQ-subscales were all moderate and significant (r between 0.352 and 0.597, p<0.001 to p<0.009). For the MS Group, both BDI and GHQ scores also correlated significantly with the MSSE and fatigue scores.

Conclusions: BDI scores may be a useful tool to compare patients with MS and other neuropsychological disorders, but its scores may lack specificity to depression in MS. Alternatives may be either to factorially discriminate and construct subscales or to use other self-reported measures for depression.

Disclosure: M Lana-Peixoto has nothing to disclose.
depressive mood state but psychotic disorders occur far less frequently. Some authors believe that although psychotic disturbances which take form of major depression or acute psychosis onset in many cases a number years before apperance of MS, they directly attributable to the organic disease. 

**Objectives:** To find a frequency of psychosis in a group of MS patients and to evaluate location and size of brain lesions detected by MRI to recognize a relation between MS and psychotic disorders. 

**Methods:** We analyzed retrospectively all the MS patients of our center (595 persons) and found those with psychosis before or after onset of neurological symptoms of MS. We excluded iatrogenic psychosis for example due to corticosteroids. The group of MS patients with psychosis and control MS group formed with respect to age, sex, disability and duration of MS, underwent MRI of the brain. We evaluated areas of lesions on T2-weighted scans in frontal and temporal lobe and in corpus callosum.

**Results:** We found 7 patients with psychosis (3 schizoaffective, 2 paranoid and 1 maniopressive psychosis and 1 paraphrenia) in 5 cases was onset before neurological symptoms (3-11 years) and in 2 cases during neurological ilnesses (1 resp. 5 years). The mean age of onset was 33 years (range 18-52). The prevalence of psychosis in MS patients was slightly higher than in common population (1.7% versus 0.5-1%) but was more often in women than men (6:1), while in common population is the same in both sex. We found statistically significant differences between the psychotic and control groups for areas of lesions in temporal lobe (p=0.04). The psychotic group had greater areas of lesions in corpus callosum, but this was not statistically significant. We obtained no differences for frontal lobe.

**Conclusions:** Psychosis is more often in MS women than in common population. In psychotic MS patients were demyelinating lesions larger in temporal lobe than in other areas of the brain. This might suggest that psychosis could be a part of MS disease process.

Disclosure: I Niedermayerová has nothing to disclose.

**P288**

**THE EFFECT OF INTERFERON BETA ON THE COGNITIVE DYSFUNCTION OF 100 IRANIAN PATIENTS WITH MULTIPLE SCLEROSIS**

**S. Pakdaman**, S. Pakdaman

*Neurology, Beheshti University, Tehran, Iran; Neurology, Beheshti University*

**Background:** Recent studies in both Europ and North America have shown the effect in cognitive are quit common even in early MS. Cognitive dysfunction is increasingly recognized an important and disabling aspect of MS for many patients.

**Objectives:** Clinic studies suggest a prevalence of cognitive dysfunction of between 54% and 65%. Cognitive dysfunction have a preivaasive influence on people’s lives adversely aff- cting mood employment, social function and rehabilitation. The literature on therapy and training interventions to improve cognitive dysfunction is scarce and no general model of effective practise exists.

**Methods:** We conducted a double blinds placebo-controlled randomized parallel group trial study on 200 patients (128 women and 72 men) were diagnosed as having MS on the Poser criteria after gave written informed consent were divided into two group 100 patients were taken one of three available interferon beta in Iran (Avonex, Rebif andBethesderon) and another 100 patients group were treated with placebo. All patients have a comprehensive neuropsychological battery before starting treatment and after two years.

**Results:** Interferon beta had a significant effect on the cognitive domain of in- formation processing and solving. More detailed analysis reveal a significantly longer time to sustained detoriation on Paced Auditory Task for interferon beta group.

**Conclusions:** these findings indicate that interferon beta had a benefical effect on both cog- nitive domain and specific test performance which are typically most affected by MS disease. Information processing and problem with memory are both sources of major disability and dandicap with MS. If the onset of significant deteriation in these cognitive are can be delayed this will enhance patient quality of life and enable to remain confident effective and productive for longer. There were no significant difference of the effect of each three interferon beta on cognitive dysfunctions.

Disclosure: H Pakdaman has nothing to disclose.

**P289**

**HOPELESSNESS IN MULTIPLE SCLEROSIS**

**Patten SP**, L. Metz

*Community Health Sciences, University of Calgary, Calgary, Alberta, Canada; Clinical Neurosciences, University of Calgary, Calgary, Alberta, Canada*

**Background:** Hopelessness ratings can predict suicide risk. In patients with multiple sclerosis (MS), suicide rates are elevated relative to the general popula- tion. In one study of interferon (IFN) beta-1b, suicide attempts were seen only in patients on active drug. Two recent, randomized, double-blind, placebo-controlled clinical trials of IFN beta-1a in MS have obtained Beck Hopelessness Scale (BHS) ratings. One of these, the PRISMS trail, evaluated IFN beta-1a 22  or 44mcg three times weekly for 2 years in relapsing-remitting (RRMS). Another, the SPECTRIMS trial, evaluated the same medication and doses in secondary progressive (SP) MS over 3 years.

**Objectives:** To describe changes in hopelessness during two random- ized, controlled trials of IFN beta-1a in MS, and to compare levels of hopelessness in patients with RRMS and SPMS.

**Methods:** Raw data from both clinical trials were obtained from the study sponsor (Serono). Median BHS ratings, and the proportions exceeding a 9/10 BHS cut-point were calculated over a 2-year (PRISMS) or 3-year (SPECTRIMS) follow-up period. Depressive symptoms were examined using the Center for Epidemiological Studies Depression Rating Scale and the General Health Questionnaire.

**Results:** Ratings of hopelessness were higher in the SPM (SPECTRIMS) than in the RRMS (PRISMS) group. Changes in hopelessness were unrelated to treatment group or IFN beta dose. Hopelessness ratings increased over time in the SPM group, but not in the RRMS group. Depressive symptom ratings did not increase over time in either study and were not related to treatment. These findings suggest that suicide risk may be greater in those with SPMS than RRMS, and that suicide risk may increase over time during the secondary progressive phase.

**Conclusions:** Hopelessness, as measured by the BHS, is a different concept than depression. Whereas levels of depression appear do not increase over time in SPMS, hopelessness ratings do. These findings indicate a need for suicide risk surveillance and psychological support that increases over time and is independent of IFN beta-1a treatment in SPMS.

Disclosure: Both SB Patten and LM Metz have received honoraria from Serono.

Funding: Supported by Serono.

**P290**

**COGNITIVE DYSFUNCTION IN MULTIPLE SCLEROSIS**

**Petkovska-Bojkova T**, Daskalovska V, Bojkovski V

*Clinic of Neurology, Skopije, Macedonia*

**Background:** Cognitive impairment affects 40 to70% of patients with MS and adversely affect the quality of life. The most frequent cognitive abnormalities in MS are subtle defects in abstraction, memory, attention and word finding.

**Objectives:** To determine cognitive dysfunction in MS patients.

**Methods:** 30 MS patients suffering from a relapsing-remitting (R-R) (12 patients), a secondary progressive (SP) (17 patients) or a primary progressive (PP) form of the disease (1 patient), were assessed using a neuropsychological battery of the main cognitive areas: general intelligence functioning, memory, attention and concentration, executive functioning, language, visual-spatial functioning, visual-motor functioning and personality.

**Results:** Memory (episodic and semantic), executive functioning, visual-spatial perception have been impaired more than visual-motor functioning and attention, whereas short term memory, language and global intellectual effi-
ciency were normal. 6 patients showed no deterioration of cognitive functioning. All of them were R-R MS patients, except one who presented PP form of MS and has no deterioration. Some cognitive disorders were present even at the early stage of the disease as worse attention and concentration, visual retention, spatial cognition.

Conclusions: According to our data cognitive impairment, with various intensity, can be presented at early stages of the disease or later.

Disclosure: T Petkovska-Boskova has nothing to disclose.

P291
A CROSS-SECTIONAL STUDY OF 21 NEWLY DIAGNOSED, RELAPSE-REMITTING MULTIPLE SCLEROSIS PATIENTS WITH PET, MRI AND TESTS OF COGNITIVE FUNCTIONS.

Tscherning T, Mathiesen HK, Jonsson A, Blinkenberg M, Rostrup E, Larsen HF, Paulson OB, Søebelg Sorensen P

Department of Neurology, MS Research Unit, Copenhagen, DK-2100 Copenhagen, Denmark; 
Danish Research Center For Magnetic Resonance, Copenhagen University Hospital Hvidovre, Hvidovre, Copenhagen, Denmark; 
Dept. of MRI, Trondheim Hospital, Trondheim, Norway

Background: Other investigators have shown a relationship between MRI and the clinical neurological evaluation as measured by EDSS. Previous studies at our department have also indicated a relationship between whole brain glucose metabolism and clinical function.

Objectives: This cross-sectional study of patients with multiple sclerosis (MS) was initiated in order to assess the correlation among cognitive functions, Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET).

Methods: Twenty-one newly-diagnosed, clinically definite, MS patients with the mean age of 35.6 [range 22-48] years old and with a mean EDSS score of 1.8 [range 0-4] were neuropsychologically tested and scanned by PET-FDG (whole brain fluorodeoxyglucose metabolic rate) and MRI (whole brain Fluid Attenuated Inversion Recovery) within 30 days. A group of 75 normal individuals, matched for age, sex and educational level, served as controls in regard to neuropsychological test results.

Results: From the cross-sectional data we show that the studied population of MS patients compares to a control group of 75 normal individuals when controlling for age and sex, but that out of 26 cognitive measures, 11 MS patients failed 0-3 tests (<1.5 SD below the normal controls), 6 patients failed 4-7 tests, 3 patients failed 8-11 tests, and 1 patient failed more than 12 tests. No correlation was found between PET-FDG (glucose metabolic rate) and MRI (measured by lesion volume or ratio of lesion volume to brain volume). The only neuropsychological test that correlated significantly with both MRI ratio of lesion volume to brain volume (rho=-0.42; p=0.059) and PET-FDG metabolic rate (rho=0.40; p=0.074) was verbal fluency (words with s in 1 minute).

Conclusions: This cross-sectional study of patients with MS showed a minor correlation between cognitive measures, MRI and PET, although almost half the MS patients had cognitive deficits compared to a matched normal control group.

Disclosure: T Tscherning has nothing to disclose.

Funding: Supported by Medical Faculty of Copenhagen University.

Experimental Allergic Encephalomyelitis (Part 2)

P292
DETECTION OF MAGNETICALLY LABELED ENCEPHALITOTIGENIC T-CELLS IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE) BY CELLULAR MAGNETIC RESONANCE IMAGING

Anderson SA, Shukaliak-Quandt J, Arab SA, Jordan EB, Martin R, McFarland HF, Frank JA

WINDS, NIH, Bethesda, Maryland, USA; LDRR/CC, NIH, Bethesda, Maryland, USA

Background: Intracranial labeling with iron oxides MR contrast agents provides the ability to monitor the trafficking of T-cells by MR microscopy (MRM). EAE in the SJL mouse is a relapsing remitting model of multiple sclerosis.

Objectives: The purpose of this study is to detect magnetically labeled encephalitogenic T-cells in an adoptive transfer EAE in the SJL mouse by MRM.

Methods: EAE was induced by proteolipid protein 139-151 specific T-cells (TC) that were intracranially labeled with superparamagnetic iron-oxide nanoparticles (SPIO). Lymph node cultures from PLP-immunized mice were labeled after 3-4 days of restimulation with antigen and then by incubation with contrast agent Ferideix (Berlex,USA) and PolyL-lysine. Labeled cells were infused into recipient mice. In vitro and in vivo studies were done in parallel to compare the phenotype, proliferative and encephalitogenic properties of magnetically labeled versus non-labeled cells. In vivo and ex vivo MRM was performed at 7 Tesla during disease exacerbation and remission with T2*w gradient-echo images. Prussian blue (PB) for the presence of iron in cells and routine histology was performed.

Results: Magnetic labeling of T-cells did not interfere with the development of EAE in the AT model. Animals with clinical score 2 at time of relapse demonstrated diffusive hypointensities of the thoracic and lumbar cord due to susceptibility effects of PLL-Feridex consistent with infiltration of labeled cells. In addition, white matter hyperintensities with focal dark can be appreciated in animals at time of remission. Histology showed extensive cellular infiltrate on H&E and PB positive mononuclear cells in nerve roots and white matter with perivascular cuffing.

Conclusions: These results provide the basis for tracking magnetically labeled encephalitogenic T-cells in EAE by MRM. This approach should further our understanding of T-cell mediated autoimmune CNS diseases and potentially visualize the host response to under various experimental conditions.

Disclosure: S Anderson has nothing to disclose.

P293
EXPRESSION OF THE ACTIVATION MARKER UROKINASE PLASMINOGEN ACTIVATOR IN CNS MICROVASCULAR PERICYTES IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS


Neurology, Wayne State University, Detroit, Michigan, USA

Background: The ability of inflammatory cells to migrate through CNS tissue involves proteolytic degradation of the extracellular matrix. At least one enzyme used for this purpose is Urokinase plasminogen activator (uPA). When bound to its receptor (uPAR) uPA is an active cell surface protease. We have previously shown that upregulation of uPAR expression is associated with macrophage, pericyte, microglial and endothelial cell (EC) activation as well as migration. Activated uPAR+ monocytes are increased in multiple sclerosis (MS).

Objectives: To study the expression of uPAR during EAE.

Methods: Lewis rats were immunized with myelin basic protein (MBP), and CNS microvessels (MV) were isolated at various times post immunization. CNS pericytes (PC) were subcultured from freshly isolated MV.

Results: uPAR were detected by 7 days post immunization (PI). Positive staining was associated with MV in cells with the morphological appearance of PC.
uPAR+ PC were detected in vivo using immunohistochemistry and uPAR specific mRNA was analyzed by RT-PCR and fluorescence in situ hybridization (FISH). uPAR protein expression was confirmed by immunohistochemistry and western analysis. The number of uPAR+ PC, as assessed by the PC: EC ratio, was decreased at 10-14 days PI. During the recovery phase, MHC Class II+uPAR+ PC were again observed in MV fragments and the PC to EC ratio was normal. Results indicate that CNS MV PC became activated during the induction phase of EAE. uPAR+ PC were detected before leukocyte infiltration.

Conclusions: The appearance of activated PC correlates with activation of the endothelium. Decreased PC: EC ratios during the clinical phase suggests that PC may have migrated from the vessel using uPAR and/or that activated PC upon interacting with T-effector cells are selectively killed. Recovery is associated with renewed pericyte coverage.

Disclosure: P Dore-Duffy has nothing to disclose.
Funding: This study is supported in part by NIH and NMSS.

P294
TREATMENT OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS WITH INTRAVENOUS IMMUNOGLOBULIN

Hume Jørgensen S*, Laursen H*, Soelberg Sørensen P*
*MS Research Unit, Copenhagen University Hospital, Copenhagen, Denmark; Laboratory of Neuropathology, Copenhagen University hospital, Copenhagen, Denmark

Background: Clinical trials have shown that intravenous administration of polyclonal immunoglobulin (IVIG) has the potential to reduce the disease activity in multiple sclerosis (MS). However, the mechanisms by which IVIG may interfere with the pathophysiology of MS are not yet fully understood. In the present study we evaluated IVIG treatment of experimental autoimmune encephalomyelitis (EAE), the primary animal model for human MS.

Objective: The objectives of the study were to assess the effects of IVIG on: 1) the incidence and severity of active EAE and 2) the EAE pathology in the central nervous system.

Methods: EAE was induced in rats by immunization with incomplete Freund's adjuvant and a spinal cord homogenate. Control rats were immunized with saline. At day 0 and 1 post immunization, animals were treated with infusions of polyclonal human IVIG (1 g/kg) or placebo (0.1% albumin in 10% maltose). The animals were weighed and observed daily, and the clinical disease severity was graded on a scale 0-6. During the acute attack at day 11, the rats were sacrificed and the brain and spinal cord dissected and cut for histological examination. The tissue sections were blinded and scored for inflammation and demyelination.

Results: Treatment with IVIG significantly suppressed the development of EAE as measured by incidence (placebo 92%, IVIG 50%), day of onset, weight loss and maximal average EAE score (placebo 2.6, IVIG 0.7). In the placebo group, the development of active disease was associated with severe inflammation and demyelination in the spinal cord, brain stem and cerebellum. When animals were treated with IVIG, the pathological changes in the central nervous system were significantly reduced as measured by the average histological score (placebo 8.8, IVIG 5.3).

Conclusions: In conclusion, IVIG treatment of EAE did not simply ameliorate the clinical symptoms of experimental autoimmune disease but had a protective effect against the pathological changes in the CNS.

Disclosure: S Humle Jørgensen has nothing to disclose.
Funding: Supported by The Novo Nordisk Foundation and The Johnsen Foundation.

P295
SUSCEPTIBILITY TO T CELL-MEDIATED CENTRAL NERVOUS SYSTEM INFLAMMATION MODULATED BY NON-MYELIN-SPECIFIC T CELLS

Jones RE*, Kay T*, Wilkins DP, Tsaknaridis L*, Bourdette DP*

Background: CNS inflammation and paralysis are induced by activation of myelin-specific T cells in EAE. Myelin-specific T cells are also associated with pathogenesis in MS, but earlier etiologic triggers which precede pathogenic changes remain uncertain. Factors governing susceptibility of the CNS to attack by myelin-specific T cells have been identified in EAE but mechanisms which modulate susceptibility prior to disease onset are not fully understood in EAE or MS.

