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Poster Sessions 2

Imaging-2

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Prospective multi-modal MRI study to examine the effect of natalizumab on tissue injury in the brain and spinal cord in patients with RRMS

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Background: Natalizumab, a monoclonal antibody targeting a4b1, is considered to be a highly effective therapy in RRMS. The efficacy of NTZ is related to its profound effect on reducing lymphocyte trafficking into the CNS. However, its effect on tissue injury in the CNS and axonal metabolic function is not well understood.

Objectives: To determine the effect of natalizumab (NTZ) on brain tissue injury and cervical cord volume in a multi-modal advanced MRI study.

Methods: A four-year, prospective, open-label study was conducted in RRMS patients initiating therapy with NTZ, who were previously naïve to NTZ therapy. Multi-modal advanced brain MRI including 3D-T1W, DTI, MTR, multi-voxel ^{1}H-MRS, and cervical cord MRI were obtained at baseline and annually thereafter. Focal pathology in the brain was also tracked longitudinally by following 3 to 5 lesions per brain. Age-matched healthy controls (HC) were also imaged annually. We are presenting the two-year interim analysis of this ongoing four-year study.

Results: Twenty-five patients with RRMS initiating therapy with NTZ participated. At year 2 mean *t*NAA/*t*Cr improved from 1.98 to 2.10 (p=0.003). Compared to baseline and HC, there was no significant loss of cortical surface volume or thalamic volume at year 2. Focal T2 lesion pathology followed longitudinally (102 non-enhancing lesions) showed mean MTR improve from 44.37% to 46.16% (p=0.0006); mean FA from 0.247 to 0.290 (p=0.001); voxel-wise MTR showed 88.4% of the lesions to be stable over 2 years. T2 lesion volume and T1 lesion volume were significantly reduced. Brain volume change (PBVC) was -0.81% from baseline to year 1 and -0.53% from year 1 to year 2. Cervical cord cross-sectional area (CSA) measured at C2 level changed from 75.76 mm^2 to 74.45 mm^2 (p=0.16). There was no significant different when comparing the net change in HC.

Conclusions: This study demonstrates the effect of NTZ on preserving axonal metabolic function, as well as improving focal pathology indicated by improving MTR and FA. There was no significant loss of thalamic or cortical surface volume, when comparing to HC. These results indicate that besides a profound effect of NTZ at the blood-brain barrier level, there may be additional effects on tissue injury and axonal metabolic function in the CNS. Further longitudinal follow-up with annual MRI scans is ongoing to investigate the anti-inflammatory and potential neuroprotective effects of NTZ in the CNS.

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Corpus callosal myelin water fraction and transcallosal inhibition in multiple sclerosis

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Background: Magnetic resonance imaging (MRI) techniques assessing T_2 relaxation can measure myelin water fraction (MWF), a marker for central nervous system demyelination in multiple sclerosis (MS). Transcallosal inhibition (TCI), elicited by transcranial magnetic stimulation (TMS), reflects functional integrity of fibres in the corpus callosum (CC).

Objectives: To characterize the relationship between MWF in the CC and TCI assessed by TMS-evoked potentials in MS subjects on glatiramer acetate. This relationship may guide the use of MWF for evaluating therapies that induce remyelination or have neuroprotective effects, and improve the design of clinical trials for more efficient development of new treatments.

Methods: Twenty-six relapsing-remitting MS patients (5M/21F, mean age 42.3: range 28-59y, EDSS range 1.0-6.0, disease duration range 1-35y) and 10 controls (2M/8F, mean age 43.4y) underwent both MRI and TMS testing. The MRI protocol included a 32 echo T_2 relaxation GRASE sequence (TR=1000 ms, 10 ms echo spacing, 20-5 mm slices). The T_2 signal was modelled via multiple exponential components and the MWF was computed as the ratio of the short T_2 component to the total area. The CC was segmented anteroposteriorly into five regions and mean MWF was calculated for each. TMS was performed using a figure-of-eight coil delivering focal stimulation over the primary motor cortex representation of the forearm extensor musculature. Single pulses were delivered ipsilaterally to elicit a transient suppression in the electromyographic activity of the forearm during a voluntary isometric grip contraction (50% max). The onset latency, duration and depth of this suppression were used to quantify levels of TCI.

Results: TCI duration was significantly longer in MS subjects (36.0 ± 5.2 ms) than in controls (25.6 ± 8.2 ms, $p = 0.016$). MWF was 35% higher in the posterior than in the anterior CC ($p < 0.0001$). Median MWF was lower in MS subjects in all five regions, though without statistical significance. In MS subjects, the log transformed TCI depth was negatively correlated with the MWF of the posterior CC midbody ($r = -0.73$, $p = 0.039$), a region previously shown to be critical in TCI transmission.

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Conclusions: The relationship between MWF in the CC and TCI assessed by TMS-evoked potentials in MS subjects improve understanding of the structure and function of myelin in MS. MWF and TMS measures may provide biomarkers of demyelination and disease progression.

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White matter and long-tract lesions play a marginal role in determining cortical atrophy

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Background: To what extent retrograde axonal degeneration induces cortical atrophy in white matter diseases is still debated. Neuroimaging studies of the sensory-motor cortex in patients with severe tract pathology, such as traumatic spinal cord injury (SCI) and neuromyelitis optica (NMO) with extensive spinal cord lesions, gave conflicting results.
Objectives: To analyse cortical atrophy in patients with severe lesions of the sensory-motor tracts (SCI and NMO), inflammatory tumour-like lesions (TLL) and relapsing remitting multiple sclerosis (RRMS) with or without spinal cord lesions.
Methods: Fifteen patients with severe SCI (mean follow-up period: 5.8 years), 10 patients affected from NMO with extensive inflammatory spinal cord lesions (mean disease duration: 6.2 years), 5 patients with TLL and 30 patients with MS (15 with and 15 without spinal cord lesions) were included in the study. MRI examination included T2, 3D T1, FLAIR, double inversion recovery (DIR) and diffusion tensor imaging (DTI) sequences. Cortical atrophy was analysed by Freesurfer.
Results: A significant thinning of sensory-motor cortex, unrelated to subcortical T2 lesion load and the presence of spinal cord lesions, was observed in RRMS ($p < 0.001$), while it was modest in SCI (p ranging from 0.08 to < 0.01) and NMO (p ranging from 0.04 to 0.02). Global and regional cortical thickness was significantly decreased in RRMS compared to both NMO and SCI ($p < 0.001$ for all comparisons). In TLL, cortical atrophy was modest in the cortex over the lesions compared to that of the contralateral hemisphere ($p=0.02$). Fractional anisotropy was significantly decreased in the cortex of RRMS while it was normal in SCI, NMO, and TTL.
Conclusions: Whether cortical atrophy in MS is the results of retrograde axonal degeneration or is mainly related to a direct (local) damage is still debated. We found that severe long-trait damage induces only a modest cortical thinning in the sensory-motor cortex, while in RRMS cortical atrophy was relevant even in patients without spinal cord lesions and with low T2 lesion load. Thus, retrograde degeneration of axons seems to plays only a marginal role in determining cortical thinning in MS. A role for a local pathological process, determining neuron loss and cortical atrophy, is also supported by the finding that FA of the cortex was normal in SCI, NMO and TLL, and significantly decreased in RRMS.