Objective: Identify pre-pathogenic hematopoietic and immune cellular elements controlling susceptibility to CNS inflammation.

Methods: Transplantation of xenogeneic rat bone marrow (BM) cells alone or with non-myelin-specific rat T cell lines into severe combined immunodeficiency mice was used to generate experimental subjects which differed greatly in their susceptibility to CNS inflammation induced subsequently by myelin-specific rat T cells. Clinical severity and number of myelin-specific T cells required to induce disease were used to assess disease susceptibility. Specific labeled monoclonal antibody staining with flow cytometry was used ex-vivo to identify CNS cells associated with elevated disease susceptibility. DNA microarray analyses were used to assess gene expression patterns in human T cell populations which differed in their ability to migrate to the CNS.

Results: Non-myelin-specific T cells rapidly entered the CNS parenchyma and caused a greatly accelerated accumulation of BM-derived, antigen-presenting cells (APC) to functionally significant levels in the CNS. Elevated numbers of BM-derived CNS APC were associated with significantly elevated susceptibility to CNS inflammation. Certain human T cells also entered the CNS and caused an increase in BM-derived CNS APC. Gene expression patterns differed between human T cells which migrated to the CNS and those which did not.

Conclusions: Non-myelin-specific T cells modulate susceptibility to CNS inflammation by regulating indirectly, through effects on CNS APC development, the threshold number of myelin-specific T cells required to initiate pathogenesis. Human T cells expressing a particular pattern of genes may modulate disease susceptibility through a similar, indirect process.

Disclosure: R Jones has nothing to disclose.
Funding: Supported by NINDS and US Department of Veterans Affairs.

P296
AXONAL PROTECTION BY FLECAINIDE THERAPY IN EAE

Bechtold DA*, Kapoor R*, Smith K*
*Neuroinflammation Research Group, GKT School of Medicine, London, United Kingdom; Department of Neurology, National Hospital for Neurology and Neurosurgery, London, United Kingdom

Background: Axonal degeneration is a major cause of permanent neurological deficit in MS. The events that cause degeneration remain unclear, but it is likely that axons are vulnerable to the effects of inflammatory mediators such as nitric oxide (NO). Indeed, we have shown that axons can degenerate if they are exposed to exogenous NO while electrically active. We have also shown recently that the sodium-channel blocking agents lidocaine and flecainide can protect axons in spinal roots from such degeneration, as expected from the mechanisms which are likely to underlie the neurotoxic effects of NO.

Objective: This study examined the possibility that treatment with flecainide could also limit the axonal degeneration which occurs within the CNS in an experimental demyelinating disease (chronic relapsing experimental autoimmune encephalomyelitis, CR-EAE, and animal model of MS).

Methods: CR-EAE was induced in dark agouti (DA) rats by immunizing with spinal cord homogenate and complete Freund's adjuvant. CR-EAE rats were randomly assigned and received flecainide (15 mg/kg twice per day, s/c) or control solution from 7 days post-inoculation (dpi), a stage at
which the animals start to show signs of neurological dysfunction. The neurological deficit was assessed daily, and the extent of axonal loss was assessed 29 dpi using immunohistochemical and electrophysiological techniques.

**Results:** Morphometric examination of neurofilament-labeled axons in the fasciculus gracilis of the spinal cord of severely affected animals revealed that significantly more axons survived in the flacainide-treated animals (97 axons/1000μm² +/- 19 axons) than controls (67 +/- 24, p<0.01). The amplitude of the compound action potentials recorded from tail nerves in response to throracic spinal stimulation was also greater in flecainide treated rats than in controls.

**Conclusions:** These findings indicate that blockade of sodium channels may be associated with axonal protection in EAE, and suggest a novel way to limit axonal degeneration, and thereby possibly the progression of disability, in patients with MS.

Disclosure: R Kapoor has nothing to disclose.

Funding: Supported by the MS Society of Great Britain and Northern Ireland.

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**P297** This abstract was also presented at the platform.

A SYNTHETIC ANDROSTENE DERIVATIVE WITHOUT GENDER-RELATED SIDE EFFECTS INHIBITS EAE CANDIDATE FOR CLINICAL TRIALS IN MS?

Offner HP¹, Zamora AB², Matejuk AR³, Auci DC, Morgan EC, Reading CC

¹Neurology, Oregon Health and Science University, Portland, Oregon, USA; ²Neuroimmunology Research, Portland VA Medical Center, Portland, Oregon, USA; ³Neurology, Stanford University, Stanford, California, USA; ⁴Neurology, University of California, San Francisco, San Francisco, California, USA; ⁵Neurology, Stanford University, Stanford, California, USA; ⁶Microbiology and Immunology, University of North Carolina, Chapel Hill, North Carolina, USA; ⁷Mercer University School of Medicine, Macon, Georgia, USA; ⁸Pulmonary Medicine, University of California, San Francisco, San Francisco, California, USA

**Background:** Experimental allergic encephalomyelitis (EAE), a Th1 polarized demyelinating disease of the central nervous system (CNS), shares many pathological and clinical similarities with multiple sclerosis (MS), and thus represents a relevant animal model for this disease. A number of observations support the idea that fluctuations in sex hormone levels are related to changes in autoimmune disease status, and estrogen and testosterone can both inhibit clinical signs of EAE.

**Objectives:** The goal of this study was to evaluate suppressive effects on EAE of fluasterone (HE2500), a synthetic androstone derivative without gender-related side effects.

**Methods:** SJL mice were immunized with PLP-139-151 peptide/CFA to induce EAE. Starting on day -7, animals were given daily injections (s.c.) of HE2500 (3.0 mg) in vehicle, or vehicle alone for 33 days.

**Results:** HE2500 significantly delayed the onset, reduced peak clinical score and cumulative disease index of EAE, and prevented or significantly attenuated relapses. Moreover, T cells from treated mice had significantly reduced PLP-alpha specificity. HE2500 and vehicle-treated animals had similar numbers of TNF-alpha and IFN-gamma producing cells in the CNS. Additionally, HE2500 significantly delayed the onset of clinical disease in HE2500-treated animals.

**Conclusions:** These findings indicate that HE2500 inhibits EAE without gender-related side effects. HE2500 represents a relevant animal model for MS, and retains the potent anti-proliferative, anti-inflammatory and immune regulatory effects of fluasterone. HE2500 is practically devoid of toxic, estrogenic, or androgenic side effects.

Disclosure: D Auci, E Morgan, and C Reading are employees of Hollis-Eden Pharmaceuticals.

Funding: Supported by Hollis-Eden Pharmaceuticals, National Multiple Sclerosis Society (RG5108-A-1), NIH (AI42576 and NS23444).

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**P298**

THE ROLE OF THE MHC CLASS II TRANSACTIVATOR (CIITA) IN CLASS II EXPRESSION AND ANTIGEN PRESENTATION BY ASTROCYTES AND IN SUSCEPTIBILITY TO CNS AUTOIMMUNE DISEASE

Stuve OP, Youssef SS, King CL, Patamroyo JC, Brickey JW, Piskurich JP, Chapman HA, Zamvil SS

¹Neurology, University of California, San Francisco, San Francisco, California, USA; ²Neurology, Stanford University, Stanford, California, USA; ³Microbiology and Immunology, University of North Carolina, Chapel Hill, North Carolina, USA; ⁴Mercer University School of Medicine, Macon, Georgia, USA; ⁵Pulmonary Medicine, University of California, San Francisco, San Francisco, California, USA

**Background:** Experimental autoimmune encephalomyelitis (EAE) is a demyelinating disease of the central nervous system (CNS) that serves as a model for multiple sclerosis (MS). EAE is mediated by CD4+ T cells that recognize CNS self-antigens (Ag) in association with MHC class II molecules. Astrocytes are the most abundant CNS glial cell population. Their contribution to class II restricted Ag presentation in vivo is unknown. CIITA, directs constitutive and IFNg-inducible expression of MHC class II genes. The potential role of CIITA in Ag processing and presentation by astrocytes has not been directly addressed in previous studies.

**Objectives:** Using CIITA-deficient mice, we tested the hypothesis that CIITA-directed class II expression is necessary for T cell activation in CNS inflammation. We also tested the possibility that constitutive CIITA-directed class II expression could promote CNS inflammatory disease.

**Methods:** Newly created GFAP-CIITA Tg mice and CIITA-deficient mice were used to examined the role of CIITA in CNS class II expression and Ag presentation by astrocytes and in EAE susceptibility.

**Results:** CIITA-deficient mice were resistant to EAE by immunization with CNS autoantigen. Adoptive transfer of wild-type CNS autoantigen-specific CD4+ T cells into CIITA-deficient mice did not induce EAE, indicating that CIITA-dependent class II expression was required for CNS Ag presentation. GFAP-CIITA-Tg mice did not develop spontaneous EAE or more severe disease than control mice when immunized with CNS peptide. Whereas IFNg-activated astrocytes could present either peptide or native Ag, CIITA-transfected astrocytes could present peptide Ag only.

**Conclusions:** Our results demonstrate that although CIITA-directed class II expression is required for CNS Ag presentation and EAE induction, CIITA-directed class II expression alone in astrocytes may not be sufficient for Ag processing and does not support induction of CNS autoimmune disease.

Disclosure: O Stuve has nothing to disclose.

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**P299**

TREOSULFAN IN MYELIN-OLIGODENDROCYTE-Glycoprotein-INDUCED EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS: POTENTIAL NEW TREATMENT FOR MULTIPLE SCLEROSIS


¹Department of Neurology, University of Tuebingen, Tuebingen, Baden-Württemberg, Germany; ²Medac, Wedel, Schleswig-Holstein, Germany

**Background:** Treosulfan (Dihydroxybutanesulfuric acid, DHBSH, L-threitol-1,4-bis [methylene sulfonate]) is a cytostatic alkylating agent with a favourable profile of side effects. Myelin-oligodendrocyte-glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE) induced in DA (RT1av1) rats resembles multiple sclerosis in many aspects since central nervous system pathology shows inflammation, demyelination and axonal loss. Moreover DA rats develop a relapsing–remitting disease course.

**Objectives:** We explored the potential beneficial effect of treosulfan in the treatment of MOG-induced EAE in DA rats.

**Methods:** EAE induction with MOG; i.p. treatment with treosulfan; disease evaluation; quantitative real time PCR for cytokines; elispot for interferon-gamma secreting antigenspecific T cells; determination of anti-MOG...
immunoglobulin serum titers; quantitative analysis of white and red blood cells, reticuloocytes and thrombocytes.

**Results:** A single i.p. administered dose of treosulfan (1000 mg/kg body weight) at the day of immunization significantly reduced disease severity compared with PBS treated controls. Three repeated injections of treosulfan on consecutive days after disease induction (each 333 mg/kg body weight) did not further increase the therapeutic benefit. After disease had evolved, a single dose of treosulfan (1000 mg/kg body weight) given i.p. on day 14 p.i. improved long term disease outcome. Treatment with treosulfan resulted in reduced mRNA expression of IL-12 and IFN-gamma in draining lymph nodes and reduced numbers of IFN-gamma secreting MOG specific T cells. Treatment did not affect MOG specific antibody titers. Myelosuppression was not observed.

**Conclusions:** Taken together treosulfan is well tolerated and has beneficial effects on the disease severity and disease course in DA rats. Based on these experimental data treosulfan should be explored in multiple sclerosis patients who have failed conventional treatment.

**Disclosure:** Joachim Baumgart and Gretel Sass are employed by Medac Pharma. The experiments were performed in the laboratory of Robert Weissert. Robert Weissert or members of his group and staff of the Department of Molecular Biology and Epigenetics of the University of Tuebingen have no financial interest. Funding: Medac Pharma to Robert Weissert Deutsche Forschungsgemeinschaft to Robert Weissert.

**Healthcare Systems**

**P300**

**COST-BENEFIT AND COST-EFFECTIVENESS ANALYSIS OF INTERFERON BETA-1A 44MCG TIW, VERSUS INTERFERON BETA-1A 30MCG QW, IN RELAPSING-REMITTING MULTIPLE SCLEROSIS (RRMS)**

Beresniak A¹, Martin M²

¹Corporate Pharmacoconomics, Serono International SA, GENEVA, Geneva, Switzerland; ²Corporate Pharmacoconomics, Serono International SA, Geneva, Geneva, Switzerland

**Background:** A cost-benefit and cost-effectiveness analysis was performed, based on the comparative, randomized, controlled clinical trial (EVIDENCE) comparing: IFN beta-1a 44 mcg sc tiw (n = 339), and IFN beta-1a 30 mcg IM qw (n = 338) in the treatment of RRMS. Over 48 weeks, 62% of 44 mcg tiw patients remained relapse-free compared to 52% of 30mcg qw patients (odds ratio =1.5, p = 0.009). 44 mcg tiw patients had a 30% reduction in the rate of first relapse relative to the rate for 30 mcg qw patients (HR=0.70, p = 0.003). Placebo relapse rate for comparisons came from the PRISMS trial comparing IFN beta-1a 44 mcg tiw and placebo at 1 year (37% relative reduction).

**Objectives:** To assess the cost-benefit and cost-effectiveness ratios of two interferon beta-1a formulations in RRMS.

**Methods:** Cost calculations integrated the cost of drugs, cost of care (physician visits, symptom-related medication, MRI, Lab tests) and specific costs during one relapse. Mean costs for one relapse was assessed by a specific cost study on managed care organization databases in the USA.

**Results:** The cost-benefit ratio was 21.57 using IFN 44 mcg tiw and 30.50 using IFN 30mcg qw (total cost per patient / costs avoided per patient). If only patients with relapses are considered, the cost avoided per relapse avoided was $3,600 using 44 mcg tiw rather than 30 mcg qw of 48 weeks (difference of total relapse costs / difference in relapse number). If the whole study population is considered a cost per relapse avoided is $28,100 taking into account all costs (drug, cost of care and relapse related costs). This value is comparable to previously reported differences between IFN beta-1b, IFN beta-1a 30 mcg qw or 44 mcg tiw and placebo (Brown MG et al, 2000, Nicholson T et al, 1999). The cost per time to first relapse was $1,100 per month with 44 mcg tiw and $1,400 per month with 30 mcg qw (IFN treatment cost / time to first relapse for 39th percentile, 48 weeks). This means that 44 mcg tiw treatment is more cost-effective in delaying time to first relapse than 30 mcg qw treatment.

**Conclusions:** This pharmacoeconomic analysis indicates that IFN beta-1a 44 mcg tiw has a better cost-benefit ratio and is more cost-effective than IFN beta-1a 30 mcg qw in RRMS.

**Disclosure:** Both authors are employees of Serono International S.A.

**P301**

**A RELAPSE OF MULTIPLE SCLEROSIS: HOW MUCH DOES IT COST IN CATALONIA?**

Casado V¹, Martinez-Yelamos S¹, Martinez-Yelamos A², Carmona O³, Hernandez JP³, Arbizu T³

¹Multiple Sclerosis Unit, C.S.U. Bellvitge, Hospitalit Llobregat, Barcelona, Spain; ²Neurology, Hospital de Viladecans, Viladecans, Barcelona, Spain

**Background:** Treatments for Multiple Sclerosis (MS) available today are effective but expensive. We consider of high relevance to estimate their efficacy.

**Objectives:** To calculate direct and indirect costs of a MS exacerbation in our cohort of patients (Baix Llobregat, Catalonia, Spain) and how they relate to immunomodulatory treatment.

**Methods:** We collected data from patient questionnaires, hospital charts, Catalonian Public Healthcare System tariffs and Catalanian Statistics Institute. The Human Capital Approach is used to estimate indirect costs, including morbidity and mortality costs attributable to MS.

**Results:** We analysed 148 patients with MS (Poser) from EDMUS database, clinically controlled in our MS Unit, with similar characteristics to general MS population. 69% were on interferon beta during the previous year. A relapse of MS needs, average, 1 visit to Emergency Ward, 1 to General Physician, 2 to Neurology outpatients and the administration of standard relapse treatment; we obtain a value of 1209.2-1079.8 euros for direct costs (hospitalization and ambulatory, respectively). Each relapse causes an average of 23.5 days missed from work, traducing a value of 1530.6 euros for indirect costs for patient and relapse. We estimate an average total cost of 2675.1 euros for patient and relapse. Patients in our MS Unit, treated with interferon beta and followed-up during 4 years, reduced their relapse-rate from 1.5 (before treatment) to 0.5. This would represent, in our cohort, saving 2675,1 euros per patient and year.

**Conclusions:** Total cost of a MS relapse -2675,1 euros- in our population is lower than previously reported in literature. Impact of MS is mostly the consequence of disability progression; cost-effectiveness of new treatments will depend on their ability to avoid not only relapses but also disease progression, which generates direct, indirect and intangible costs.

**Disclosure:** V Casado has nothing to disclose.

**P302**

**AN AUDIT OF HEALTH SCREENING ISSUES IN MS PATIENTS IN GENERAL PRACTICE. HAWKINS S(1), MAULCRUG K(1), WHITTINGTON D(2), EVASON E(2) & REILLY P(1). QUEEN’S UNIVERSITY, BELFAST(1) & UNIVERSITY OF ULSTER, JORDANSTOWN(2), BELFAST, NORTHERN IRELAND.**

Hawkins SA¹, Maclurg K¹, Whittington D², Evason E², Reilly P¹

¹Queens University, Belfast, Northern Ireland, United Kingdom; ²University of Ulster, Jordanstown, Belfast, UK, United Kingdom

**Background:** There is a perception that disabled patients, particularly those with MS are inhibited from, or unable to participate in health screening programmes. Cheng et al. BMJ 2001 showed impairments in mobility related to a reduced use of preventive services. We wished to investigate how patients in a different system of healthcare complied with our NHS screening standards.

**Objectives:** To audit how a representative sample of MS patients in the community, registered with general medical practitioners, comply with a range of health screening standards.

**Methods:** There is a network of general practices in Northern Ireland with computerised diagnostic registers - the N I General Practices Framework. They are situated in inner city, suburban & rural settings, having 193,300 patients - about 13% of the total population of N Ireland. All practices were asked to identify all
P303

THE ECONOMIC BURDEN OF RELAPSE IN MULTIPLE SCLEROSIS: DIRECT MEDICAL COSTS PER EPISODE IN THE UNITED STATES

O’Brien J, Patrick A, Duran P, Caro J
Caro Research Institute, Concord, Massachusetts, USA

Background: In today's health care environment it is necessary to consider economic, as well as clinical consequences when making management decisions related to multiple sclerosis.

Objectives: To determine the cost of managing a relapse of multiple sclerosis.

Methods: A combination of direct data analysis and cost modeling was employed to derive typical resource use profiles and direct medical costs in 2002 US dollars, from the perspective of a third-party payer responsible for comprehensive health-care. Place of care and scope of resource use defined the relapse management level. A high relapse management level consists of acute hospitalization and post-acute care (i.e., sub-acute inpatient care, rehabilitation, home health services, outpatient follow-up, related readmissions). Outpatient care including symptom-related medications. consultations; physical, occupational and speech therapy; tests; emergency room and observation unit stays comprise a moderate relapse management level. A mild relapse management level is defined by physician visits and symptom-related medications only. Data were obtained from many sources including all payer inpatient, ambulatory and emergency room databases from several states, physician and laboratory fee schedules, government reports and surveys, and literature. All charges were adjusted using cost-to-charge ratios.

Results: The average cost per person for high management level was $12,719, based on an analysis of 4,634 hospital cases (mean age 48 years, 73% female). Hospital care comprised 71% of that cost. At discharge, 36% required inpatient sub-acute care, rehabilitation or home care. The typical cost per moderate episode was $1,847 and mild episodes were $243.

Conclusions: The effectiveness of treatment. Decision makers concerned with evaluating new technologies may be especially concerned with avoiding relapses will have a major impact on the cost-effectiveness of treatment. Decision makers concerned with evaluating new therapies for this disease may consider this.

Disclosure: J Jaime is a consultant for Serono International SA. A Beresniak is a speaker for Serono International SA.
Funding: Supported by Serono International SA.

P304

BURDEN OF NURSING CARE FOR HOSPITALIZED PATIENTS WITH MULTIPLE SCLEROSIS

TISSOT E, RUMBACH L, LIMAT S, BERGER E, MONNIN C, LAVIER A, WORONOFF-LEMSI M

Background: Patients with MS are frequently hospitalized and require nursing care during their hospital stay. Little is known about this care load.

Objectives: To assess the burden of nursing care for hospitalized patients with MS and to analyze the potential relationship between this burden and sex, age, disease severity, MS form and duration since MS diagnosis.

Methods: During 18 months, all consecutive patients hospitalized in our Department of Neurology (Besançon University Hospital, France) were included. The daily burden of nursing care of each hospitalization was prospectively evaluated with a direct explicit Canadian method: the Nursing Research Project software. Patients were classified into 3 groups, according to the Expanded Disability Severity Scale (EDSS): I (EDSS 0 to 3.5), II (4.0 to 6.0) and III (6.5 to 9.0). The daily burden of nursing care in relationship to sex, age, disease severity, MS form and duration since MS diagnosis was compared by an analysis of variance.

Results: 129 patients were included: sex ratio M/F = 39/90, mean age (y) = 44.9±12.1; mean duration (y) since MS diagnosis = 12.5±9.7; and average EDSS = 5.3±1.9. 33 (64%) patients had a relapsing-remitting form, 36 (26%) secondary progressive form, 6 (5%) primary progressive form and 4 (3%) suspected MS. 389 hospitalizations were analyzed: mean number of hospitalizations for each patient was 3.0±2.6 and the average length of each hospitalization was 3.4±3.8 days. The mean total daily burden of nursing care was 200±126 minutes per patient: nursing care secondary to physician’s prescription = 60±33 min, secondary to nurses’ own activity = 70±75 min and secondary to the communication relationship with the patient = 70±20 min. The total daily burden of care increased significantly with disease severity [group I = 147±134 per patient; group II = 207±120 min; group III = 220±121 min (p=0.0002)] and was significantly different according to the MS form [relapsing-remitting = 186±124 min; secondary progressive = 219±128 min; primary progressive = 222±110 min (p=0.03)], but not according to sex, age and duration since MS diagnosis.

Conclusions: This study suggests that the nursing care of hospitalized patients with MS require many human resources. It should help to increase the quality of this care.

Disclosure: L RUMBACH has nothing to disclose.
Funding: ARSEP

P305

THE NEW ZEALAND BETA-INTERFERON PROGRAM. AN APPROACH TO EQUITABLE PROVISION OF TREATMENT FOR MS WHERE FUNDING IS RESTRICTED

Willoughby EW, Abernethy DA, Wright AR, Anderson NE

Background: Since 2000, in New Zealand (NZ), funding for beta-interferon (BIF), either Avonex or Betaferon, has been provided for a limited number of patients with MS - approximately 10 - 15% of the total. Applications for BIF are reviewed by a national panel of 3 neurologists who also review progress annually. Strict criteria have been set: Starting: relapsing MS (secondary progressive may be present) with 2 significant relapses in the previous year and EDSS score of 3.0 to 6.5. Stopping: stable or increased relapse rate or sustained increase in EDSS by 1 point or worsening to EDSS 7.0. The starting criteria are considerably more restrictive than those used in the major treatment trials, especially with respect to the frequency and duration of relapses (must persist more than 1 week) and the EDSS range. The rationale, with funding limited,
New Clinical Trials (Part 2)

P306

THE INDEPENDENT COMPARISON OF INTERFERON (INCOMIN) TRIAL: MRI ANALYSIS OF THE NEW PROTON DENSITY/T2 LESIONS.

DURELLI L*, PIPIERI A*, VERDUN E*, BARBERO P*, INCOMIN G*
*Neurosciences, TURIN University, TURIN, Italy; 1INCOMIN, GROUP, Italy.

Background: Controlled trials have demonstrated efficacy of once-a-week IFN beta-1a or of on-alternate-day IFN beta-1b compared to placebo. Recently, we evaluated the clinical comparison of the two drugs.

Objectives: To compare and analyse at the MRI the frequency occurrence of new PD/T2 lesions in the RRMS treated with two IFN beta preparations.

Methods: Prospective 2-years follow-up of 188 consecutive relapsing-remitting patients randomized by 15 MS centers to receive IFN beta-1a (30 mcg intramuscularly once a week) or IFN beta-1b (8 MIU subcutaneously on alternate days), 79% of these patients had baseline and every year MRI. The scans were evaluated in blind at the end of 2-year of follow-up.

Results: At the end of the 1-year of the study follow-up the mean number of new PD/T2 lesions was 0.79±1.9 in the IFN beta-1b group and 1.97±2.74 in the IFN beta-1a (p=0.003), in the second year of follow-up the mean number of new PD/T2 lesions with concomitant enhancement 0.35±0.9, lesions that were new PD/T2 lesions without concomitant enhancement 1.32±2.2, enhancing lesions 0.94±2.2 and enhancing lesions that were not new lesions on PD/T2 scans 0.6±1.7. For IFN beta-1a group the mean of new PD/T2 lesions with concomitant enhancement 0.91±1.6, lesions that were new PD/T2 lesions without concomitant enhancement 1.3±3, enhancing lesions 1.8±2.9 and enhancing lesions that were not new lesions on PD/T2 scans 0.8±1.4. Secondary outcome measures were all significantly (p<0.05) better for IFN beta-1b group except for the mean of enhancing lesions that were not new lesions on PD/T2 scans (p=0.05).

Conclusions: The results of MRI analysis showed that IFN beta-1b reduced signs of disease activity more effectively than IFN beta-1a and confirmed the clinical results.

Disclosure: L DURELLI has nothing to disclose.

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BETAFERON® / BETASERON® (INTERFERON Beta-1B) IN EARLY TREATMENT OF MULTIPLE SCLEROSIS: THE BENEFIT STUDY

*Division of Neurology, Ottawa Hospital, Ottawa, Ontario, Canada; None, Rennes, France; None, Dusseldorf, None, Germany; None, Geneva, None, Switzerland; None, London, None, United Kingdom; None, Barcelona, None, Spain; None, Amsterdam, None, Netherlands; None, Berlin, None, Germany.

Background: Evidence from clinical studies indicates that treatment of patients with a first demyelinating event suggestive of MS and an abnormal MRI with once weekly interferon beta produces effects on clinical and MRI parameters. However, the long-term impact of interferon beta treatment at the first episode and the benefits of a high dose/high frequency treatment have yet to be evaluated. A high dose/high frequency treatment was recently shown to be more efficacious than a once weekly treatment in established RR MS (INCOMIN, EVIDENCE).

Objectives: BENEFIT (Betaferon®/ Betaseron® in Newly Emerging MS for Initial Treatment) is a randomised, double blind, placebo-controlled, parallel group, multicenter, phase III study in patients with a first demyelinating event suggestive of MS and an abnormal MRI.

Methods: The trial is designed to: (i) investigate the efficacy of high dose/high frequency interferon beta-1b relative to placebo, assessed by onset of a second clinical event, development of new MRI lesions, changes in EDSS and MSFC, and quality of life; (ii) explore long-term effects beyond those on the second event and MRI lesion development; (iii) reveal the relationship of the two primary endpoints (time to diagnosis of MS according to the Poser criteria, and time to clinically definite MS (CDMS) according to the McDonald criteria) in the presence or absence of immunomodulatory treatment; and, (iv) compare treatments initiated before and after conversion to CDMS and evaluate the prognostic relevance of new lesions on follow-up MRI scans.

Results: Approximately 400 patients from Europe and Canada will be enrolled, 150 to receive placebo and 250 to receive 250 µg (8 MIU) interferon beta-1b every other day (eod). The maximum duration of the double blind phase will be 2 years, or until both primary endpoints are reached (i.e. after CDMS). Patients with CDMS, and all patients completing the 2-year study, will enter a long-term follow-up study, and be offered open-label interferon beta-1b eod for at least 3 years.

Conclusions: It is the goal to maintain all patients in the extension study regardless of therapy.

Disclosure: M Freedman has nothing to disclose.

P308

PLACEBO-CONTROLLED DOUBLE-BLINDED DOSE RANGING STUDY OF FAMPRIoine-SR IN MULTIPLE SCLEROSIS

*Neurology, University of Rochester, Rochester, New York, USA; 1Acorda Therapeutics, Inc., Hawthorne, New York, USA; 1Mellen Center, Cleveland Clinic, Cleveland, Ohio, USA; 1Neurology, Washington University, St. Louis, Missouri, USA; 1Neurology, Yale University, New Haven, Connecticut, USA.

Background: Previous studies of the potassium channel blocker, fampridine (4-aminopyridine), in MS have reported finding improvement in various aspects of neurological function. However, the safety and efficacy of higher doses are not known.

Objectives: The primary aim of this trial was to determine the safety and tolerability of escalating doses of a sustained release (SR) formulation given orally to patients with MS. The secondary aim was to explore efficacy over a broad dose range using measures of fatigue and motor function.

Methods: Inclusion criteria were clinically definite MS with EDSS < 6.5 and Fatigue Severity scores > 4. Thirty-six patients at 4 centers were randomized to
Results: The most common adverse effects reported at least once at any dose level in the fampridine-treated group were dizziness (36%), insomnia (36%), and paresthesia (32%). Five subjects withdrew because of seizure (2), tremor (1), dizziness and nausea (1), and leg pain (1). Adverse effects tended to be more severe at doses of 50 mg/day and higher including the 2 occurrences of seizure (at doses of 60 and 70 mg per day). The fampridine–SR group showed statistically significant improvement from baseline compared to placebo in functional measures of mobility (timed 25 walking speed; p=0.04) and lower extremity strength (manual muscle testing; p=0.01). Dose response curves showed increasing benefit in both measures in the 20 to 50 mg/day range. No other measures showed significant treatment effects.

Conclusions: The safety profile of fampridine SR was consistent with previous experience. Doses above 50 mg added little benefit and included adverse effects. There was significant improvement in measures of mobility and muscle strength.

Disclosure: A Goodman, J Cohen, M Rizzo, and T Tolliner are consultants for and have received grant support from Acorda Therapeutics. A Cross has received grant support from Acorda Therapeutics. A Blight and M Katz are full time employees of Acorda Therapeutics.

Funding: Supported by Acorda Therapeutics.

P309

DISABILITY AND QUALITY OF LIFE (FLAIR STUDY) AND NEUROPSYCHOLOGY (COBRA STUDY) IN RELAPSING MS PATIENTS

Jongen P*, Carton H*, Sindic C*, Tinbergen P*, Wesnes K*, FLAIR S*

*Multiple Sclerosis Centre Nijmegen, Gelderland, Netherlands; *Multiple Sclerosis Centre Melsbroek, Melsbroek, Flanders, Belgium; University of Louvain, Louvain, Flanders, Belgium; Biogen International, Hoofddorp, North-Holland, Netherlands; Cognitive Drug Research, Reading, England, United Kingdom

Background: It is not known 1) how disability relates to quality of life (QoL), and 2) how neuropsychological functions change in relapsing MS patients treated with interferon-beta (INFb) in general neurological practice.

Objectives: To study 1) the relationship between disability and QoL (FLAIR study) and 2) neuropsychological functions (COBRA study) in relapsing MS patients before and during treatment with INFb-1a (30 micrograms/week i.m.).

Methods: Patients with relapsing MS fulfilling indication criteria for INFb treatment and decided to start INFb-1a 30 micrograms/week i.m. were eligible. Evaluations at baseline and months 6, 12, 18, and 24 included EDSS and MSFC (disability), and MSQoL and AMSQoL (QoL) (FLAIR), and attention (reaction time, digit vigilance), short term memory (numeric and spatial working memory), long term memory (word recall, word, picture, and face recognition), and complex semantic processing (logical reasoning) (COBRA).

Results: Recruitment ended December 2001. 284 patients were included in the FLAIR study in 37 centres, located in the Netherlands (17), Belgium (17), UK (1), Ireland (1), and Luxembourg (1). 27 centres in general hospitals, 9 centres were academic or specialised MS centres. Forty-four FLAIR patients were enrolled in the COBRA study. Both studies are ongoing. Analysis of baseline FLAIR data include: 1) relation between EDSS and MSFC, 2) interrelationships between MSFC, QoL, and clinical and demographic patient characteristics, 3) comparison between general hospitals and academic/MS centres, 4) comparison with patient characteristics of the pivotal fase 3 INFb trials. Analysis of baseline COBRA data include interrelationships between neuropsychological measures, disability, QoL, and patient characteristics.

Conclusions: A large cohort of 284 relapsing MS patients, treated with INFb-1a 30 micrograms/week i.m., is under study for disability and QoL, with a subgroup being studied for neuropsychological changes.

Disclosure: The Multiple Sclerosis Centre Nijmegen received fees for the coordinating activities of PJH Jongen in the FLAIR and COBRA studies, from Biogen International, the Netherlands. J Tinbergen is medical director of Biogen International, the Netherlands. Funding: Biogen International, the Netherlands.

P310

TREATMENT OF EAE AND MS BY RAISING SERUM URATE LEVELS THROUGH ADMINISTRATION OF INOSINE.

Spitin S, Hooper D, Scott GS, Kropowski H

Microbiology and Immunology, Thomas Jefferson University, Philadelphia, Pennsylvania, USA

Background: The administration of uric acid (UA), a peroxynitrite (ONOO-) scavenger, has been shown to have therapeutic effect in experimental allergic encephalomyelitis (EAE). Several independent researchers have also shown that serum urate levels are inversely correlated with the incidence of MS in humans. Attempts to raise serum urate in humans by oral administration of UA to MS patients have not been successful. This prompted us to look for alternative approaches.

Objectives: To develop a methodology to raise serum urate levels by administration of UA precursors such as inosine and inosinic acid and evaluate therapeutic potentials of such approaches in MS and EAE.

Methods: ONOO- scavenging properties of UA and its precursors were evaluated by the ONOO- dependent oxidation of dihydrorhodamine 123 and by Western analysis of tyrosine nitration of bovine serum albumin. Inosine was administered to MS patients orally. Inosine and inosinic acid were administered to mice orally and i.p. Serum urate concentrations were measured by HPLC or by colorimetric assay.

Results: Both inosine and inosinic acid were efficiently taken up when administered orally to humans or mice and rapidly metabolized appearing in the serum as urate. Neither of the UA precursors inhibited ONOO- mediated activities in a series of in vitro tests. UA precursors were shown to have beneficial effect in EAE as well as in MS in a limited phase I human clinical trial.