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Characteristic of orbit magnetic resonance imaging in neuromyelitis optica and multiple sclerosis patients presenting with optic neuritis

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Background: Patients with neuromyelitis optica (NMO) and multiple sclerosis (MS) both can present with optic neuritis (ON). There have been several reports on neuroimaging findings of ON in NMO and MS, but the results have been contradictory. Previous studies reported patients with chiasmal involvement developed clinically definite MS, whereas recent studies suggested there was a trend toward more posterior location within the anterior visual pathways in NMO-associated ON.
Objectives: We aimed to determine whether there were magnetic resonance imaging (MRI) characteristics of the anterior visual pathways in acute ON to distinguish NMO/NMO spectrum disorders (NMOSD) from MS.
Methods: We included 26 NMO/NMOSD patients (34 affected eyes) and 48 MS patients (71 affected eyes) who presented with ON. The NMO/NMOSD patients fulfilled the revised diagnostic criteria and showed seropositivity for NMO-IgG. MS patients met 2010 McDonald diagnostic criteria. We analyzed orbit MRIs obtained within 60 days of ON onset. The MRI parameters assessed were as follows: extent and location of affected optic pathway (retrobulbar, canalicular, intracranial, optic chiasm, and optic tract), severity of optic nerve swelling and enhancement, and presence of involvement of adjacent brain structures such as hypothalamus.
Results: The female percentage was 84.6% in the NMO/NMOSD group and 79% in the MS group. The mean age (SD) was 36 years (13.6) and 39 years (13.9) in NMO/NMOSD patients and MS patients, respectively. The number of patients with chiasmal involvement was significantly higher in the NMO/NMOSD group (n=8, 20.0%) than in the MS group (n=3, 3.8%) ($p=0.006$). The optic tract was more frequently affected in NMO/NMOSD patients (n=4, 10.0%) than in MS patients (n=1, 1.3%) ($p=0.042$). NMO-associated ON have more extensive lesion beyond the three segments of optic pathway. Hypothalamic involvement was exclusively seen in NMO-associated ON (n=3, $p=0.035$). There were no significant differences in the severity of swelling and enhancement.
Conclusions: Based on our results, NMO/NMOSD-associated ON shows more extensive lesions than MS-associated ON and involves more posterior parts within the anterior visual pathways. Concurrent involvement of hypothalamus is a unique feature in NMO/NMOSD-associated ON. These findings may be related to the distribution of sites of high AQP4 expression and help to distinguish ON in NMO/NMOSD from MS.

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High field spinal cord imaging in multiple sclerosis at 7 Tesla

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Background: Spinal cord is a major site of involvement in progressive MS. Imaging of the spinal cord is challenging due to its small size and location in a bony canal. Conventional MRI does not detect the diffuse damage occurring in grey matter and normal appearing white matter.

Objectives: Our purpose is to evaluate the sensitivity of 7T to detect SC lesions in MS patients compared to lower field (3T), clinically standard MRI evaluation. We also show the improvement in gray (GM) and white matter (WM) visibility allows for a discrimination of GM lesions, which are known to exist from histopathological studies, but have not been shown at 3T.
Methods: MR scans were performed at 7T (Philips Healthcare) with a novel 16-channel cervical spinal cord receive coil. High resolution T1- and T2*-weighted scans were acquired at 0.5x0.5x5mm3 resolution in 9 minutes with the following parameters: T1 - 3D FFE, TR/TE/flip angle = 30ms/4ms/60°, T2*-weighted - multi-slice FFE, TR/TE/flip angle = 305ms/9ms/25°. Conventional T2*-weighted turbo spin echo scans were obtained in the same patients at 3T using a standard clinical protocol. 7T scans were obtained in 15 healthy controls and 23 MS patients. The SNR and CNR were calculated.
Results: We were able to obtain T1, T2*, PD weighted axial sequences, as well as T2 w sagittal sequences, in under 15 minutes. On the other hand, the high SNR of 7T allows us to aim for high in-plane resolutions. While clinical resolution scans can be performed in 2 minutes, we were able to get twice the clinical resolution, 0.5 x 0.5 mm, in a 3 minute scan.WM SNR and WM-Lesion CNR were 2.1 and 1.8 times higher at 7T compared to 3T. In sagittal T2 scans, 4.5 +/- 1.5 lesions were detected at 7T compared to 2.5 +/- 2.2 at 3T. Spinal cord cross sectional area at C2/3 space was 75.34 +/- 9.36 sq mm in MS patients, compared to 83.87 +/- 4.87 sq mm in healthy controls.
Conclusions: In this first ever report of 7T MRI in large numbers of MS patients, we show significant gains in in-plane resolution (more accurate determination of cord atrophy) and lesion detection. Due to the higher SNR and CNR afforded at 7T, evaluation of the SC in MS shows a significant improvement in lesion visualization compared to the clinical standard at 3T. Finally, 7T SC MRI allows for more accurate assessment of the magnitude and extent of SC involvement in patients with MS, which may provide greater confidence in diagnosis and treatment monitoring.

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Multi-center upper cervical spinal cord areas obtained from brain MRI scans at 3T in patients with MS

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Background: Upper cervical cord area (UCCA) is strongly associated with physical disability in MS patients, particularly in advanced stages of disease. Many sites do not routinely acquire MRI that can provide reliable measures of UCCA. We performed a 3T MRI study using standard brain high-resolution 3D T1-weighted (T1W) volumes that included the upper cervical cord to provide estimates of UCCA.
Objectives: To compare the multi-site utility of UCCA determined from brain T1W MRI to direct spinal cord MRI-obtained UCCA in patients with MS.
Methods: Brain MPRAGE sequences (sagittal 1mm isotropic voxels) were acquired at the Brigham Women's Hospital (BWH, n=12) and UCSF (n=68), as part of the SUMMIT consortium. Both sites used the same Siemens Skyra 3T MRI, 20-channel head and neck coil, and pulse sequence. UCCA was determined using JIM software, independently at the 2 sites. UCCA was compared within each site with spinal cord specific sequences (T2W images at BWH and PSIR at UCSF). A subset at UCSF (n=12) was matched for sex, EDSS, and disease duration for comparison of MPRAGE-derived UCCAs and correlation with disability to evaluate multi-center data integration.
Results: BWH: N=12, 8 females, mean (SD): 17 (15) years disease duration, 3.0 (2.1) EDSS, 10 RRMS, 1 SPMS, 1 PPMS. UCSF: N=68, 44 females, 17 (11) years disease duration, 2.9 (1.8) EDSS, 50 RRMS, 15 SPMS, 3 PPMS. Brain MPRAGE UCCA were BWH: 72(7) mm2 and UCSF: 71(10) mm2. Strong correlations were found between the UCCA from MPRAGE and spinal cord specific sequences (Pearson BWH R2=0.63, UCSF R2=0.96). Correlation coefficients for EDSS predicted by UCCA from MPRAGE were BWH: Spearman r=-0.74 p=0.006; Pearson r=-0.66, -0.90: -0.14 CI and in the matched UCSF cohort: r=-0.73, p=0.007; Pearson r=-0.64, -0.89: -0.11 CI. Combining the datasets we found (N=24) Spearman r= -0.69, p<0.001; Pearson r=-0.63, -0.83: -0.31 CI). The correlations from the cord specific sequences were similar or lower. Statistical power (alpha=0.05) for the combined MPRAGE-UCCA data was 0.96 for N=24 while power for the individual sites were 0.71 and 0.67 for N=12 and predicted power at N=24 was 0.96 and 0.95.
Conclusions: Across sites, concordant UCCA values and correlations with EDSS were found. No loss of power due to combining data was observed. Such data potentially provide readily accessible UCCA estimates for integration in large multi-site trials and can provide an estimate of spinal cord atrophy without direct cord images.

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Identification of tissue-specific MRI markers to assess protection and repair in response to fingolimod

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Background: While the immunological effects of Fingolimod are well established, there is controversy regarding its role in promoting tissue repair. Advanced MRI techniques such as diffusion tensor (DTI) and diffusional kurtosis imaging (DKI) provide information about tissue microstructure. Using DKI, and a diffusion model of white matter (WM) is possible to derive indices of axonal degeneration (axonal water fraction) and de and re-myelination (tortuosity).
Objectives: Using a combined DKI and histological analysis to assess the impact of Fingolimod on WM and GM brain tissue repair in experimental models with minimal immunological component: Lysolecithin-induced de- and re-myelination and AdIl-1-induced GM injury.

Methods: WM demyelination was induced by stereotaxic injection of 1% lysolecithin into the corpus callosum (n=4 treated with Fingolimod (3mg/kg); n=4 treated with vehicle and n=4 controls injected with PBS). GM lesions were induced by stereotaxic injection of AdIL1 into the cortex (n=4 treated with Fingolimod; n=4 treated with vehicle). In-vivo brain MRI was performed on a 7.0 Tesla Bruker scanner. DKI data were acquired using EPI sequence with 30 gradient directions (b-values: 1.25 and 2.5ms/μm^2) at 7, 14 and 28dpi for lysolecithin-injected mice and 7, 14 and 21-dpi for AdIL1-injected mice. After MRI, mice were perfused with 4% PFA and brains were processed for cryostat embedding. 20μm sections were immunostained using standard protocols. Primary antibodies were used at 1:200 for Olig2 and CD11b. GFAP was used at 1:500. Samples were examined with a Leica Microsystems confocal microscope.
Results: At 7 dpi, ROI analysis showed no significant changes in WM integrity parameters in lysolecithin-injected mice treated with Fingolimod compared to PBS-injected control mice. Significant changes were observed in AdIl-1 injected mice when compared to control PBS-injected mice (p< 004). Immunohistochemistry showed an increase of Olig2+, Cd11b, and GFAP cells in lysolecithin-injected brains at 7dpi whereas a decrease in all these cell types and in NeuN was detected in AdIL-1-injected brains.
Conclusions: Our preliminary results suggest that Fingolimod might exert a protective effect thus reducing the severity of tissue brain damage 7 dpi after injections of lysolecithin or AdIL-1

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Spinal cord and brain atrophy in neuromyelitis optica: a comparative study with MS and healthy controls

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Background: Both spinal cord and brain atrophy in neuromyelitis optica (NMO) was reported. However how is the relationship between brain and spinal cord pathology in NMO compared with MS, and which one can sever as a biomarker for correlating with clinical disability and for clinical trials is unclear.
Objectives: To investigate the spinal cord and brain volume in NMO, and its relationship with other MRI measurements and clinical disability, compared with well-matched multiple sclerosis (MS) patients and healthy controls (HC).
Methods: We recruited 35 NMO patients, 35 MS patients and 35 healthy controls (HC) with both spinal cord and brain images at 3 Tesla MRI. Mean upper cervical cord cross-sectional area (MUCCA), brain parenchymal (BPF), grey (GMF) and white matter fraction (WMF), spinal cord and brain lesion loads were measured and compared among groups. Multivariate associations between spinal cord and brain volume measurement and clinical variables were assessed by partial correlation and multiple linear regression model.
Results: Both NMO (0.73±0.08cm^2) and MS (0.75±0.09 cm^2) showed smaller MUCCA than HC (0.79±0.07 cm^2) (p< 0.001), while no significant difference was identified between MS and NMO. The NMO patients showed lower BPF and WMF than HC, however with no significant difference in GMF. MS patients had lower BPF and GMF than NMO patients. MUCCA was correlated with total lesion length (r=-0.55, p=0.001), a higher number of relapses (r=-0.435, p=0.011) and EDSS (r=-0.75, p< 0.001) in NMO, while in MS MUCCA was correlated with WMF (r=0.450, p=0.009) and EDSS (r=-0.438, p=0.011). MUCCA was the only independent variable for predicting clinical disability measured by EDSS in NMO (R^2=0.22, p< 0.001) and MS (R^2=0.16 p=0.019).
Conclusions: NMO showed predominately spinal cord atrophy with mild brain atrophy mainly in WM, while MS demonstrated much more severe brain atrophy especially in GM. MUCCA is the essential MRI-derived parameter for explaining clinical disability in NMO and MS, and may serve as a potential biomarker for further clinical trials especially in NMO.