Conclusions: Serum urate levels in MS patients and mice with EAE can be readily and safely elevated by administration of the UA precursors inosine and inosinic acid, both of which are approved for human consumption as food supplements. Since neither inosine or inosinic acid showed effects on chemical reactions associated with ONOO- or the activity of inflammatory cells implicated in the pathogenesis of EAE we conclude that the therapeutic effects of these precursors are manifested through their metabolism to UA.

Disclosure: S Spitin has nothing to disclose.

Funding: Supported by in part by a grant from the National Multiple Sclerosis Society and by a grant from the Commonwealth of Pennsylvania to the Biotechnology Foundation Laboratories.

P311

INTERFERON-B TREATMENT IN RELAPSING-REMITTING MULTIPLE SCLEROSIS: RESULTS OF AN OBSERVATIONAL STUDY IN SOUTHERN ITALY


*Neur. Psychiatr. Sci., University of Bari, Bari, Italy; **Neurology Section, University of Foggia, Foggia, Italy

Background: Pivotal phase III trials of three interferon beta (IFNb) products showed that each agent produces similar reductions of relapse-related measures in relapsing-remitting multiple sclerosis (RRMS). Recent head-to-head clinical trials seem to not confirm these results.

Objectives: To evaluate whether the results of an observational study on a large cohort of mildly disabled (EDSS 0-3.5) RRMS patients confirm the magnitude of effects observed in randomized, controlled clinical trials of IFNb products.

Methods: A total of 1,033 patients from 15 MS centers in Southern Italy participated in the study. Relapse rate, EDSS, adverse events, presence of neutral-
Materials and methods: DNA from more than 300 patients of the Austrian Genetics in MS Study (AgoMS) was genotyped for the TNFRII polymorphism using a restriction fragment length polymorphism (RFLP) approach. Only one of the previously described RFLP alleles was found (based on our in silico analysis). The proportion of relapse-free patients resulted not different (p=0.10) among the three treatment groups (53.7% for Betaferon, 54.4% for Avonex and 49% for Rebif 22mcg) after the first year, and between the two treatment groups (37.5% for Betaferon and 33% for Avonex) after two years. All IFNβ products reduced (p<0.001) relapse rate at 12 months and 24 months compared to baseline. There were no differences among the three IFNβ (p=0.17) groups in mean changes in relapse rate and EDSS scores at 12 or 24 months. In general, IFNβ-1b patients had a higher incidence of adverse events during the first 3 months of treatment compared with IFNβ-1a patients (p=0.05), and more patients discontinued treatment with IFNβ-1b (10%) compared to Avonex (5%) and Betaferon (1%) after 24 months. The frequency of NAB+ patients (with a titre higher than 20 IU/ml in at least two consecutive samples, 3 months apart) resulted of 14.8% in Betaferon, 2.2% in Avonex and 6.7% in Rebif groups (p=0.00005).

Conclusions: The results of the present observational study confirm those of the controlled phase III trials of IFNβ in RRMS, and they also are in agreement with those of other presented, independent European open-label studies on large populations. Discussion: M Trojano has nothing to disclose.

P312

A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY OF INTRAVENOUS IMMUNE GLOBULINS (IVIG) IN COMBINATION WITH INTRAVENOUS MethylPREDNISOLONE (MP) IN THE TREATMENT OF RELAPSES IN PATIENTS WITH MULTIPLE SCLEROSIS (MS)

Visser LH*, Beekman R+, Tijsse CC+, Uitdehaag BM*, Movig C+, Lenderink B*

*a neurology, St. Elisabeth hospital, Tilburg, Noord-Brabant, Netherlands; *pharmacology, St Elisabeth Hospital, Tilburg, Noord-Brabant, Netherlands; *neurology, VU University, Amsterdam, Zuid-Holland, Netherlands

Background: Treatment with MP increases the rate of recovery of acute relapses in patients with MS, however some patients do not show a clear improvement after treatment.

Objective: Our trial was designed to compare the efficacy of the combination of IVIG and MP with the standard treatment of MP alone in promoting recovery from acute moderate to severe relapses in MS.

Methods: MS patients with a relapse with at least one increase in EDSS in comparison to the pre-attack EDSS were randomized to IVIG-MP or MP treatment. Neurological disability was assessed at entry, week 1, 2, 4, 8, 12 and 6 months by EDSS, Scarrps neurological rating scale (SNR), 9-HPT and ambulation index. The primary outcome was the difference in EDSS grades at 4 weeks between the two treatment groups. The predetermined interim analysis was performed after inclusion of 19 consecutive patients to accurately calculate the number of patients needed to treat to show a difference between the treatment groups.

Results: The baseline characteristics were similar in both groups. The primary outcome showed that both groups had improved 1 point on the EDSS 4 weeks after start of treatment (p=0.81). By this outcome one of the stopping rules at interim analysis was reached. Also the median time to improve 1 or more EDSS points, the difference in SNR, 9-HPT and ambulation index did not differ between the treatment groups at any endpoint. Logistic regression revealed that the patients with a more severe relapse were more likely to improve at least 1 point on the EDSS in comparison to those with a milder relapse (p=0.0028). During follow-up of 6 months five patients in the IVIG-MP group had a new relapse in comparison to two patients in the placebo group.

Conclusions: Although based on a small number of patients our study, stopped after interim analysis, shows that IVIG-MP and MP are equally effective in the treatment of acute moderate to severe relapses in MS.

Disclosure: L Visser has nothing to disclose.

P313

EFFECTS OF INTERFERON BETA-1B DOSE TITRATION ON EFFICACY AND TOLERABILITY

Wroe S

Neurology, Ipswich Hospital, Ipswich, United Kingdom

Background: Interferon beta-1b (Betaferon® / Betaseron®) is an effective treatment for relapsing remitting (RR) and secondary progressive multiple sclerosis (MS). Initial dose titration is an effective approach for limiting the occurrence of adverse events associated with interferon beta-1b therapy.

Objective: To establish whether a slower titration regimen (62.5, 125, and 187.5 µg subcutaneously [sc] every other day [eod] sequentially over 4 weeks, then 250 µg sc eod) offers improvements in tolerability over the standard titration regimen (125 µg interferon beta sc eod for 2 weeks, then 250 µg sc eod), we conducted a 3-month, double-blind, placebo controlled, multicenter study in patients with relapsing remitting MS.

Methods: Of the 98 patients recruited to the study, 65 received interferon beta-1b (31 standard titration, 34 slow titration) and 33 received placebo. Patients in the placebo group underwent the same titration regimens.

Results: The frequency of adverse events was generally similar between the two titration regimens. Significantly fewer patients receiving interferon beta-1b experienced relapses relative to those receiving placebo (9.2% vs 27.3%, p=0.0204). Patients receiving interferon beta-1b by the standard titration method had significantly fewer relapses compared with placebo (6.5% vs 33.3%, p=0.0291). However, the reduction in the number of patients experiencing relapses in the slow dose titration group was not significant (11.8% vs 22.2%, p=0.4248).

Conclusions: Rapid and significant improvements in relapse rates compared to placebo are achieved using the standard dose titration method. In contrast, the slow dose titration regimen does not reduce the frequency of adverse events and may reduce efficacy. Patients should therefore receive high frequency interferon beta-1b at the full dose as soon as possible to gain maximum benefit.

Disclosure: S Wroe has nothing to disclose.

Genetics (Part 2)

P314

This abstract was also presented at the platform.

TUMOR NECROSIS FACTOR RECEPTOR II POLYMORPHISM IN PATIENTS WITH MULTIPLE SCLEROSIS

Ehing R*, Gassner C*, Fazekas F*, Kollegger H*, Kristofferitsch W*, Reindl M*; Berger T*

*a Neurology, University of Innsbruck; *Blood Transfusion, University of Innsbruck, Innsbruck, Austria; *Neurology, University of Graz, Graz, Austria; *Neurology, University of Vienna, Vienna, Austria; *Neurology, SMZ Ost, Vienna, Austria

Background: Several studies have suggested a role for tumor necrosis factor (TNF) -a in the pathogenesis of multiple sclerosis (MS) and recently, the TNFRII (p75 TNF-a) receptor, which is a mediator of TNF effect, was shown to play an essential role in pathology and progression in experimental autoimmune encephalomyelitis (EAE). So far, MS association with TNFRII polymorphism has only been investigated using microsatellite markers. Our main purpose was to type TNFRII polymorphism directly and to correlate our findings with clinical data of MS patients in comparison to healthy controls (HC).

Objective: To investigate the role of TNFRII polymorphism in MS, genomic DNA from more than 300 patients of the Austrian Genetics in MS Study

Funding: IVlg was partially sponsored by Baxter Benelux.
Group, 25 samples of patients with systemic lupus erythematoses and samples of 191 white blood donors, which served as healthy controls, was genotyped.

Methods: Genomic DNA was purified from peripheral blood leukocytes. Nucleotide sequencing of the TNFRII gene revealed the presence of 5 polymorphic sites, which have been previously reported: exon 6 nucleotide (nt) 676 T/G, exon 6 nt 783 G/A (both of the latter are associated with nonconserved amino acid substitution), exon 10 nt 1663 G/A, exon 10 nt 1688 T/G and exon 10 nt 1690 T/C. All DNA samples were genotyped using sequence-specific priming (SSP). Data were statistically analyzed using Mann-Whitney U test.

Results: Our group is the first to present data on TNFRII polymorphism in association with MS. Clinical characteristics of MS patients were correlated with the investigated polymorphism. We are able to confirm the allelic frequencies of the investigated polymorphism found by other study groups. In addition, analyzing TNFRII polymorphism regarding disease progression, exon nt 676 T homozygous individuals were found to have a pronounced aggressive progression.

Conclusions: For the first time we report a potential role of TNFRII polymorphism in the pathogenesis of MS. It seems possible that TNFRII polymorphism play a role in MS disease progression.

Disclosure: T Berger has nothing to disclose.
Funding: This study was supported by a research grant of the Austrian Multiple Sclerosis Society (2001).

P315
ANTIBODY RESPONSE TO MYELIN OLIGODENDROCYTE GLYCOPROTEIN AND MYELIN BASIC PROTEIN DEPEND ON FAMILIAL BACKGROUND AND ARE PARTIALLY ASSOCIATED WITH HUMAN LEUKOCYTE ANTIGEN ALLELES IN MULTIPLEX FAMILIES AND SPORADIC MULTIPLE SCLEROSIS
Lutterotti A*, Reindl M*, Gassner C*, Poukta K*, Schanda K*, Deisenhammer F*, Berger T*
*Neurology, University of Innsbruck; †Blood Transfusion, University of Innsbruck, Innsbruck, Austria

Background: Increased intrathecal immunoglobulin synthesis in multiple sclerosis (MS) patients and the raised incidence of antibodies against myelin oligodendrocyte glycoprotein (MOG) and myelin basic protein (MBP) in CSF and sera of MS patients suggests a relevant biological role for these antibodies in MS. In experimental autoimmune encephalomyelitis, an animal model of MS, an antibody response to MOG and MBP was shown to be under genetic control.

Objectives: To elucidate a possible genetic influence on the humoral immune response to MOG and MBP we analyzed the sera of MS patients and members of 12 multiplex families for antibodies against these antigens, and typed this cohort for HLA class II antigens.

Methods: The MS cohort included 41 patients with sporadic MS (sMS, 29 females, 12 males; mean age 35.4 years) and 24 patients (fMS; 19 females, 5 males; mean age 43.7 years) with 33 healthy/asymptomatic relatives (HR, 13 females, 20 males; mean age 47.8 years) and 65 healthy controls (HC, 38 females, 27 males, mean age 39.8 years). Antibodies against MOG and MBP were detected by western blot analysis. HLA genotyping was performed by PCR technique.

Results: We found a significantly increased IgM and IgA antibody response to MOG and IgM antibody response to MBP in MS patients without any difference to HR. Anti MOG IgM was significantly increased in sMS patients. There was a significantly raised incidence in antibody response to MOG in combination with MBP when comparing sMS and HR with HC and sMS respectively. HLA DRB1*04 was associated with IgM reactivity to MOG in MS patients, and DRB1*15 and DRB5 with anti-MOG IgA in HR.

Conclusions: We conclude that antibody responses to MOG and MBP depend on familial background. Moreover the humoral immune reactivity against MOG is partially under control of certain HLA class II alleles. However, whether the MS phenotype in these multiplex families reflects a reaction to common environmental triggers or is controlled by shared genes in and outside the HLA in remains to be elucidated.

Disclosure: T Berger has nothing to disclose.

Funding: This work was supported by a grant of the Austrian Federal Ministry of Science (Nr. GZ 70.059/2-Pv/499).

P316
LACK OF ASSOCIATION BETWEEN CTLA-4 GENE POLYMORPHISMS AND MULTIPLE SCLEROSIS IN SARDINIAN PATIENTS
Cocco EE, Fadda EE, Rolesu MM, Melis CC, Solla EE, Schirru LL, Costa GG, Murru MM, Murru RR, Marroso MM
neuroscience, University of Cagliari, Cagliari, Italy

Background: Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), expressed exclusively on activated CD4 and CD8 T cells, functions to down-regulate T cell activity. The human CTLA-4 gene maps to chromosome 2q33; its structure comprises 3 exons and a leader sequence. Genetic variations of the CTLA-4 locus have been implicated in human autoimmune diseases. Conflictting results of the CTLA-4 role have been reported in various studies in MS. Thus, its possible that an increased risk is carried by CTLA-4 variants only in some ethnically different populations.

Objectives: In order to test a relationship between two CTLA-4 gene’s polymorphisms and MS susceptibility, we performed an association study on Sardinian MS patients from a large set of trios families (one affected offspring and both healthy parents). Sardinian population is a genetically different and homogeneous one, having a high prevalence of MS and particular HLA-DR3 and DR-4 MS association.

Methods: Peripheral blood DNA was extracted from 416 MS trios families. Both polymorphisms, one located at the -318 position of the promoter region and the second one at the 49 position of the exon 1, were typed using standard PCR allele specific dot-blot hybridization. HLA DRB1-DQA1-DQB1 analysis was performed using a high molecular typing with AS0 probes and dot blot hybridization. The transmisio disequilibrium test (TDT) was used to analyze deviation allele from the expected 50: 50 transmission.

Results: No deviation in transmission of C-318T and A49G alleles and the C/T and A/G genotype was found in these samples of families. Moreover, stratification of patients and controls according to predisposing/not predisposing HLA-DRB1, DQA1, DQB1 haplotypes did not reveal differences.

Conclusions: The analysis of CTLA-4 C-318T and A49G polymorphisms failed to demonstrate a significant contribution to MS susceptibility in Sardinian families. Moreover, stratification of sample by HLA predisposing/not predisposing haplotypes did not reveal possible association, confirming that Sardinian CTLA-4 does not influence susceptibility to MS.

Disclosure: E Cocco has nothing to disclose.

P317
A49G CTLA-4 GENE POLYMORPHISM IN SARDINIAN PATIENTS WITH MULTIPLE SCLEROSIS AND TYPE 1 DIABETES
Cocco EE, Fadda EE, Rolesu MM, Melis CC, Solla EE, Schirru LL, Fadda LL, Sanna S, Marroso MM
neuroscience, University of Cagliari, Cagliari, Italy

Background: Cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) has been investigated in vivo in both animal model and in human autoimmune diseases starting from biochemical and physiological functions of the molecule. In autoimmune insulin- dependent diabetes (T1D) from Italian and Spanish populations, an association between T1D and A49G CTLA-4 exon 1 polymorphism has been reported. However, in the same study no association was found in a group of Sardinian T1D families. Recently we reported that Sardinian multiple sclerosis (MS) patients are at high risk for T1D. In Sardinia, an Italian island, a very high prevalence of MS and T1D has been described.

Objectives: We analyzed the A49G CTLA-4 polymorphism in 28 patients having both MS and T1D, all Sardinian and of Sardinian ancestry, to test the hypothesis of a role of the CTLA-4 in susceptibility to both the diseases.

Methods: Peripheral blood DNA was extracted from 28 patients with MS and T1D, 788 MS patients and 145 healthy control (HC). Moreover, A and G alle-
les were evaluated in 954 control chromosomes using the affected family based control (AFBAC). The 49 position exon 1 was typed using standard PCR allele specific dot-blot hybridization. Association was tested by two side chi square test.

Results: The AA genotype was found in 46.4% of MS and T1D patients, in 61.3% MS patients and in 58.6% of HC, without differences between groups. Conversely, the AG genotype was found in 46.4% of MS and T1D subjects, in 32.2% of MS and in 35.9% of HC. The GG genotype was found in 7.1% of MS and T1D subjects, in 5.2% of MS and in 5.5% of HC. The A allele was found in 69.6% of MS and T1D, in 78.2% of MS, in 76.5% of HC and in 79.6% of AFBAC, while the G allele was found in 30.3% of MS and T1D, 21.8% of MS, 23.4% of HC and 23.4% of AFBAC.

Conclusions: The analysis of CTLA-4 A49G polymorphisms did not demonstrate a role of this gene in susceptibility to the co-morbidity of MS and T1D in a group of Sardinian patients having both the diseases, confirming data observed in patients having only one of the two autoimmune conditions.

Disclosure: E Cocco has nothing to disclose.