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MR frequency shift imaging as a sensitive measure of longitudinal changes in multiple sclerosis lesions

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Background: Multiple sclerosis (MS) is characterized by the appearance of focal and diffuse lesions in the brain and spinal cord due to demyelination, episodic inflammation and axonal damage. Magnetic resonance (MR) frequency images contain quantitative information related to tissue magnetic susceptibility and microstructure.
Objectives: As shown in a previous serial study (Wiggermann et al., Neurology 81, 2013), MR frequency increases sharply when new lesions appear and remains elevated for at least 6 months. Here, we present long-term follow up (LTF) data from the previous short-term study cohort. We hypothesize lesions will demonstrate a reduction in the MR frequency signal due to axonal destruction and axonal loss, in agreement with theoretical predictions.
Methods: 8 subjects with relapsing-remitting MS (at LTF: mean age=44.5yr (range: 34-57yr), median EDSS=2.5 (range: 1-4.5), mean disease duration=15.4yr (range: 7-30yr)) were scanned monthly over 6 months and received one LTF scan after 3.2-5.6 years on a 3T Philips Achieva system. 3 healthy controls (age=38-48yr) were scanned at months 0, 6 and LTF. FLAIR (Fluid Attenuated Inversion Recovery) and Gd-enhanced T_1w images were acquired for lesion detection. Frequency shift images were acquired using a 3D single Gradient Echo sequence (FOV=240x166x64mm^3,voxel size =0.43x0.43x1mm^3, TR/TE=40/20ms) and registered using FSL´s FLIRT. Regions of Interest were manually defined on MR frequency images for enhancing lesions, normal- appearing white matter (NAWM) and normal WM (NWM) in controls. Statistical analysis was performed using a linear mixed effect model.

Results: Visually, new MS lesions seen at months 0-6 nearly disappeared on MR frequency shift images at LTF. After the initial frequency increase at month 0-6, the frequency decreased over time while control regions remained constant (NAWM in subjects, p=0.3; and NWM in controls, p=0.8). The decrease in frequency of new lesions at LTF was significant compared to months 2-6, which showed elevated frequency shortly after first appearance (p=0.004).
Conclusions: Our data is in good agreement with theoretical predictions (Yablonskiy et al., PNAS 109, 2012) of frequency shifts in MS lesions. The preliminary results demonstrate the sensitivity of frequency shifts to measure microstructural changes in MS lesions at high spatial resolution, which occur due to the destruction of the myelin sheath and axonal damage. Remyelination could also explain the observed decrease in frequency at long-term follow up.

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Distinction between neuromyelitis optica and multiple sclerosis using multi-voxel pattern classification

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Background: Neuromyelitis optica (NMO) is often the alternative diagnosis in patients misdiagnosed with multiple sclerosis (MS), because patients with both diseases share some similarities. NMO patients show absence of demyelination and lack of cortical lesions, but presence of cortical thinning in the visual and motor cortices, suggesting that imaging measures related to grey matter (GM) may distinguish them from MS.
Objectives: To automatically classify MRI scans in either MS or NMO based on pattern of GM changes.
Methods: We used multi-voxel pattern analysis (MVPA) which is performed in 2 steps: first it learns the differences in GM intensity between the 2 groups using a training set, and then it classifies previously unseen scans. We included 97 subjects from 2 centers:

(i) From Tehran,Iran: 25 MS patients [mean disease duration (DD)=8.0 yrs and age=32.8yrs, median EDSS=2.5] and 30 NMO [mean DD=6.1yrs and age=33.6, median EDSS=3.0] and
(ii) From Padova, Italy: 24 MS [mean DD=8.1yrs and age=36yrs, median EDSS=4.0] and 21 NMO [mean DD=7.5yrs and age=42.5yrs, median EDSS=4.5]. MR protocols were performed using a 3T in Tehran and 1.5T scanner in Padova. They included high-resolution T1-weighted scans, FLAIR, and T2-weighted scans.

To extract GM probability maps,we performed the following pre-processing:

1) Binary lesion segmentation on FLAIR images
2) Filling of white matter T1 hypo-intense lesions
3) Construction of a study-specific template from all the T1 scans
4) GM segmentation in native space
5) Normalization of all subjects´ GM maps to study-specific template.

The normalized GM probability maps were assigned to either a training (n=49) or a validation set (n=48). Principal components analysis was used to limit the analysis to 4 components with the highest variance. We trained the model on training set and assessed its performance on validation group, with reiteration on 1000 bootstrap samples.
Results: Average accuracy (± standard deviation) of the model in classifying NMO vs MS was 84% (± 0.09) with GM maps, and 87% (± 0.10) when also including white matter lesion load. The most important brain regions whose GM probability contributed to the correct classification were the bilateral hippocampi, insula, orbitofrontal gyri and cerebellar cortices.
Conclusions: We showed that different pattern of GM changes between MS and NMO can be used to automatically classify patients. Our results have important implications for improving and facilitating the differential diagnosis between NMO and MS in the clinical practice.

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FLAIR* for the non-invasive histological diagnosis of MS

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Background: Current magnetic resonance imaging (MRI) criteria to support the diagnosis of multiple sclerosis (MS) are based on dissemination in time (DIT) and space (DIS) of central nervous system (CNS) white matter lesions (WML). However, application of these criteria is not always straightforward and may delay diagnosis in patients who do not develop the required number of WML. An alternative approach to support a diagnosis of MS using MRI may be to detect a histological feature characteristic of MS *in vivo*. One such feature, the ´central vein´ in WML, has become accessible through application of T_2* based techniques. FLAIR* is a post-processing algorithm combining T_2* with an established sequence for WML detection - fluid attenuated inversion recovery (FLAIR).
Objectives: To explore whether FLAIR* could be superior to current MRI DIS/DIT criteria with respect to making a diagnosis of MS using MRI datasets acquired at a single time point and at a standard MRI field strength (3T).

Methods: Seventeen people (11 men; age 39± 8.6 years; disease duration 7± 5.3 years) with relapsing MS (pwRMS) had MRI using a 3T system to acquire 3D FLAIR (after Gadolinium injection) 3D T_2 and T_2*. FLAIR* images were constructed using MIPAV (mipav.cit.nih.gov) and JIST (nitrc.org/projects/jist/) image processing software. FLAIR* images were assessed by two observers independently, unaware of patients´ clinical information. WML >3mm were identified, their location and the presence of a central hypointensity suggestive of a vein (central vein sign; CVS) recorded. The proportion of CVS positive (CVS+) WML was calculated for each patient. A proportion of >40% CVS+ WML was considered diagnostic for MS. The McNemar test was used to compare diagnoses based on a proportion of >40% CVS+ WML with diagnoses made using DIS/DIT criteria *applied at a single time point* on the same MRI datasets.
Results: In 17 pwRMS, 239 WML were identified. Inter-observer agreement for the presence of the CVS was good ($\kappa = 0.63$). 88% of WML were CVS+. All pwRMS met the diagnostic criterion (CVS+ in >40% of WML). All pwRMS met current MRI DIS criteria, but only 1/17 met DIT criteria using MRI acquired at a single time point.
Conclusions: FLAIR* reliably enables detection of a characteristic histological feature of MS WML, the central vein, *in vivo*. This may simplify the diagnosis of MS. Prospective studies in people with clinically isolated syndrome suggestive of demyelination are needed to confirm the diagnostic value of CVS detected using FLAIR*.