P318

INFLUENCE OF MHC/HLA ALLELES IN MULTIPLE SCLEROSIS CLINICAL VARIABLES


Background: Despite a general agreement about the association between multiple sclerosis (MS) predisposition and certain HLA class II alleles, the involvement of these genetic markers and clinical variable influencing MS course is discussed. Recently, we reported an independent association between MS predisposition and several alleles located in the MHC region, either at the class II (DRB1, DQB1, DPB1) or telomeric to the class I locus, this last marked by D6S1683 microsatellite.

Objectives: To examine the relationship between several clinical variables and presence of haplotypes and genotypes at the MHC/HLA loci in Sardinian MS patients.

Methods: 889 MS patients were typed for DPB1, DRB1, DQB1, D6S1683. Logistic regression analysis was assessed to describe the relation between predisposing/not predisposing alleles at these loci and several covariates: sex, age at onset, time to reach EDSS 6 from onset, bout onset (relapsing remitting and secondary progressive) or primary progressive course, presence of MS in first degree relative. DRB1-DQB1 predisposing alleles were analyzed considering either each single associated haplotype (namely DRB1*1303-DQB1*0301, DRB1*0405-DQB1*0301, DRB1*0301-DQB1*0201, DRB1*1501-DQB1*0602 and DRB1*0405-DQB1*0302) or grouping together all positively associated haplotypes and genotypes. Conversely, we considered the positively associated DPB1*0301 and the positively associated D6S1683 allele 4 (186 bp) and negatively associated (protective) allele 3 (184 bp).

Results: An association with several MHC/HLA allele was observed only in patients having other relatives with MS (familial disease). In these patients, we found an association with the D6S1683 allele a (p=0.03). Lower age at onset (p=0.001) and progressive course (p=0.01) was associated with DRB1*0301-DQB1*0201 haplotype. Early onset was increased by a factor=1.94 in patients carrying 2 predisposing DRB1-DQB1 haplotypes. Lower age in reaching EDSS 6 was associated (p=0.01) with one of the five DRB1-DQB1 predisposing haplotypes. Finally, in women an association (p=0.02) with the DPB1*0301 allele was found.

Conclusions: MHC/HLA loci seem to influence age at onset, type and severity of course in familial MS, suggesting that genes contributing to MS predisposition might also modulate several clinical variables.

Disclosure: E Cocco has nothing to disclose.

P319

A GENOME-WIDE LINKAGE SCREEN IN TURKISH MULTIPLEX FAMILIES WITH MULTIPLE SCLEROSIS

Eraksoy M1, Kurtuncu M2, Sawcer SJ, Akesson E3, Akman-Demir G4, Compston AD5, Turkish Multiple Sclerosis Genetics Study Group 1Neurology, Istanbul University, Faculty of Medicine, Istanbul, Turkey; 2Neurology, University of Cambridge, Cambridge

Background: Epidemiological findings support a multigenic hereditary predisposition to multiple sclerosis. Turkey lies at the intermediate zone in terms of the risk of developing multiple sclerosis between Northern Europe and Asia. Turkish population may be considered special from the point of view of multiple sclerosis because of consanguinity and the ethnic genetic background.

Objectives: This study was designed to reveal special genetic features of the Turkish multiplex families with multiple sclerosis.

Methods: A genome-wide screen for linkage in 43 Turkish multiplex families with multiple sclerosis 13 of which are consanguineous families.

Results: In this study, there was no region of linkage with genome-wide significance, but these data reveal suggestive linkage in the 2 regions, in particular myelin basic protein region on 18q and 13p.

Conclusions: Further analysis of the region of interest with additional families and markers with multiple sclerosis may provide useful information.

Disclosure: M Eraksoy has nothing to disclose.

Funding: Supported by Multiple Sclerosis Societies of Great Britain and Northern Ireland. Supported by The Turkish Neuroimmunology Society. Supported by The Turkish Brain Research Society.

P320

MOLECULAR ANTHROPOLOGICAL VIEW IN A POPULATION ANALYSIS OF BRAZILIAN INDIVIDUALS DURING A MULTIPLE SCLEROSIS GENETIC STUDY OF MLA DRB1-DQB1-DQA1

Leon SV, Alvaranga RP, Caballero A, Alonso A, Fernandez O 1Neurology, Universidade do Rio de Janeiro, Rio de Janeiro, Brazil; 2Neurology, Hospital Universitário Clementino Fraga Filho - UFRJ, Rio de Janeiro, Rio de Janeiro, Brazil; 3Neurology, Hospital Universitário Carlos Haya, Málaga, Andalucía, Spain; 4Neurology, Hospital da Lagoa, Rio de Janeiro, Rio de Janeiro, Brazil

Background: To appraise the studies of HLA class II in Brazilian population, it is necessary to understand the history of the transatlantic slave trad. Portuguese arrived in Brazil in April 1500 and were followed by other European populations. Brazilian black population originated from three million immigrants from different regions and tribes of Africa. Almost 30% of Brazilian MS patients are African-Brazilian.

Objectives: To determine the allelic distribution of HLA DQB1, DQA1 and DRB1 loci association in African-Brazilian and White Brazilian population and discuss probable association or protection of these alleles with diseases as multiple sclerosis according to the ethnic background.

Methods: 132 African-Brazilian and 147 White individuals from Rio de Janeiro City enrolled in a genetic study for multiple sclerosis were analyzed. Typing of HLA class II DRB1, DQB1 and DQA1 genes was made by amplification of the DNA using PCR followed by hybridization with SSOP for the DRB1 and DQB1 loci and PCR-SSP for the DQA1 locus.

Results: DQA1*0201 and 0301 were significantly associated with White Brazilians (p=0.0000004 and p=0.000004). DQB1 0303-0404-0503-0504-0604-0697-0608 alleles were absent among African-Brazilian population, but present at White population, with relatively high frequency for DQB1*0303 (21.08%), DQB1*0401 (7.48%), and DQB1*0604 (10.88%). No significant difference with DQB1 alleles was observed. DRB1*1501 allele was significantly present in White Brazilians (p=0.00001), and conversely, the DRB1*1503 was significantly present in African-Brazilians (p<0.000001).

Conclusions: Positive association with DQB1*0602 in both groups can reflect the admixture of American colonization. DRB1*1501, common in
European and in DR2 haplotype was present almost exclusively in White Brazilian group. DRB1*1503, unusual in both Europeans and African-Americans was significantly associated with African-Brazilians. Our African-Brazilian population differs from African-American samples with no presence of DR2 haplotype.

Disclosure: S Leon has nothing to disclose.

P321
HISTOCOMPATIBILITY CLASS II DR*, DQ*, DP* ANTIGENS ASSOCIATION WITH MULTIPLE SCLEROSIS IN A POPULATION OF THE RIO DE JANEIRO CITY, BRAZIL

Santos CC, Emmerick M, Liem AM, Frugulhetti F, Leon SV, Quirico-Santos T

*Neurology, Hospital Universitário Clementino Fraga Filho - UFRJ, Rio de Janeiro, Rio de Janeiro, Brazil;  †Neurology, Universidade do Rio de Janeiro;  ‡Department of Cellular and Molecular Biology, Universidade Federal Fluminense, Niterói, Rio de Janeiro, Brazil

Background: Multiple sclerosis (MS) is a chronic inflammatory disease of the human central nervous system of putative autoimmune origin characterized by multifocal demyelination. Genetic and environmental factors are thought to be involved in the pathogenesis of MS, with the disease considered rare in tropical countries, including Brazil. Which gene(s) present in this region are responsible for MS susceptibility in the Brazilian population is still an unsettled issue. Heterogeneity distribution of HLA class two alleles was previously showed.

Objectives: This study aimed to analyze the frequencies of HLA class II alleles (DQA1*0102, DQB1*0602, DPA1*0301, DRB1*1501, DRB1*1503) in a new group of MS patients from the Rio de Janeiro city

Methods: It was included 42 individuals (73.8% female and 26.2% male) with clinically definite MS, age range of 15 to 55 years. In relation to ethnic background, MS patients were 72.6% White/Caucasian and 23.8% African-Brazilian descendants. Age-matched healthy control group consisted of 53.6% (female), 46.4% (male), 58.3% White/Caucasian (CA) and 41.7% African-Brazilian (AF).

HLA typing was performed by amplification of the DNA isolated from peripheral leukocytes with Polymerase Chain Reaction (PCR) followed by SSO hybridization.

Results: MS patients showed a positive association for alleles DQB1*0602 (P=0.021; RR=2.40) and DQA1*0102 (P= 0.0041; RR=3.30). Likewise, increased frequencies of DQB1*0602 (45.2%) and DQA1*0102 (35.7%) endowed strong correlation of this allele combination with disease susceptibility in CA patients. DR2 (DRB1*1501, DQA1*0102, DQB1*0602) haplotype frequencies among healthy individuals and MS group was low.

Conclusions: Since both ethnic groups of MS patients showed very low frequencies of DRB1*1501 and DRB1*1503 it is suggested that another DRB allele association may be conferring susceptibility to MS in this population. Finally the very low frequency (RR<0.36) of DPA1*0301 allele consistently observed in our MS patients indicate that such allele may rather have a protective role against MS.

Disclosure: S Leon has nothing to disclose.

Funding: Supported by CNPQ, CAPS (Brazilian Research Foundation).

P322
PRIMARY ASSOCIATION OF A TUMOR NECROSIS FACTOR GENE POLYMORPHISM WITH MS SUSCEPTIBILITY

Rafael A, Virginia D, Alfonso M, Ana R, Xavier M, Emilio G

*Neurology, Hospital Clínico San Carlos, Madrid, Spain; †Immunology, Hospital Clínico San Carlos, Madrid, Spain; ‡Neurology, Hospital Vall d’Hebron, Barcelona, Spain

Background: MS is a complex disease with a polygenic mode of inheritance. Genomic scans have shown that the main susceptibility locus is located within the Major Histocompatibility complex (MHC), where the HLA-DRB1*1501 allele has been seen consistently associated with MS in different populations. TNF-alpha is an important inflammatory cytokine and TNF gene, located in the MHC, could be a second susceptibility gene.

Objectives: To determine whether TNF genetic markers might modulate susceptibility conferred by HLA-DRB1*1501

Methods: We studied 2 groups of Spanish MS. First 286 patients and 340 ethnically matched controls. Sixty-three families were studied together with both parents, and the 4 parental haplotypes were identified for each family. The second study comprised 100 patients, all primary progressive MS (PPMS). HLA-DRB1 and TNFαb microsatellites were typed.

Results: As expected HLA-DRB1*1501 was found to be associated with MS (33% vs 19%; p=0.001; OR=2.08). We also observed that HLA-DRB1*1501 was in linkage disequilibrium with TNFα1b4 (66% of DRB1*1501+ controls carried TNFα1b4 vs 14% DRB1*1501+; p<0.001; OR=6.86), reflecting their coexistence in the common Ancestral Haplotype AH1.7. We then analyzed the susceptibility of HLA-DRB1*1501 in individuals positive and negative for TNFα1b4, and a striking difference was observed. The association of HLA-DRB1*1501+TNFα1b4+ with MS was weaker (16% vs 12% in controls; p=0.18; OR=1.36) than the association found in HLA-DRB1*1501+TNFα1b4− individuals (16% vs 6%; p<0.001; OR=2.08). These results were confirmed in a second group of 100 white Spanish PPMS patients and 101 ethnically matched controls. Susceptibility of DRB1*1501+TNFα1b4− individuals was higher (19% vs 3%; p=0.001;
OR=7.66) than that of DRB1*1501+TNFa11b4+ (16% vs 9%; p=0.13; OR=1.95). Moreover, 63 MS family trios were included in the study. Again, transmission disequilibrium was higher in HLA-DRB1*1501+TNFa11b4+ haplotypes (11T vs 2NT; p=0.01) than in DRB1*1501+TNFa11b4+ haplotypes (10T vs 8NT; p=ns).

Conclusions: Our data indicate that TNFa11b4 microsatellite alleles are a marker for a gene modulating MS susceptibility conferred by HLA-DRB1*1501. Haplotype studies suggest that this gene is carried in the 7.1AH.

Disclosure: A Rafael has nothing to disclose.

P324
IL10 GENE AND RESPONSE TO IFN BETA IN MS

de las Heras V*, Rafael A*, Martinez A*, Rubio A*, G de la Concha E*
*Neurology, Hospital Clinico San Carlos, Madrid, Spain; †Immunology, Hosp Clinico San Carlos, Madrid, Spain

Background: IL-10 is an anti-inflammatory cytokine and IL-10 gene could be a susceptibility gene for MS. Several studies on IL-10 polymorphisms associations with MS have already been published. It has been reported also an association between IL-10 and a susceptibility gene for MS. Several studies on IL-10 polymorphisms and a more severely unbalanced towards a Th1 response. To know whether the IFN-β treatment. This is true at least for IL-G12 allele, and probably for the IL-10G12/ACC haplotype, the presence of which determines a worse response. These polymorphisms could provoke a downregulation of IL-10 production and a more severely unbalanced towards a Th1 response. To know whether the IFN-β-mediated modulation of the cytokine network, and therefore its efficacy, is dependent on IL-10 genotype would deserve further study.

Disclosure: A Rafael has nothing to disclose.

P325
ASSOCIATION OF APOLPOLYPEPTIDE E AND MYELOPEROXIDASE GENOTYPES WITH THE CLINICAL COURSE OF FAMILIAL AND SPORADIC MULTIPLE SCLEROSIS

*Neurology, Medical Academy of Warsaw, Warsaw, Warsaw, Poland; †Department of Neurodegenerative Disorders, The Medical Research Centre, Polish Academy of Sciences, Warsaw, Warsaw, Poland

Background: Recently the importance of apolipoprotein E (APOE) and myeloperoxidase (MPO) genotypes in the clinical characteristics of multiple sclerosis (MS) has been emphasized.

Objectives: These results prompted us to re-examine in a large group of Polish Caucasian patients the hypothesis that allelism in APOE and MPO genes influences the course of the disease. Genotypes were determined in 117 MS patients (74 females and 43 males; 99 sporadic and 18 familial cases) with mean EDSS of 3.6, mean age of 44.1 years, mean duration of the disease - 12.8 years and mean onset of MS of 31.2 years.

Methods: The relationship between the APOE and MPO genes polymorphism and the MS activity (increase in EDSS during 2 years of follow up) as well as the presence and the degree of cognitive function changes and defect of remyelination on MRI were analyzed.

Results: The APOE epsilon 4 allele presence was not related to the disease course or the APOE epsilon 2 - to the degree of the demyelination on MRI. The MPO G-G allele was found in all familial MS and in 56 sporadic cases. It was related to more pronounced brain atrophy on MRI (p<0.05). The MPO G-G subpopulation was characterized by significantly higher proportion of SP MS (p<0.05) and by significantly higher value of EDSS.

Conclusions: According to our results the MPO allele is frequently found among Polish MS patients. The MPO G-G form is probably related, by the mechanism of accelerated oxidative stress, to more severe nervous tissue damage and may determine worse clinical course of MS. We postulate that the MPO G-G allele may be one of genetic factors having a major impact on the progression of disability in MS patients.

Disclosure: B Zakrzewska-Pniewska has nothing to disclose.

Immunotherapy (Part 2)

P326
RAPID ONSET MITOXANTRONE-INDUCED CARDIO TOXICITY IN SECONDARY PROGRESSIVE MULTIPLE SCLEROSIS

Aysarala JR, Cross AH, Clifford DB, Siegel B, Abbey EE
Neurology, Washington U School of Medicine, St Louis, Missouri, USA

Background: Mitoxantrone is a recently approved drug for patients with secondary progressive multiple sclerosis (SPMS). Cardiac side effects limit its use and lifetime cumulative dose should not exceed 140 mg/m2. Mitoxantrone is contraindicated in patients with a baseline left ventricular ejection fraction (LVEF) of <50%. Prior treatment with anthracyclines, mediastinal radiotherapy and pre-existing cardiovascular disease are some of the potential risk factors that could contribute to cardiac dysfunction in patients on mitoxantrone treatment.

Objectives: We opted to follow LVEF evaluations at baseline and at the end of one year of treatment since the potential side effects of mitoxantrone therapy in SPMS patients is largely unknown.

Methods: SPMS patients on mitoxantrone received baseline LVEF evaluations by radionuclide ventriculography (RVG) and just prior to their 4th dose. We followed 31 (21 F; 10 M) patients with SPMS being treated with i.v. mitoxantrone at the approved dose of 12 mg/m2 every 3 months. Patients receiving mitoxantrone for SPMS ranged in age from 37-54 years; the mean disease
duration was 13.2 years. Baseline LVEF was above > 60% in 27/31 patients and < 57% in 4/31 patients.

Results: We found that 4/14 patients (28.5%) had a significant drop in LVEF. No patient who had a drop on their repeat LVEF had any cardiac risk factors. The mean age (40 vs 43.2), gender, and duration of MS (10 vs 9.4 yrs) did not differ between those who had a significant drop in LVEF and those that did not.

Conclusions: We suggest that in patients receiving mitoxantrone, more stringent cardiac monitoring than currently accepted standards is necessary.

Disclosure: J Avasarala has nothing to disclose.

Funding: J. Avasarala is a fellow of the National MS Society.

P327

ANTI MOG AND ANTI MBP ANTIBODY SUBCLASSES IN MULTIPLE SCLEROSIS PATIENTS DURING INTERFERON BETA THERAPY


Neurology, University of Innsbruck

Background: Antibodies against MOG and MBP seem to play a role in a subset of MS patients. Own previous data suggested a partial influence of interferon-beta on the antibody response against MOG and MBP, measured at a single time point, in a large cohort of 261 MS patients.

Objectives: The aim of this prospective study was to investigate whether interferon-beta treatment influenced the antibody response against MOG and MBP after one year of treatment.

Methods: Until yet we have analysed IgG, IgG1, IgG2, IgG3, IgM and IgA serum antibodies against MOG and MBP in 22 MS patients (mean age 32.8 years; mean duration 5.5 years; disease course: 1 PPMS, 2 SPMS, 19 RRMS) before and after one year of interferon-beta therapy. The control group consisted of 12 MS (mean age 34.7 years; mean duration 5.4 years; disease course: 12 RRMS) patients without any immunomodulatory therapy. None of the patients had suffered a relapse or received any immunosuppressive treatment within 3 months before blood samples were taken.

All antibody isotypes and subclasses were detected by western blot analysis. IgG and IgM isotypes were additionally analysed by ELISA.

Results: We found significantly raised IgA antibodies in the control group before and after 1 year. No significant differences were found for any other antibody response between the treatment and control group. No significant differences in the frequency of antibody response to MOG and MBP were found within each group comparing baseline and 1 year data. However, there was a trend towards a reduction of IgM antibodies after 1 year of interferon-beta treatment when performing a paired t-test. Furthermore, disease courses, relapse rates and EDSS are currently analysed.

Conclusions: In this prospective 1 year follow-up study we could not demonstrate a significant reduction of the anti-MOG and/or anti-MBP antibody response after one year of interferon-beta therapy in a group of yet analysed 22 MS patients. However as we see a trend towards a reduction of anti-MOG IgM antibodies, we are reluctant to completely exclude any influence of interferon therapy on the anti-MOG antibody response. An extension of the number of patients and of the follow-up period might reveal an influence of interferon-beta on immune responses against MOG and MBP.

Disclosure: T Berger has nothing to disclose.

Funding: This study was supported by an unrestricted research grant from Schering Austria GmbH.

P328

GLATIRAMER ACETATE (GA)-REACTIVE T-CELLS PRODUCE BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF)

Chen M, Dhib-Jalbut S

1Neurology, Univ of Maryland; 2Neurology, Department of Veterans Affairs, Baltimore, Maryland, USA

Background: Glatiramer acetate (Copaxone) therapeutic effect in multiple sclerosis (MS) is believed to be mediated by anti-inflammatory GA-reactive Th2 cells that enter the brain, cross react with myelin antigens and produce bystander suppression. Experimental and MRI studies suggest a neuroprotective effect possibly mediated by neurotrophic factors. BDNF is produced by cells from both the nervous and immune systems.

Objectives: To examine BDNF production in GA-reactive T cells.

Methods: BDNF levels were examined by ELISA and confirmed by RT-PCR. BDNF production was examined in 73 GA- and 33 MBP-reactive short-term T-cell lines from 12 MS patients. Mean level in GA-TCL was 38.67±5.18 pg/ml compared to 20.42±11.11 pg/ml in MBP-TCL (p=0.046). In contrast nerve growth factor levels (examined as a control neurotrophic factor) did not differ between the two groups. Six of 73 GA-TCL but none of the MBP-TCL produced BDNF levels two standard deviations above the mean. All six GA-reactive TCL were Th2 as determined by the IL-5/IFNγ levels ratio. RT-PCR analysis confirmed BDNF expression in the GA-reactive TCL. Regression analysis showed a positive correlation between BDNF and IL-5 (Th2 indicator) (p=0.006) but not with IFNγ levels in GA-TCL derived from GA treated MS patients.

Conclusions: While autoreactive T-cells may have a dual role in autoimmunity and neuroprotection, GA-reactive Th2 TCL are more likely to produce BDNF, which is consistent with a neuroprotective effect for the drug.

Disclosure: Dr. Chen’s fellowship is Supported by TEVA Neuroscience. Dr. Dhib-Jalbut research is Supported by and has received honoraria from TEVA Neuroscience.

Funding: Supported by Research grants from TEVA Neuroscience and the National Institutes of Health.

P329

IMPORTANCE OF CONTINUOUS IMMUNOMODULATORY TREATMENT IN MULTIPLE SCLEROSIS

Csépány T, Csiba L

Department of Neurology, University of Debrecen, Debrecen, Hungary

Background: The most important therapeutic aim of any disease-modifying treatment of MS is to prevent or postpone long term disability. Clinical trials study patients for only short periods of time (2 or 3 years) and, use only short-term outcome measures to assess efficacy. There are no clinical data to support the possible importance of continuous treatment.

Objectives: Five year follow up of 23 relapsing-remitting multiple sclerosis (MS) patients treated with interferon beta-1b in our study (group A: n=12, subcutaneously (s.c.) injected 8 MIU every other day, mean age: 38 years, duration: 3.3 years) and copolymer-1 (group B: n=11, s.c. injected 20 mg every day, age: 41.5 years, duration: 5.5 years).

Methods: We compared the yearly outcome by measuring the EDSS scores, the number of exacerbations 2 years prior the initiation of therapy and follow up to 5 years. While group A patients had continuous treatment, the other group of patients was treated for 3 years and withdrawn for a year and restarted again.

Results: EDSS scores were not increased in the first two years in neither group after the immunomodulatory treatment, whereas it was increased (p<0.05) in group B in the next 2 years. The relapse rate was less during the follow up than that in the pretreatment period, but it was markedly increased during the withdrawal phase in group B (p<0.005).

Conclusions: Our data support the continuous favourable effect of immunomodulatory treatment by modifying the course of multiple sclerosis. The possibility that the withdrawal of efficent immunomodulatory therapy can increase the progression of MS needs to be proved by studying a larger number of patients.

Disclosure: T Csépány has nothing to disclose.
**P330**

**EFFECT OF ORAL GLATIRAMER ACETATE (COPAXONE) IN MULTIPLE SCLEROSIS: REDUCTION OF INTERFERON GAMMA PRODUCTION**


*Neurology, CHRU de Lille, Lille, Nord, France; ‡Immunology, CHU de Lille, Lille, Nord, France; §Neurology, CHU de Clermont-Ferrand, Clermont-Ferrand, Centre, France; ¶Neurology, CHU de Rennes, Bretagne, France; ¶Neurology, Hopital Saint-Philibert, Lomme, Nord, France

**Background:** Glatiramer Acetate (GA) (Copaxone) is active in suppressing experimental autoimmune encephalopathy (EAE) and in the treatment of multiple sclerosis (MS) when injected parenterally. Recently, Copaxone has also been demonstrated to be active when administrated orally in an EAE model.

**Objectives:** to evaluate whether oral administration of Copaxone can shift from a proinflammatory to an anti-inflammatory in vitro cytokine profile in MS patients.

**Methods:** As a part of a phase III trial, we performed a prospective study in 31 MS patients. We evaluated interferon gamma and interleukine (IL) 10 production by mononuclear cells on contact with different myelin antigens by an ELISPOT method. Twenty-one patients were treated with oral Copaxone (11 with 50 mg per day and 12 with 5 mg per day). The remaining eight patients were treated by placebo. Dosages were performed at baseline, month 2 (M2) and month 14 (M14).

**Results:** We did not observe any modification in both placebo and 50 mg oral Copaxone groups at M2 and M14. Moreover, we did not observe any modification with 5 mg oral Copaxone at M2. By contrast, at M14, we observed that patients treated with 5 mg oral Copaxone had a significant decrease in term of reactive T-cells producing interferon gamma against the entire MBP (p=0.009). We did not found any significant modification of IL-10 production through the study.

**Conclusions:** Our study demonstrates, for the first time, an in vitro decrease of interferon gamma secretion of T-cells in presence of MBP in MS patients treated with 5 mg oral Copaxone but only after 14 months of treatment. These results argue for a delayed effect of Oral Copaxone on immunological evaluation in MS.

Disclosure: J de seze has nothing to disclose.
Funding: Supported by Teva Pharma.

**P331**

**A RETROSPECTIVE COMPARATIVE ANALYSIS ON THE EFFICACY OF THREE INTERFERON BETA TREATMENTS IN MULTIPLE SCLEROSIS**

Giray S, Demirkiran M, Sarica Y

Neurology, Çukurova University, Adana, Turkey

**Background:** Although all three interferon beta treatments are effective immunomodulating therapies in multiple sclerosis, a comparison of the three regarding the relative efficacy of each is lacking.

**Objectives:** To analyse and compare the effects of different interferon beta treatments on clinical parameters and MRI findings in multiple sclerosis.

**Methods:** The effects of three different interferon beta therapies on 40 patients with relapsing-remitting multiple sclerosis have been retrospectively analysed and compared. Twenty two patients received interferon beta-1b (Betaferon) 8 MIU every other day subcutaneously, 18 patients were treated with interferon beta-1a, 12 of them with Rebif 22 mcg three times a week sc and 6 of them with Avonex 30 mcg once a week intramuscularly. These three groups were compared according to relapses frequency and disability scores before and after treatment, relaps-free time after treatment and the MRI findings. The patients were followed for a mean of 18 months.

**Results:** Relaps-free time after initiation of interferon treatment was a mean of 17 months for Betaferon, 12 months for Rebif and 10 months for Avonex. Although it did not reach statistical significance, the frequency of relaps-free patients was higher in Betaferon and Rebif groups than in Avonex group (respectively 63.6%, 83.3%, 33.3%). Furthermore, the number of relapses after treatment was lower in patients on Betaferon and Rebif than it was in Avonex by 25% and 10%, respectively. Betaferon was found to be effective on disability scores; the difference in disability scores before and after treatment was found to be statistically significant (P=0.006). Moreover, 25% of the patients on Betaferon therapy improved 1 point in their disability scores, while there was no considerable improvement in either of the other treatment groups. All treatment groups had positive effects on MRI findings and there were no statistical differences among them on this aspect.

**Conclusions:** These findings should be interpreted cautiously due to the retrospective nature of this study, to the small number of patients, especially on Avonex and also to the differences in the follow-up durations of each treatment group. Randomized controlled and prospective studies can provide conclusive data on the relative efficacies of these interferon beta therapies.

Disclosure: M Demirkiran has nothing to disclose.

**P332**

**CLINICAL AND MRI IMPACT OF MITOXANTRONE IN 111 SECONDARY PROGRESSIVE MULTIPLE SCLEROSIS PATIENTS**

GREGORY T, Emmanuelle L, Emmanuelle L, Eric S, Marc C, Gilles E

CHRU Pontchaillou, Rennes, Bretagne, France

**Background:** The clinical benefit of Mitoxantrone (Mito) for worsening Relapsing-remitting and secondary progressive multiple sclerosis (MS) has been supported by 2 controlled trials.

**Objectives:** This study sought to assess clinical and MRI follow-up of 111 consecutive secondary progressive multiple sclerosis (SPMS) patients treated with Mito over a median duration of 3 years.

**Methods:** In our MS center, 111 SPMS had received Mito according to 2 different protocols: 53 SPMS, were treated monthly with Mito IV, 20 mg, and methylprednisolone 1 g IV for 6 months (cumulative dose: 77 mg/m2), followed 3 months later by a maintenance therapy (13 patients by Mito every 3 months, 8 patients by Interferon Beta, 14 patients by Methotrexate, 8 patients by Azathioprine). 58 other patients were treated with Mito, 20 mg, every 3 months (cumulative dose: 60 mg/m2), followed by other maintenance therapies (9 patients by Interferon Beta, 3 patients by Methotrexate, 1 patient by Azathioprine). The median duration of MS and the median duration of progressive course, before initiating Mito, were respectively 14 years and 8 years. The mean worsening of EDSS within the 24 months before Mito was 1.5. 67 patients (60%) had superimposed relapses within 2 years prior Mito. 36% had gadolinium enhanced lesions on MRI. Clinical and MRI data were collected yearly in EDMUS.

**Results:** The mean EDSS didn’t change significantly from baseline: from 6.0 to 6.1 at 1 year (108 patients), from 6.0 to 6.2 at 2 years (74 patients) and from 6.9 to 6.2 at 3 years (39 patients). 1 point EDSS worsening at time point from baseline was 23% of patients at 1 year, 18% of patients at 2 years, and 20% of patients at 3 years of follow-up. the mean annual relapse rate decrease from baseline: from 0.7 to 0.1 at 1 year, from 0.9 to 0.2 at 2 years and from 1.0 to 0.3 at 3 years of follow-up. 10% of Mito after discontinuing Mito, has gadolinium enhanced lesions. Data according to the 2 different protocols will also be presented.

**Conclusions:** This open trial supported the conclusion of the previous controlled trials, that Mitoxantrone has a clinical and MRI impact on the inflammatory markers of the disease.

Disclosure: T GREGORY has nothing to disclose.
of neutralizing antibodies (NAB) in some patients could inhibit the biological activity of IFNB.

Objectives: To determinate whether the development of NAB on long-term therapy with IFNB-1b in relapsing-remitting MS (RRMS) is associated with treatment failure.

Methods: Serum samples from 90 RRMS patients receiving IFNβ-1b at least for two years were collected before, at 3 months and every 6 months after initiating treatment. ELISA and immunoblotting were performed to detect binding antibodies to IFNβ-1b. Positive sam-ple s were tested for NAB by viral cytopathic effect inhibition assay. Results were re-lated with clinical response parameters.

Results: 77/90 patients developed antibodies to IFNβ-1b. NAB developed in 27/90, generally during the first year, with higher titles during the second year. NAB disappeared in all but 9/65 patients in the fourth year of treatment, with a mean time of disappearance of 29 months. In the second year, 16/27 patients with NAB were bad responders to IFNβ-1b therapy. Interestingly, only 11/63 patients without NAB were bad responders (p<0.001). In the fourth year, 4/9 patients with sustained high levels of NAB were bad responders. In contrast, only 7/56 patients without NAB were bad responders (p=0.038).

Conclusions: Most MS patients developed anti-IFNβ-1b antibodies. Only a small group of patients developed NAB. Since the frequency of bad response to IFNβ-1b was greater in this group, alternative treatments may be considered in patients with sustained high levels of NAB.

Disclosure: J Hernández-Regadera has nothing to disclose.

P334

LONG-TERM TOLERABILITY OF INTERFERON BET-ALa IN RELAPSING-REMITTING MULTIPLE SCLEROSIS: 6-YEAR SAFETY FOLLOW-UP OF THE PRISMS STUDY

Kappos L1, Stam Moraga M2
1University Hospitals Kantonsspital, Outpatient Clinic Neurology-N euro-surgery, Basel, Switzerland; 2Sorono International S.A., Geneva, Alabama, USA

Background: Patients completing PRISMS-4 were offered to continue on dose-blinded treatment for up to 2 years (PRISMS-6) to provide long-term safety data.

Objectives: To assess the long-term safety of IFN beta-1a (Rebif, Serono).

Methods: Adverse events (AEs) were assessed and laboratory tests performed every 3 to 6 months. Serious (S)AEs and terminations due to AEs were documented. Safety comparisons focused on patients on 22mcg (n=273) or 44mcg (n=271) at any point in the 6 years.

Results: 560 patients enrolled in the original cohort. 79% of 560 patients initially enrolled completed 4 years on study (77% on treatment). 57% entered year 5, and 48% completed 6 years on dose-blinded therapy (many patients started on marketed product and are not included in this analysis). Total exposure was 1050 patient-years for 22mcg 1002 patient-years for 44mcg. In years 5 to 6, the most frequent IFN-related AEs were injection-site inflammation (50% on 22mcg vs. 58% on 44mcg, p=0.22), headache (42% vs. both groups), injection-site reactions (29% both groups), fatigue (27% both groups), and influenza-like symptoms (25% vs. 19%, p=0.23). All values are lower than reported during the placebo-controlled phase. Most common laboratory abnormalities reported as AEs were lymphopenia (11% for 22mcg vs. 18% for 44mcg, p=0.08) and elevated liver enzymes (9.6% vs. 6.5%, p=0.41). Over 6 years, 55/544 patients (10.1%) stopped therapy due to AEs; 35/271 (12.9%) for 44mcg, p=0.08) and elevated liver enzymes (9.6% vs. 6.5%, p=0.41). Over 6 years, treatment adherence was nearly identical between doses. Dose-related AEs show a trend only for lymphopenia. Although incidence of all AEs appears to decrease over time, reduced patient numbers and less frequent visits may partly contribute to this finding. An ongoing Phase IV study that will include the original cohort will also collect efficacy data including MRI and EDSS assessments. PRISMS investigators are listed in: Neurology 2001;56: 1628.

Disclosure: LK’s work is Supported by the Swiss MS Society; LK has received honoraria and compensation as speaker, consultant, member of various com-mittes and study investigator for the sponsor of this study and many other pharmaceutical companies; these payments were used exclusively for support of research activities at his department. MSM is employed by Serono.

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Funding: Serono International S.A.

P335

ALGORITHM FOR LONG-TERM TREATMENT OF EARLY MULTIPLE SCLEROSIS

Koechler I, Wicht S, Hey W, Holger S
Neurology, Johannes-Gutenburg University, Mainz, Rheinland-Pfalz, Germany

Background: Current studies support the impression of different therapeutic power of different interferon-beta drugs in several stages of MS. Furthermore, different efficacy of interferon-beta-1a could be demonstrated in patients with clinical defined MS. Nevertheless, a concept for individual long-term treatment strategies is needed.