P502

A longitudinal study of spinal cord atrophy in progressive multiple sclerosis

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Background: Spinal cord atrophy is strongly associated with physical disability in multiple sclerosis (MS) and has previously been used as a secondary outcome measure in clinical trials in progressive MS. However, the routine implementation of this measure has been limited by technical constraints, particularly poor reproducibility and insensitivity to small changes. We have recently reported a reproducible method for measuring upper cervical cord cross-sectional area (UCCA) in MS that combines 3D phase sensitive inversion recovery (PSIR) imaging and an active surface model (ASM).
Objectives: To measure spinal cord atrophy by using this new methodology in a progressive MS cohort at one-year follow-up, and assessing its association with physical disability.
Methods: We recruited 31 progressive patients: 18 with secondary progressive (SP), 13 primary progressive (PP) MS and ten controls. Physical disability was estimated at baseline and one-year follow-up using the expanded disability status scale (EDSS). All subjects had 3T magnetic resonance imaging (MRI) of their cervical cord at both time points. The MRI protocol included a 3D-PSIR acquisition centred at C2/C3 with resolution of 0.5 x 0.5 x 3 mm^3 and UCCA was measured from these images using the ASM. To measure differences between MS and controls and changes from baseline to follow-up, unpaired and paired t-tests were used; univariate correlations between UCCA and EDSS were calculated using Spearman's rank correlation coefficient.
Results: At baseline, progressive MS subjects (PP and SPMS combined) had a smaller UCCA than controls (68.13mm^2 ± 10,89 vs. 83.21mm^2 ± 7,92, p = 0.0002). There was a significant progression of clinical disability in MS patients (p= 0.0001) and a significant decrease in UCCA in both patient groups over one year (decrease in PPMS: 1.44mm^2, 2.02%, SPMS: 1.33mm^2, 2.03%). A reduction of UCCA in healthy controls was not detected. Thirteen of the 31 patients had an increase in their EDSS during the 12-month period, and they exhibited a greater reduction in cord area during the year (decrease in UCCA: 2.08 mm^2 ± 4,16 vs 0.87 mm^2 ± 0,35; p = 0.023) compared with the 18 patients whose disability status was unchanged.
Conclusions: This newly developed method detects change in UCCA over the relatively short period of one year in both SP and PPMS patients. These results support the use of this imaging biomarker as a potential primary endpoint in future trials of neuroprotection in progressive MS.

P503

Development of gray matter atrophy is associated with disability progression in patients with CIS: a 4 year follow up study

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Background: Although multiple sclerosis (MS) was originally considered to be a disease affecting predominantly the white matter (WM), pathological changes of gray matter (GM) are increasingly recognized as an important determinant of neurological sustained disability progression (SDP) and increased relapse activity in MS patients.
Objectives: To investigate the association between the development of GM atrophy and clinical disease progression in patients with clinicaly isolated syndrome (CIS).
Methods: This prospective, observational, 4-year follow-up study examined 210 CIS patients treated with 30 µg of intramuscular interferon beta-1a once a week. MRI and clinical assessments were performed at baseline, 6, 12, 24, 36 and 48 months. Associations between clinical worsening [24-weeks SDP and occurrence of a second clinical attack] and longitudinal changes in lesion accumulation and brain atrophy progression were

investigated by mixed-effect model analysis after correction for multiple comparisons.
Results: SDP was observed in 32 (15.2%) CIS patients, while 146 (69.5%) were stable and 32 (15.2%) showed disability improvement. 112 CIS patients (53.3%) developed clinically definite MS (CDMS). CIS patients who developed SDP showed increased lateral ventricle volume ($p < .001$), decreased whole brain ($p = .025$), GM ($p = .011$) and cortical ($p = .001$) volumes compared to patients who remained stable or improved in disability. Converters to CDMS showed increased rate of progression of number of new/enlarging T2 lesions ($p < .001$), decreased whole brain ($p = .007$) and increased lateral ventricle ($p = .025$) volumes.
Conclusions: This study showed that cortical GM atrophy is associated with development of the SDP in and conversion to CDMS in patients with CIS patients on a standard disease-modifying therapy (DMT). To the best of our knowledge, this is the first follow-up study reported to date on the evolution of GM pathology and development of SDP in a homogenous sample of CIS patients treated with DMT. Further research is needed to clarify the nature and extent of GM pathology in CIS and to investigate the effect of newly introduced DMTs on prevention of GM pathology.

P504

Microstructural white matter damage in fatigued multiple sclerosis patients: a DTI-TBSS study

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Background: Fatigue is one of the most common and disabling symptoms in Multiple Sclerosis (MS). Yet the relationship between fatigue and normal appearing white matter (NAWM) damage is still unclear.
Objectives: to investigate the microstructural damage of the NAWM and its relationship to fatigue in relapsing-remitting MS (RRMS).
Methods: Sixty-three RRMS were enrolled in the study and, after the evaluation by the Fatigue Severity Scale (FSS), classified as fatigued (F-RRMS; FSS score > 45; N=33) and non-fatigued (NF-RRMS; FSS score < 36; N=30). Thirty-one age- and sex-matched, non-fatigued, healthy controls (HC) were used as control group. A clinical evaluation, including the EDSS score, was obtained in all RRMS patients . Al subjects underwent a 3T MRI including conventional and DTI sequences. Gray (GM) and white matter (WM) atrophy were estimated using SIENAX software. WM focal lesions were identified and lesion volume (LV) and Lesion Probability Maps (LPM) were computed. The microscopic NAWM damage was explored by Tract Based Spatial Statistic (TBSS) analysis, using LPM to exclude voxels were WM lesion frequency was higher than 5%.
Results: F-RRMS and NF-RRMS did not show any significant difference in age, gender, disease duration, EDSS, LV and GM/WM atrophy measures. TBSS-derived metrics (mean diffusivity [MD], fractional anisotropy [FA] and radial diffusivity [RD]) of the reconstructed NAWM skeleton were significantly and diffusely altered when comparing RRMS with HC. A widespread FA reduction and MD increase was found in the NAWM of F-RRMS when compared to NF-RRMS (uncorrected threshold, $p<0.001$). These findings mostly located at the level of thalamus as well as motor and pre-motor cortices, predominantly in the right hemisphere. In the correlation analysis a significant relationship was found between DTI-derived measures (MD and RD) of the NAWM skeleton, particularly at the level of fronto-parieto-insular regions, and FSS scores.
Conclusions: a widespread microstructural NAWM damage, especially located in the right motor/pre-motor cortex and thalamus, might be a critical factor in determining fatigue in RRMS patients. The relevance and specificity of such finding is further emphasized by the lack of significant differences in GM/WM atrophy and LV between F-RRMS and NF-RRMS.