Objectives: The aim of this analysis was to outline an algorithm which can support individual long-term treatment strategies in early stages of MS.

Methods: Based on peer reviewed papers and latest data of interferon-beta studies in MS an algorithm was designed. Single steps within this algorithm were linked to the studies on which they based. In case of unclear data all options were listed.

Results: It is evident that long-term treatment in early MS is useful in case of first relapse and distinct MRI abnormalities. Interferon-beta-1a (Avonex) is the primary drug which is approved for this stage of disease. If this therapy fails we suggest to favour the treatment with Rebif 22 3 times a week as an escalating therapy followed by Rebif 44 3 times a week. Interferon-beta-1b or Glatiramer acetate data are not available for patients in this stage of MS. MRI-follow-up should be done every 6 to 12 month. Progress of initial normal electrophysiological recordings could be used also for follow-up investigations.

Conclusions: Criteria for long-term treatment in early MS are present in literature. Avonex seems to be the first choice after the first relapse and distinct MRI abnormalities (McDonald criteria). Frequent follow-up investigations by MRI or electrophysiological studies are necessary to detect non responder. Rebif 22/44 should be the alternative for escalating therapy regime. In a current ongoing study interferon-beta-1b is proofed with respect to efficacy in patients with risk of developing CDMS (Benefit). Furthermore treatment options in CDMS were discussed in this algorithm.

Disclosure: J Koehler has nothing to disclose.

P336

THE BRAZILIAN EXPANDED CONSENSUS ON TREATMENT OF MULTIPLE SCLEROSIS

Lana-Peixoto MA, Callegaro D, Moreira EA, Marchiori PE, Lino A, Gabbai AA, Souza AM, Campos GB, Rocha FC, Gama PD, tosta E, Ataide L, Brito L, Bacheshi LA
CJEM M/NS UFMG Center for Investigation of Multiple Sclerosis, Brazilian Committee for Treatment and Research in Multiple Sclerosis, Belo Horizonte, MG, Brazil

Background: The increasing number of therapeutic trials has prompted neuro-scientific societies in some countries to discuss guidelines for its treatment. As the presently available DMA have a high cost and a modest effect a number of questions has been risen about their prompt use. Treatment reimbursement may...
represent an extra burden to developing countries. In 2000 BCTRIMS published guidelines which have oriented Brazilian Health Authorities to define a reimbursement policy for the country. An Expanded Consensus has recently been approved.

**Objectives:** To describe the main points of the Brazilian Expanded Consensus on Treatment of MS

**Methods:** Brazilian MS experts reviewed the literature on MS treatment and suggested general guidelines for treatment. Papers on MS treatment were searched from MEDLINE and analyzed according to classes of evidence and types of recommendation as set out by the American Academy of Neurology. Information about the cost of the DMA was obtained from the Department of Health. A Consensus Meeting was attended by 56 Members of the BCTRIMS.

**Results:** An Expanded Consensus for Treatment of MS, suited for Brazil prevailing economical and health conditions was approved. Its main points are: (1) treatment may be prescribed only to patients who fulfill the International Panel criteria; (2) MS diagnosis and treatment must be conducted only by neurologists with expertise in the area in referral centers; (3) decision regarding the onset of treatment must be taken in an individual basis; (4) there is no immunomodulating agent of choice and neurologists are encouraged to take account of the cost of the different DMA; (5) use of immunomodulating agents must be restricted to ambulatory patients (EDSS 6.5 or less). Other items are similar to those previously published.

**Conclusions:** The Brazilian Expanded Consensus for Treatment of MS emphasizes concerns about the diagnosis of MS, decision about the onset of treatment and the financial cost of the DMA. It may turn out to be a model in other developing countries.

**Disclosure:** M Lana-Peixoto has nothing to disclose.

**P337**

**INDUCTION TREATMENT WITH MITOXANTRONE DURING 6 MONTHS IN WORSENING RELAPSGING REMITTING MULTIPLE SCLEROSIS: A RESCUE THERAPY FOR SUB-OPTIMAL RESPONDERS TO INTERFERON BETA 7.4 PILOT STUDY**


**Background:** In the French and British Mitoxantrone Trial, Mitoxantrone (MITOX) as induction therapy for 6 months, reduced dramatically radiological and clinical parameters of disease activity in patients with severe relapsing-remitting multiple sclerosis (RRMS) (Edan et al., 1997). In the open follow-up retrospective study of 100 RRMS patients treated in Rennes on the basis of this trial, the benefit initially observed was maintained during a period of 4 years.

**Objectives:** To assess the potential action of MITOX in RRMS patients experiencing a sub-optimal response to interferon beta 1a or 1b (IFN) compared with patients naive of disease modifying therapy (DMT) prior to MITOX

**Methods:** From the open retrospective study of 100 RRMS patients followed in Rennes after an induction therapy with MITOX 20mg monthly for 6 months, 11 patients treated by IFN for at least 12 months but who failed to respond were compared with 50 patients naive of DMT for at least 12 months prior to MITOX.

**Results:** The 11 IFN non responders and the 50 DMT naive patients started MITOX at a mean age of 33.3 vs 34.5 years, after a mean disease duration of 6.6 vs 5.7 years, respectively (NS). Clinical and radiological parameters of disease activity during the 12 months preceding MITOX were similar in IFN non responders and DMT naive patients with an annual relapse rate (ARR) of 3.1 vs 2.8; a mean EDSS deteriorated of 1.4 vs 2 points and raising a score of 4.4 vs 4.1 at MITOX onset, respectively. Gadolinium enhancing (GD+) lesions were present in 7/11 IFN non responders and 40/50 DMT naive patients. One year after MITOX onset, clinical and radiological benefits were similar in IFN non responders and DMT naive patients with an ARR of 0.27 vs 0.20 (reduction of 91% vs 93%); 80% vs 76% of relapse free patients; a mean EDSS improved of 1 point; 6/11 (55%) vs 28/50 (56%) of patients improved, 5/11 (45%) vs 19/50 (38%) of patients stabilised and MRI activity reduced by 86% vs 90%, respectively.

**Conclusions:** MITOX may provide a new treatment option in RRMS patients who experience sub-optimal response to INF beta

**Disclosure:** E Le Page has nothing to disclose.
subcutaneously with glatiramer acetate in a dose of 20 mg daily. Blood samples were taken before the onset and after 6 months of therapy. IL-18 serum levels from MS patients and controls (15 patients with tension headache) were measured by ELISA (R&D Systems, USA).

**Results:** IL-18 levels in sera of MS patients were significantly higher in comparison with control group. After 6 months of therapy with glatiramer acetate in MS patients a statistically significant decrease of IL-18 serum levels has been found (p<0.05).

**Conclusions:** The therapy of relapsing-remitting MS patients with glatiramer acetate is associated with a significant decrease of serum IL-18 levels. We show for the first time that glatiramer acetate may in vivo influence the production of IL-18 in relapsing-remitting MS patients.

Disclosure: J Losy has nothing to disclose.

**P340**

**THERAPEUTIC POTENTIAL OF STATINS IN RELAPSING-REMITTING MULTIPLE SCLEROSIS**

Markovic-Plese S, Powell AW, Cortez A, Vollmer TL

Neurology, Yale University School of Medicine, New Haven, Connecticut, USA

**Background:** Statins, the inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, are cholesterol-lowering agents extensively used in medical practice. Recent studies have reported on their multiple previously unrecognized immunomodulatory effects.

**Objectives:** To study in vitro immunomodulatory effects of Simvastatin on immunocompetent cells derived from patients with relapsing-remitting multiple sclerosis (RR MS).

**Methods:** Mechanisms of action of Simvastatin were studied on peripheral blood mononuclear cells (PBMCs) isolated from RR MS patients. We enrolled 12 patients that were untreated, age 18-60, EDSS 1-5.5, with at least one Gadolinium-enhancing lesion on the recent MRI scan. The effect of Simvastatin was measured on PBMCs stimulated with immobilized aCD3 and human recombinant IFN-g for 48 hours in a cholesterol-free media. A panel of surface markers was evaluated in untreated and Simvastatin-treated cultures on gated CD4+ and CD8+ lymphocytes, CD14+ monocytes, and CD19+ B-cells. IL-2, IFN-g, TNF-a and IL-4 cytokine production was measured in supernatants by ELISA. In order to simultaneously capture treatment-induced changes in the expression of multiple cytokine and apoptosis-related genes, untreated and treated PBMC cultures are assessed using gene arrays and cDNA microarrays. Cluster analysis was used to identify genes that show a significant change in expression.

**Results:** Simvastatin-treated monocytes exhibit a significant decrease in IFN-g inducible expression of MHC class II DR molecules (p<0.01). In contrast, the treatment did not affect constitutive DR expression on CD19+ B lymphocytes. In vitro treatment of aCD3-activated PBMCs with Simvastatin induced a decrease in TNF-a production (p<0.05). Production of the other Th1 type cytokines IL-2 and IFN-g was decreased, however the difference was not statistically significant.

**Conclusions:** Immunomodulatory effects of Simvastatin detected in this in vitro study will be utilized as immunological outcome measures in Phase II clinical trial of Simvastatin in RR MS patients.

Disclosure: S Markovic-Plese has nothing to disclose.

**P341**

**EXPRESSION OF CHEMOKINES AND CHEMOKINE RECEPTORS BY GLATIRAMER ACETATE-REACTIVE T-CELL LINES**

Neuhaus O*, Bartosik-Psiuk H*, Kiesieker BC, Wiendl H, Hartung HP

*Dept. of Neurology, Karl Franzens University, Graz, Austria; ‡Dept. of Neurology, Medical Academy, Lublin, PL, Poland; †Dept. of Neurology, Heinrich Heine University, Düsseldorf, D, Germany; ‡Dept. of Neurology, Eberhard Karls University, Tübingen, D, Germany

**Background:** The family of chemokines (chemoattractant cytokines) is characterized by their capacity to induce migration and activation of leukocytes.

**Methods:** The secretion of the chemokines RANTES (regulated upon activation, normal T-cell expressed and secreted cytokine) and IP-10 (interferon-gamma inducible protein 10) by GA-reactive TCL was investigated by ELISA. Surface expression of chemokine receptors was measured by FACS, transcription of chemokine receptor mRNA by real-time PCR. The TCL were selected using the split-well cloning technique, and the cytokine profile (TH1 versus TH2) was determined using intracellular FACS methods.

**Results:** RANTES was upregulated predominantly in TH2 rather than in TH1 GA-reactive TCL. IP-10 was secreted both by TH1 and TH2 TCL. The chemokine receptors CXCR3 and CXCR4 were expressed both on TH1 and TH2 TCL. CCr1, CCr2, CCr3, CCr6, CXCR2 and CXCR5 were not detectable at the protein level, whereas CCr5 mRNA was transcribed by TH1 TCL and not by TH2 TCL.

**Conclusions:** These findings suggest that RANTES and IP-10 are secreted by anti-inflammatory TH2 GA-reactive T cells and thus can be involved in chemooattraction of (i) other regulatory TH2 cells, (ii) pathogenic inflammatory cells in the CNS. Both pathways would increase the local bystander suppressive effect of TH2 cytokines secreted by the GA-reactive T cells. Expression of further chemokines and chemokine receptors by TH1 and TH2 GA-specific T cells and their putative role in the mechanisms of action of GA are currently investigated.

Disclosure: O Neuhaus has received grant support by Teva pharmaceutical industries. Funding: Supported by Teva pharmaceutical industries.

**P342**

**ACUTE MYELOID LEUKAEMIA (AML) INDUCED BY MITOXANTRONE**

Radu TD, Marc D, Herve V

Neurology, Nancy Central Hospital, Nancy, 81, France

**Background:** We encountered the case of a 48-year-old man with multiple sclerosis (MS) treated by Mitoxantrone who developed an AML.

**Methods:** The patient presented a progressive relapsing form of MS with onset at the age of 26 (right optic neuritis). Twelve years later (1992) he had presented three attacks with incomplete remission in one year. The EDSS was 5. A treatment by Cyclophosphamide 600 mg/m2 twelve months reduced EDSS to 4. Between 1992 and 1998 he had no new neurological signs. This silence period was followed by two attacks with severe brainstem signs (EDSS 6). The MRI performed at this time showed multiple supra- and infratentorial signals, with contrast enhancement. The patient refused Interferon. Six Mitoxantrone monthly cures were administered (120 mg). The treatment was well tolerated and showed no cardiotoxicity. The clinical and MRI evaluation revealed significant improvement (EDSS 4.5). The patient was lost until may 2001, when two other attacks occurred (mixed sensitive-motor signs in legs, left optic neuritis). The MRI showed the same lesions but contrast enhancement. He received Mitoxantrone intravenously (20mg) in April and August 2001. The blood samples were normal. However, the blood exams performed November 2001 showed 50200 leucocytes with 80% blasts, 6.1 g/l Haemoglobin, 36000 Thrombocytes. It exists a translocation (8; 21). The immunotyping of blasts revealed myeloid origin (CD 13, CD 33, CD 34+). An AML type I diagnosis is advanced. A treatment by AraCytine and Idarubicine dramatically reduced haematological signs, leading to complete remission.

**Results:**

**Conclusions:** This is the second case reported in France of acute leukaemia in MS patients treated by Mitoxantrone.

Disclosure: T Radu has nothing to disclose.
**P343**

**GLATIRAMER ACETATE AS AN IN VITRO TOOL TO FOLLOW THE LONG-TERM EFFECT OF IMMUNOMODULATORY THERAPIES ON T CELL RESPONSES IN PATIENTS WITH MULTIPLE SCLEROSIS**

Schmied MP, Reindl MP, Auff E, Vass K

*Neurology, University of Vienna, Austria; Neurology, University of Innsbruck, Innsbruck, Austria*

**Background:** Current immunomodulatory treatment regimes for multiple sclerosis (MS) are aiming to diminish proinflammatory T cell responses that are associated with inflammatory activity in brain lesions. In vitro data described antiproliferative effects as well as induction of TH2 T cell responses induced by immunomodulatory therapies with IFNb and GA. As an universal antigen GA induces in vitro antigen specific responses in T cells and can be used as a read out for changes of T cell reactivity induced by immunomodulatory therapies.

**Objectives:** To further investigate the immunomodulatory effects of glatiramer acetate (GA) and interferon-beta (IFNb) on in vitro antigen specific T cell responses against GA and myelin antigens.

**Methods:** Short term T cell lines against glatiramer acetate, human myelin basic protein (MBP) and myelin oligodendroglial glycoprotein (MOG) p 1-111 were generated from 16 multiple sclerosis patients before and in three months intervals over 12 months during treatment with GA (8) or IFNb (8). Split well assays were performed to measure antigen specific 3H-thymidine incorporation and the cytokines IFN-g, IL-13 and IL-5 were measured from supernatants by standard ELISA methods. Primary proliferative responses of peripheral blood leukocytes against GA and tetanus toxoid were followed.

**Results:** GA specific T cell lines were established from peripheral blood leukocytes from all MS patients and control subjects. In accordance to earlier in vitro studies during the treatment with GA T cell lines showed a treatment related decrease in their in vitro proliferative response and IFN-g secretion with an increased T cell susceptibility to apoptosis and with clinical response to treatment. We could detect a transient decrease in the in vitro reactivity of GA specific T cell lines from IFNb treated patients with no clear shift to a TH2 cytokine response. In those patients who showed reactivity to myelin antigens the frequency of antigen specific T cell lines appeared to be influenced with a trend to a more sustained effect during treatment with GA.

**Conclusions:** In vitro T cell reactivity against GA appears to reflect the effect of immunomodulatory therapies on T cell responses in patients with MS.

**Disclosure:** M Schmied has nothing to disclose.

**Funding:** Aventis Pharma.

**P344**

**REDUCED EXPRESSION OF THE INHIBITOR OF APOPTOSIS PROTEINS IN T CELLS FROM PATIENTS WITH MULTIPLE SCLEROSIS FOLLOWING INTERFERON-BETA THERAPY**

Semra YK, Sharief MK

*Dept of Neuroimmunology, GKT School of Medicine, Guys Hospital, London, United Kingdom*

**Background:** Treatment with interferon-beta reduces clinical exacerbations in MS through several immunomodulatory mechanisms that involve the downregulation of cellular IAP proteins expression. The recently identified family of inhibitor of apoptosis (IAP) proteins is a potent regulator of cell death. The expression of IAP-1, IAP-2, and X-linked IAP (XIAP) is upregulated in mitogen stimulated T lymphocytes from MS patients, and this expression correlates with MS disease activity.

**Objectives:** To evaluate the effect of interferon-beta on cellular expression of IAP proteins and other apoptosis regulatory molecules.

**Methods:** In a prospective study, we evaluated the expression of IAP proteins, the anti-apoptosis Bcl-2 protein, and the death receptor Fas in in vitro stimulated T lymphocytes from MS patients, before and serially after treatment with interferon-beta. We also investigated the long-term effects of interferon-beta on cellular expression of these proteins and T lymphocyte apoptosis in a cross-sectional study of MS patients receiving drug therapy for a mean of 4.8 years.

**Results:** Treatment with interferon-beta reduced the expression of IAP-1, IAP-2 and XIAP in stimulated T lymphocytes. This reduced expression correlated with increased T cell susceptibility to apoptosis and with clinical response to treatment. In contrast, interferon-b therapy did not alter cellular expression of Bcl-2 protein or the death receptor Fas. This downregulatory effect of interferon-beta on cellular expression of IAP proteins was maintained following long-term therapy.

**Conclusions:** Our findings suggest that interferon-beta therapy exerts a regulatory effect on peripheral T lymphocytes through an anti-apoptosis mechanism that involves the downregulation of cellular IAP proteins expression.

**Disclosure:** MK Sharief has received honoraria from Serono International.

**P345**

**THE EFFECT OF LIVER TRANSPLANT COMBINATION IMMUNOSUPPRESSION ON MULTIPLE SCLEROSIS AFTER 18 MONTHS**

Vorobeychik GP, Yoshida E, Proust A

*Neurology, UBC, Vancouver, British Columbia, Canada; Gastroenterology, UBC, Vancouver, British Columbia, Canada*

**Background:** To date no patient with multiple sclerosis (MS) and liver transplantation has been reported in literature and there is little published experience with immunosuppressive medications used.

**Objectives:** To describe the course of MS in a patient who was treated with tacrolimus, mycophenolate mofetil and tapering prednisone after liver transplantation for acute liver failure.

**Methods:** Case report and literature review.

**Results:** The patient is a 60 y.o. female with onset of relapsing-remitting MS at age of 57. She has 3 relapses in the first year after initial presentation, all including cerebellar and brainstem dysfunction, and reached Extended Disability Score (EDSS) 5.0. Her MRI and CSF analysis were compatible with MS. She developed fulminant hepatitis and required liver transplantation 2 months after she was started on 11 µg of interferon β-1a. Her post-transplant immunosuppression consisted of delayed, lower dose tacrolimus secondary to hepato-renal syndrome, mycophenolate mofetil and tapering prednisone. Her postoperative period was complicated by CMV infection (transmitted from donor) and acute rejection reaction. She has not had any relapses during the 18 months since transplantation and her MS is symptomatically improved compared to pretransplant period. Her EDSS decreased from 5.0 to 2.5 and the burden of disease (BOD) on MRI remains unchanged (143 mm² before treatment with interferon β-1a to 113 mm² 18 months later). She is currently maintained on low dose tacrolimus monotherapy with normal graft and renal function.

**Conclusions:** The post-transplant experience in MS is largely unknown. Our experience suggests that post-transplant immunosuppressive therapy with low dose tacrolimus, mycophenolate mofetil and tapering prednisone may be associated with excellent clinical outcome with little drug toxicity.

**Disclosure:** G Vorobeychik has nothing to disclose.
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Multiple Sclerosis
LB1
VALIDATION OF DIAGNOSTIC MRI CRITERIA FOR MS AND RESPONSE TO TREATMENT WITH INTERFERON-BETA-1A
Barkhof F, Rocca M, Francis G, van Waeberghe J, Uitdehaag BM, Hommes O, Hartung H, Durelli L, Edan G, Fernández O, Seeliger F, Sorensen P, Margrie S, Comi G, Filippi M, aMS-MRI centre, VU medical centre, Amsterdam, Europe, Netherlands; bIRCCS San Raffaele, Milan, Italy; cSenora Laboratoires, Rockland, Massachusetts, USA; dEuropean Charcot Foundation, Nijmegen, Netherlands; eKarls-Franzens Universität, Graz, Austria; fUniversity of Turin, Turin, Italy; gUniversité de Rennes, Rennes, France; hHospital Carlos Haya, Malaga, Spain; iC.H.U. de Charleroi, and Hôpital Erasme, Brussels, Belgium; jRighihostaople, Copenhagen, Denmark; kQuintiles Pty Limited, Sydney, Australia

Background: In the recently adopted diagnostic criteria for MS by Mc Donald, the modified criteria of Barkhof have been adopted.

Objectives: To prospectively test the validity of the modified Barkhof criteria and their predictive value for IFN-β1-a1a treatment response in the ETO study.

Methods: The ETO MS study was a randomised, double-blind, placebo-controlled study of IFN-β1-a1a (i.m.) once weekly in 309 patients with a first episode consistent with demyelinating disease. Baseline MRI was assessed for the presence of gadolinium-enhancement (or 9 T2 lesions), juxtacortical, infratentorial, and 3 periventricular lesions. Conversion to CDMS was used as the outcome parameter.

Results: Conversion to CDMS occurred in 41% of patients with gadolinium-enhancement during the first 2 years of follow-up, 21% of those without (p = 0.017); similar comparisons were 44% vs. 31% for juxtacortical (p = 0.026), 40% vs. 35% for juxtacortical (p = 0.413), and 41% vs. 17% for more than 3 periventricular lesions (p = 0.034). For the cumulative number of modified Barkhof criteria, the rate of conversion to CDMS was 25% for 1 abnormal criterion, rising to 47% with 4 abnormal criteria. For a cut-off of 3 positive criteria, the hazard ratio for time to CDMS, and suggests that treatment with IFN-β1-a is more cost-effective in patients with more abnormal criteria.

Conclusions: This study confirms the validity of the modified Barkhof criteria for conversion to CDMS, and suggests that treatment with IFN-β1-a is more cost-effective in patients with more abnormal criteria.

Disclosure: Most authors were consultants to Serona

Funding: Supported by the European Charcot Foundation and Serona

LB2
ANTI-MOG ANTIBODIES PREDICT EARLY CONVERSION TO CLINICALLY DEFINITE MS IN PATIENTS WITH A FIRST DEMYELINATING EVENT
Berger T, Rubner P, Schautzer F, Egg R, U Immer H, Mayringer I, D iltz E, Deisenhammer P, Reindl M, aNeurology, University of Innsbruck, Innsbruck, Austria; bNeurology, County Hospital, Villach, Austria; cBiostatistics, University of Innsbruck, Innsbruck, Austria

Background: 90% of MS patients present at onset with a clinically isolated syndrome (CIS). Although up to 80% of these patients will convert to clinically definite MS (CDMS), the further MS disease course is unpredictable at onset for individual patients.

Objectives: N ew neurological findings, e.g. antibody-mediated demyelination, and the concept of epitope spreading in the early disease phase, prompted us to investigate whether the presence of serum anti-MOG and anti-MBP antibodies (abs) in patients with CIS predicts the further disease course.

Methods: 103 consecutive patients with a CIS, confirmed by MRI and positive oligoclonal bands in CSF, were included and followed for at least 12 months. Anti-MOG and anti-MBP abs were measured as previously described (Reindl et al, 1999).

Results: 73 females and 30 males (mean age at disease onset: 32.0 years; mean disease duration: 50.9 months, range 12–96 months). 22 patients (21%) had serum abs against MOG and MBP 42 (41%) were serocontrols for anti-MOG abs only and 39 (38%) were seronegative. Relapses occurred in only 9 (23%) seronegative patients, but in 95% of patients with abs against MOG and MBP. Serocontrols had their first relapse after a mean relapse free interval of 45.1 months (range 25–83 months). In contrast, patients with initial seronegativity for anti-MOG and anti-MBP abs developed their first relapse after only 7.5 months (range 1–18 months, P<0.001). Quantitation of MRI showed higher mean numbers of T2 and Gd-enhancing T1 lesions in patients with anti-MOG and anti-MBP abs compared to serocontrols. However, the number of MRI lesions varied in individual patients, irrespective of their antibody status, from 2 to 9 T2 lesions and 0 to 4 Gd-enhancing T1 lesions.

Conclusions: Analysis of antibodies against MOG and MBP in patients with CIS represent a rapid, inexpensive and precise method to identify patients with either a high or low risk for early conversion to CDMS. This may have implications for counseling and management in patients with a first demyelinating event suggestive of MS.

Disclosure: T Berger has nothing to disclose.

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LB3
NEUROREHABILITATION IN MULTIPLE SCLEROSIS CONTRIBUTES TO FUNCTIONAL RECOVERY ACCOMPANIED BY CHANGES OF BRAIN ACTIVITY ON fMRI—PRELIMINARY RESULTS.
Rasova K, Krasensky J, Havrdova E, O benberger J, Zalisova M, Seidl Z, Department of Neurology, Charles University

Background: Although MS is an inflammatory demyelinating disease, which can lead to the axonal injury and loss, neurorehabilitation may contribute to functional recovery accompanied by the changes in brain activity.

Objectives: To show changes in brain activity on fMRI and their correlation with functional recovery.

Methods: 18 outpatients with MS were evaluated before and after individualized neurorehabilitation treatment (two sessions per week, 30 weeks) for impairment (EDSS), disability (BI), handicap (ESS), quality of life (MSQoL), and amplitude of signal in the primary sensorimotor cortex (ASPSMC) using serial fMRI during the performance repetitive index-thumb opposition.

Results: There were 6 men and 12 women, EDSS was 4.19, age 41.11 yrs and illness duration 11.5 yrs. 8 patients had relapsing-remitting, 4 primary progressive and 6 secondary progressive MS. The therapy led to functional recovery and positively influenced the impairment (EDSS from 4.19 to 3.63: p<0.01), the disability (81 from 94.16 to 98.05: p<0.05), the handicap (ESS from 7.30 to 4.25: p<0.05) and quality of life (MSQoL from 152.5 to 161.33: trend shown). The functional recovery was accompanied by changes in ASPSMC. There was a trend towards a decreased ASPSMC after therapy (ASPSMC for right hand from 7.82 to 7.20% for left hand from 8.16 to 7.71%). We found two different responses to therapy in two thirds of patients the ASPSMC decreased, while in one third of patients it increased. We have found no relationship between functional recovery and changes in the brain activity. It was shown that when ASPSMC in left hand changed, it changed in right hand as well during the performance of the paradigm (correlation coefficient 0.56). The therapy was not aimed at improving function of the hands, but control of the whole body. Nevertheless, the function of the hands and ASPSMC during the performance of the paradigm changed. It seems that neurorehabilitation influences the function of the whole brain.

Conclusions: There is very little scientific basis for the therapy that is designed to help damaged brain circuits recover. These preliminary results show that neurorehabilitation in MS contributes to functional recovery and can be accompanied by changes of brain activity.

Disclosure: K Rasova has nothing to disclose.

LB4
TIGHT JUNCTION ABNORMALITY IN MS AFFECTS ALL CalIBRES OF VESSEL AND CORRELATES WITH LESION ACTIVITY.
Kirk J, Plumb J, Mirakhur M, O Cqaid S, aNeurology, Queen’s University, Belfast, Northern Ireland, United Kingdom; bNeurology, Royal Victoria Hospital, Belfast, Northern Ireland, United Kingdom

Background: Increased blood-brain barrier (BBB) permeability observed in MS has been linked to pathological change in the tight junctions (TJ) and vesicular transport of vascular endothelium.

Objectives: This study quantifies the pathological changes in TJs which we have recently reported in MS, including their uneven distribution and the relation between abnormal TJ and BBB leakage.

Methods: Frozen sections from plaque and normal appearing white matter (NAW M) in 14 post-mortem(PM) cases of MS were studied together with white matter from 6 neurological and 5 normal controls. Using single and double immunofluorescence and confocal microscopy the TJ-associated proteins zonula occludens-1 (ZO-1) and occludin were examined across lesion types and tissue categories, and in relation to fibrogenic leakage. Confocal image datasets were analysed for 2198 MS and 1062 control vessels.

Results: Significant differences in the extent of TJ abnormalities (i.e. beading, interruption, absence or redistribution of fluorescence signal, separation or opening of junctions) were detected between the different lesional types in MS and between MS and control white matter. They were frequent in oil-red O (OR O) ‘active’ plaques, affecting 42.5% of vessels, but less frequent in OR O ‘inactive’ plaques (22.8%) or NAW M (13.1%) and both normal (3.9%) and neurological controls (9.5%). A similar pattern was found irrespective of the size of vessels examined. In both NAW M and inactive lesions, dual-labelling showed that those with the most TJ abnormality had the greatest fibrogenic leakage. This was most apparent in active lesions where 41% of vessels showed severe leakage.

Conclusions: TJ abnormality affects vessels of all sizes, suggesting a diffuseable chemical (cytokine) cause. It occurs in lesional and non-lesional white matter, being most severe where there is evidence of active demyelination. Disruption of TJs, affecting both paracellular and transcellular pathways probably contributes to the BBB leakage detected in this study. The finding of TJ abnormality and BBB leakage in ‘inactive’ lesions points to a failure of effective and complete TJ repair or to the continuation of a pathological process. In NAW M it suggests either pre-lesional change or white matter damage secondary to remote lesions.

Disclosure: J Kirk has nothing to disclose.

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Funding: J Kirk has nothing to disclose.

The entire text has been transcribed and formatted into a natural language representation, preserving the structure and content of the document as accurately as possible. If there were any specific formatting styles or unusual characters, they have been preserved to maintain the integrity of the original document. The text is presented in a readable format, suitable for further analysis or citation, while maintaining the integrity of the original content.
LB5

SINGLE CENTRE, DBPC, RANDOMISED TRIAL OF INTERFERON β-1B IN PRIMARY PROGRESSIVE AND TRANSITIONAL PROGRESSIVE MULTIPLE SCLEROSIS: AN EXPLORATORY PHASE II STUDY.


-<Clinical Neuroimmunology Unit, Hospital Universitari Vall d, Barcelona, Spain; *Magnetic Resonance Unit-IDI, Hospital Universitari Vall d, Barcelona, Spain

Background: Beneficial effects of interferon β-1b have been shown only for patients in the relapsing-remitting phase of MS as its role in the treatment of SPMS patients still remains controversial. The single phase II randomized controlled trial on PPMS using IFN β-1a (M) shows no significant treatment effect on EDSS though some effect on T2 lesion load.

Objectives: To investigate safety and efficacy hints of interferon 1b given to patients with primary and transitional progressive multiple sclerosis (PPMS and TPMS).

Methods: 13 patients (49 PPMS, 24 TPMS) with EDSS scores of 3.0 to 7.0, were randomized to receive 8 million IU of IFN β-1b or placebo every other day, subcutaneously for 2 years. Safety parameters including the Ashworth spasticity, Krupp fatigue and Depression inventory scales and blood tests were performed three monthly. Clinical outcomes (EDSS and MS Functional Composite – MSCF) were also performed three monthly and the Sickness Impact Profile six monthly. MRI measures (T2 and T1-weighted brain lesion load, brain parenchymal fraction, active lesions, spinal cord atrophy, MTR and spectroscopy) and neuro-psychological assessment (BRN B) were done annually.

Results: Adverse events significantly associated with IFN β-1b included injection-site reaction, flu-like symptoms and lymphopenia. No patient on placebo died of pulmonary infection. In all, 96% of the patients reached study end and 93% completed the treatment period. Treatment groups were comparable on all baseline variables. The proportion of patients with confirmed progression measured by EDSS at 3 months was 27.8% in the IFN arm and 37.8% in the placebo arm (p = 0.3135). Statistically significant differences were found for MSCF (PA-SAT 3”, 9-HPT and A1 (p = 0.03), T2 (p = 0.006) and T1 (p = 0.001) lesion load and number of active lesions (p = 0.005) in favor of the IFN-treated group.

Conclusions: IFN β-1b is safe in treating patients with PPMS and TPMS. Our study seems to be the first indicating a beneficial effect of IFN β-1b in these patients.

Disclosure: X Montalban has nothing to disclose.

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LB6

SUCCESSFUL TREATMENT WITH IFN-β1B IN RR MS PATIENTS IS ASSOCIATED WITH AN INCREASE IN THE NUMBER OF IL-10 PRODUCING (REGULATORY) CD4+ T CELLS.

van Boeschoten-Dezaire A.*, Smits M*, Uitdehaag B*, Polman C*, Naegelerken L.

-Division of Immunological and Infectious Diseases, TNO Prevention and Health, Leiden, Netherlands; *Department of Neurology,Vrije Universiteit Medical Center, Amsterdam, Netherlands

Background: Although IFN- β-1b is now widely used for treatment of MS, its mode of action still remains unclear; recent studies do not support a shift in the Th1/Th2 balance. In vitro studies show that type 1 interferons induce the differentiation of Tregulatory-1 (Tr1) cells and facilitate international standardization.

Methods: Based on EDSS-progression and the number of relapses and steroid interventions in the 2 years before initiation of IFN-β1b treatment compared with those in the 2 years after initiation of treatment, 24 RR MS patients were classified as responders (15) and non-responders (9). Using intracellular cytokine staining techniques, the effect of IFN-β1b after 0, 3 and 6 months of treatment was studied on the number of IL-10 producing CD8+ T cells, CD4+ (CD25+) T cells and monocytes.

Results: N numbers of IL-10 producing CD4+ T cells were significantly decreased prior to treatment. Remarkably, after 3 and 6 months of treatment a significant increase in the number of such T cells could be found in the clinical responders. In contrast, treatment decreased numbers of IL-10 producing monocytes in both responders and nonresponders and did not affect numbers of CD8+ T cells that produced IL-10. In a subgroup of the responders (7 out of 15), the effect of IFN-β1b treatment was also studied on CD4+CD25+ T cells. Notably, a significant increase in the number of IL-10 producing CD4+CD25+ T cells could be observed after 6 months of treatment.

Conclusions: Enhancement of the number of CD4+CD25+ T cells that produce IL-10 may be an important mechanism in the therapeutic effect of IFN- β-1b in RR MS.

Disclosure: A van Boeschoten-Dezaire has nothing to disclose.

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