P505

Quantitative evaluation of "invisible" MS brain tissue damage in GM and WM using Gradient Echo Plural Contrast Imaging

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Background: For many years, multiple sclerosis (MS) has been considered as a "white matter (WM)" disease. However, recent studies show that MS is a "whole-brain" disease. Although conventional MRI techniques have good capability of detecting WM lesions, they can barely detect "invisible" damages in normal appearing white matter (NAWM) and cortical gray matter (GM). Previously we demonstrated that MRI-based Gradient Echo Plural Contrast Imaging (GEPCI) technique, allowing simultaneous generation of naturally co-registered multi-contrast images (T1-weighted, $T2^*$ or $R2^*$ maps and frequency maps) from a single MR scan, can provide important information on tissue damage in MS lesions.
Objectives: In this paper we use GEPCI technique to quantitatively assess those "invisible" tissue damages in NAWM and GM of MS subjects with different subtypes.
Methods: All studies were approved by local IRB. High resolution ($1x1x3mm^3$) brain GEPCI data (T1 weighted and R2* maps) were acquired from 10 relapsing remitting (RM), 10 primary progressive (PP) and 10 secondary progressive (SP) MS subjects using a multi-gradient-echo sequence with 10 echoes on a 3T SIEMENS Magnetom Trio. By using a previously developed theoretical model, $R2^*$ was separated into two components: cellular part ($R2^*_c$) and vascular part (R2'). Standard clinical MPRAGE images were also collected and put into "FreeSurfer" to generate brain segmentation. By using "FLIRT" tool in "FSL", MPRAGE images were registered to GEPCI-T1-weighted images, which are naturally co-registered with GEPCI $R2^*/R2^*_c/R2'$ maps. Median values of $R2^*/R2^*_c/R2'$ were calculated for all FreeSurfer regions in each subject. A previously acquired healthy baseline data was used to calculate z-scores of MS subjects in all FreeSurfer regions.

Tissue in regions that have low z-scores (< -1.96) was considered damaged.
Results: We found that 30% of RR, 50% of PP and 50% of SP subjects have damage in NAWM. For cortical GM, the percentages are 20%, 40% and 70% correspondingly. For the globus-pallidus region R2* values are higher than normal: 50% of RR, 20% of PP and 10% of SP have z-score > 1.96.
Conclusions: The results in this study shows that quantitative GEPCI $R2^*/R2^*_c$ measurements can be considered as robust biomarkers to evaluate "invisible" tissue damages in NAWM and cortical GM, which can be difficult to evaluate using conventional methods. This new analysis clearly shows possible damages in the whole brain for subjects with different MS subtypes, thus has a great potential in clinical use.

P506
Functional neuroimaging of inattentional blindness in multiple sclerosis
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Background: Inattentional blindness (IB) refers to the failure to notice a salient stimulus in one's field of vision while conducting an attention-demanding task. Although patients with MS often show cognitive deficits, evidence suggests that they are paradoxically less likely to exhibit IB compared to healthy controls. However, the neural correlates underlying IB in MS patients are not known.
Objectives: To explore the neural correlates of IB in MS patients using functional magnetic resonance imaging (fMRI).
Methods: 26 patients with confirmed MS completed an IB task while undergoing fMRI. On each trial, subjects were asked to maintain fixation while identifying visual targets in their periphery. On critical trials, an unexpected stimulus simultaneously appeared with the targets. Trials were presented under conditions of IB, i.e. without priming for the unexpected stimulus and under conditions during which subjects were primed that the unexpected stimulus might appear and that they should watch for it. A questionnaire was also given post-test to determine each subject's awareness of the unexpected stimulus. This design allowed for 2 contrasts: Paradigm 1) A within-subjects comparison of activity on critical trials between the non-primed and primed conditions (n=9), and Paradigm 2) A comparison between subjects who always noticed the unexpected stimulus irrespective of priming (non-IB; $n = 13$) versus those who did not see the unexpected stimulus in the non-primed trial only (IB; $n = 9$). All other subjects were excluded from the analysis.
Results: Across all subjects increased activity in bilateral frontal eye fields (FEF) was found during critical trials in Paradigm 1 (FWE-corrected, $p < .005$), consistent with the role of the FEF in goal-directed attention. Comparing neural activity in Paradigm 2 between IB and non-IB subjects, increased activity in the anterior cingulate cortex (ACC)/medial prefrontal cortex (mPFC) was found in the IB patients (FWE-corrected, $p < .005$).
Conclusions: Within MS patients, IB evokes activity in similar neural regions to that of healthy controls: namely, increased activity in ACC/mPFC, which have been implicated in non-conscious processing of stimuli. Future work is required to determine the mechanism underlying MS patients' decreased susceptibility to IB, likely reflective of their reduced attention resources.

P507
The nature of white matter tract injury in relapsing-remitting multiple sclerosis: a diffusion-tensor imaging study in relation to disease duration
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Background: Diffusion-tensor imaging (DTI) studies in multiple sclerosis (MS) reveal white matter (WM) injury with disease progression. Our approach utilizes whole-brain and voxelwise approaches to extensively investigate alterations in white matter and observe their progression over space and time.
Objectives: To evaluate microstructural white matter damage in relapsing-remitting multiple sclerosis (RRMS) as measured by brain DTI in patients with varying periods of disease duration.
Methods: Axial DTI data was acquired along 31 directions at 2.6mm slices with single shot echo-planar imaging and 2 images without diffusion weighting (b=0) for 90 RRMS patients (age 37.6±1.0 years, 66 females) and 25 healthy controls (age: 35.1±2.2 years; 14 females) on a 3.0T scanner. Patients were grouped to short (< 1 year), moderate (1-6 years) and long (6-10 years) disease duration periods. Whole-brain and voxelwise analyses of fractional anisotropy (FA) data were carried out using tract-based spatial statistics (TBSS). Threshold-free cluster enhancement (TFCE) was performed for voxelwise analysis alongside the JHU ICBM-DTI-81 atlas for major WM tract identification. Student two-tailed unpaired t-test was performed on FA in each significant region´s clusters for between-group analyses.
Results: Whole-brain WM FA measurements showed a significant decrease between healthy controls and the short disease duration group (0.47±0.02 versus 0.44±0.02, $p < 0.001$), as well as between medium and long disease duration groups (0.44±0.02 versus 0.43±0.02, $p < 0.01$), but failed to show significance between short and medium disease duration groups (0.44±0.02 versus 0.44±0.02, $p = 0.95$). On further inspection, voxelwise analysis revealed diffuse FA reduction spanning a skeletal area of 60,784 of 195,350 total voxels and affecting 38 major WM tracts when comparing healthy controls and the short duration group. FA reduction was also shown in 30 major WM tracts when comparing the long and medium duration groups, affecting 42,212 of 192,208 skeletal voxels.
Conclusions: DTI data revealed significant disruptive injuries related to disease progression in RRMS patients. Initially, an acute episode of widespread alterations in WM tracts is seen in the first year after diagnosis; however, these changes slow to a plateau in patients with medium disease duration and later demonstrate significant, widespread WM changes in patients with long disease duration. Our findings signify a distinct, non-linear temporal pattern to changes in WM microstructure.

P508

Regional cortical thickness in African Americans with multiple sclerosis

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Background: African Americans (AA) have a more aggressive disease process, poorer response to disease modifying therapy, and accumulate more clinical disability as compared to Caucasians (CA). These observations suggest differences in the underlying disease process in AA patients. Previous studies have correlated cortical atrophy with both clinical disability and disease progression. In addition, a specific regional pattern of focal atrophy has been identified in MS patients, which differs from normal aging and implies a disease-specific mechanism for cortical neuronal loss.
Objectives: This study was designed to examine the differences in regional cortical thickness between AA and CA MS patients.
Methods: Fifty-five AA and 55 CA patients matched for age, disease duration, treatment, and gender were assessed in this study. Using Freesurfer, white matter and grey matter were segmented from the T1 images and were checked for misclassification. Global cortical thickness was measured using Freesurfer. Regional cortical thickness was computed using Qdec, a Freesurfer group analysis tool optimized for display of results on the cortical surface. Monte Carlo simulation was used to control for multiple comparisons set at $p < 0.001$.
Results: Disability scores (EDSS) were significantly higher in our AA (2.25±2.16) vs CA patients (1.31±1.48) ($p=0.0094$). AA had more global cortical atrophy in comparison to CA patients ($p=0.022$). Racial differences in regional cortical thickness were most evident in the right hemisphere, with the superiortemporal ($p=0.0001$) and precuneus ($p=0.04$) areas being significantly thinner in AA. In the left hemisphere, the lingual ($p=0.032$) and inferiortemporal ($p=0.0046$) regions were significantly thinner in AA as compared to CA.
Conclusions: We observed significant regional differences in cortical thickness between AA and CA patient cohorts, which were mainly in the temporal lobe, precuneus and lingual area. Our data coincides with previously reported areas of thinning in MS patients in comparison to controls, which suggests a similar disease process in AA and CA patients. The difference in severity could be explained by higher neuronal loss and dendritic atrophy, which may justify the higher clinical disability witnessed in AA in comparison to CA patients.

P509

Can we really differentiate spinal cord ischemia from the myelitis of neuromyelitis optica with MRI in the acute setting?

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Background: Both spinal cord infarct and the acute myelitis associated with Neuromyelitis Optica (NMO) have similar clinical presentations and can be associated with longitudinally-extensive central cord lesions. Clinical management of the two diseases is very different so reliably differentiating these two entities in the acute setting is of paramount importance.
Objectives: To retrospectively identify useful distinguishing imaging features for differentiating SCI from NMO myelitis during an acute clinical presentation.
Methods: We used a case-control design to compare acute radiographic presentation findings from patients at a tertiary medical center with retrospective definitive diagnoses of NMO (N=9) or angiographically-proven spinal cord infarct (N=12). Patient demographics, clinical presentation and MRI features such as the location, craniocaudal extent and area of cord lesions were categorized and quantified by 2 radiologists. Contrast and diffusion-weighted MRI were not obtained in all subjects.
Results: The age at presentation for acute myelitis did not differ between groups (mean 51 yrs, $P>0.05$), but cord infarct patients were much more likely to be male and Caucasian ($P< 0.05$). 50% of all acute NMO lesions clustered specifically at the cervicomedullary, cervicothoracic and thoracolumbar junctions. 50% of spinal cord infarcts were located below T6 and near the conus. Both groups demonstrate cord lesions originating within the central cord with mild expansion, but NMO lesions were more likely to extend into peripheral white matter. The "snake eye" sign, which is classically described as being pathognomonic for spinal cord infarct, was seen in multiple NMO patients. No lesion differences were present between NMO and SCI respectively for craniocaudal length (8.9 ± 4.0 vs 6.1 ± 2.4 cm) or cross-sectional area relative to cord at the midpoint (31.8 ± 8.8 vs 34.8 ± 5.9%).
Conclusions: Our results indicate that there is a great deal of radiologic overlap between acute spinal cord lesions from NMO or infarct. NMO lesions tended to cluster at spine junction zones, while SCI was mostly see in lower spinal cord. Classic "pathognomonic" MRI features, such as 'snake eyes' in SCI, were not useful for differentiating between the two entities. Patient characteristics and specific features of presentation may prove to be more important clues to diagnosis than conventional MRI findings.

P510

Lower brain glutamate levels in patients with relapsing MS compared to healthy controls

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Background: Glutamate is the primary excitatory neurotransmitter in brain and is converted to glutamine upon uptake by astrocytes. Abnormal levels of glutamate reflect the quantity and integrity of synapses and are a promising biomarker of neurodegeneration. Magnetic resonance spectroscopy (MRS) can non-invasively and quantitatively measure the concentrations of glutamate and other brain metabolites.
Objectives: The goal of this study was to use an advanced MRS analysis technique to extract glutamate-specific concentrations, as well as other brain metabolite concentrations, in patients with relapsing multiple sclerosis (RMS) compared to healthy controls.

Methods: 26 RMS subjects participating in a Phase III clinical trial of ocrelizumab (OPERA) were scanned at baseline. In addition, 40 age and gender-matched healthy controls were included. Spectra were measured from a 6.5x4.5x1.8cm^3 white matter voxel and fit with LCModel version 6.3. Concentrations of glutamate, N-acetyl-aspartate (primary role: neuronal integrity), creatine (energy storage), choline (membrane synthesis), and myo-Inositol (glial marker), were calculated relative to the water peak and corrected for voxel compartmentation and relaxation to obtain institutional mM values. The Wilcoxon rank sum was used to test group differences with the Holm-Bonferroni method to address multiple comparisons.

Results: Glutamate was the only metabolite which showed a significant difference between RMS subjects and controls. The glutamate concentration was [median (25th and 75th percentiles)] 6.4 (6.0 - 6.9) mM in RMS] and 7.0 (6.4 - 7.3) mM in healthy controls (p=0.006, uncorrected, significant). There was a trend to lower N-acetyl-aspartate values in RMS subjects as compared to controls: 6.7 (6.3 - 6.9) mM versus 6.9 (6.7 - 7.2) mM, respectively (p=0.021, uncorrected, not significant).

Conclusions: Lower levels of glutamate were found in RMS subjects compared to age and gender matched healthy controls. Lower glutamate levels in RMS subjects may indicate decreased synapse function or density. This result concurs with our previous finding of a decline in both glutamate and glutamine over 2 years in patients with secondary progressive MS. Taken together, these studies suggest that abnormal glutamate homeostasis is a common feature of different MS sub-types and could be potential biomarker of neurodegeneration. These brain metabolites will be measured longitudinally in the OPERA Phase 3 RMS clinical trials.

P511

No change in venous, arterial, nor CSF flows in MS patients after 1 year of disease modifying drugs

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Background: Venous dysfunction has been recently hypothesized to contribute to the pathological mechanisms of Multiple Sclerosis (MS). Phase Contrast (PC) -MRI is a non-invasive technique providing reliable quantification of CSF (cerebrospinal fluid) and blood flows, in both intracranial and cervical levels. We have previously demonstrated that these flows are comparable in MS patients and aged-matched healthy controls.

Objectives: Our aim in the current study was to re-analyze these flows in MS patients 1 year after the first quantification, and to evaluate their evolution with disease modifying treatments.

Methods: Twenty MS patients (7 CIS, 11 RR, 2 SP) underwent 3T cerebral MRI. Fast cine PC-MRI sequences were performed in 3 slice locations, at the levels of: i: the Sylvius Aqueduct with flow encoding velocity (Venc) for CSF at 10 cm/s; ii: the spinal canal with Venc at 10 cm/s for CSF and 80 cm/s for internal carotid arteries (ICA), Vertebral arteries (VA), and internal jugular veins (IJV); and iii: coronal plane (Venc at 80 cm/s) for superior longitudinal sinus (SLS), straight sinus (SS) and both transverse sinuses (TS). Images were analyzed using a home-made software to extract CSF oscillations and calculate arterial and venous flows curves during the cardiac cycle. Stroke volumes of CSF, and mean arterial and venous individual flows were calculated. The same imaging protocol was performed 1 year later, and results were compared using a Wilcoxon test.

Results: At 1 year, 7 patients were treated with Natalizumab (Nzb), and 6 with Fingolimod (Fgd). Among patients with CIS, 4 were treated with immunomodulatory drugs, 3 remained untreated. Two relapses occurred during the follow-up of patients treated with second line drugs (Nzb and Fgd), with a significantly reduced relapse rate as compared to pre-treatment period (p=0.01). No new T2 nor enhancing T1 lesions were observed.Venous flows demonstrated high heterogeneity and right-sided dominance. At 1 year, no significant change was observed for flow quantification. Vascular and CSF flow values were comparable at both time points for all patients ($p > 0.05$). No correlation was found between the presence/absence or the type of treatment, and the evolution of flows, particularly venous flows.

Conclusions: Our study is the first to follow venous drainage in a MS population. The absence of correlation between venous flows and drug-response in MS adds evidence against the implication of CCSVI in the underlying mechanisms of MS.

P512

Corticospinal tract integrity measured using transcranial magnetic stimulation and magnetic resonance imaging in NMO and MS

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Background: Neuromyelitis optica (NMO) is a demyelinating disease which until recently was considered to be a subtype of multiple sclerosis (MS). NMO is characterized by episodes of inflammation and damage to astrocytes that can result in optic neuritis and myelitis. Unlike MS, the immune attacks in NMO are believed to be mediated by antibodies targeting the protein aquaporin 4. While MS and NMO differ immunologically, they have extensive similarities in terms of conventional imaging exams and clinical manifestations that make clinical differentiation difficult. However, prognosis and optimal treatment for these diseases are very different.

Transcranial magnetic stimulation (TMS) provides a functional method for assessing the integrity of central motor pathways. Advanced MRI measuring myelin water fraction (MWF) may be employed to quantitatively monitor demyelination.

Objectives: To use TMS and MWF to better understand the common and distinct pathophysiology related to myelin and central motor conduction of NMO and MS.

Methods: Ten MS patients (EDSS=0.0-6.0, mean age=42y, mean disease duration=8.7y, 3M/7F), 10 NMO patients (EDSS=2.0-6.0, mean age=43y, mean disease duration=7.4y, 3M/7F), and 10 healthy controls (mean age=42y, 2M/8F) underwent MRI and TMS.

TMS was performed using a figure-of-eight coil delivering focal stimulation over the primary motor cortex. Motor evoked potentials (MEPs) were measured using surface electromyography of the extensor carpi radialis bilaterally. Short-interval intracortical inhibition (SICI) was quantified using paired pulses with a 2ms interval. For the MEP recruitment curve, single pulses were delivered (105, 115, …, 155% active motor threshold) during isometric contraction (20% maximum).

The MRI protocol included a 32-echo T_2 relaxation GRASE scan (TR=1000ms, 10ms echo spacing, 20-5mm slices). MWF values were obtained for the corticospinal tract (CST).

Results: Individuals with MS had significantly greater SICI (0.60±0.15) compared to NMO (0.88±0.26, p=0.01) and controls (0.83±0.17, p=0.004). The shape of the MEP recruitment curve for NMO was different from MS and controls. The NMO group had significantly lower MWF in the CST (0.17±0.02) compared to MS (0.19±0.02, p=0.01) and controls (0.20±0.02, p=0.003).

Conclusions: The combined results suggest that there are both neurophysiological and neuroanatomical changes that may be novel biomarkers to help distinguish individuals with MS from those with NMO and to better understand the underlying pathophysiology of each disease.

P513

Regional brain atrophy in early stages of single patients with rrMS analysed by automated quantified MRI

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Background: To detect regional brain atrophy due to axonal loss even in the early stage of the disease it requires a precise cross sectional individual single subject MRI analysis. For this purpose we developed and validated a biomarker to quantify T1 lesion load combined with regional brain atrophy in brain WM and GM using routine MRI sequences (Spiess et al. 2013.

Objectives: To determine the regional brain atrophy of white matter (WM) and gray matter (GM) in single patients with early stages of rrMS. Our automated quantified MRI image analysis is operator independent and applicable in clinical routine.

Methods: In a controlled, prospective and blinded design 17 consecutive patients with a rrMS disease duration maximum of 4y (11 female, 6 male; mean age 39y; mean disease duration 2.2y; mean EDSS 1.26) were enrolled. 28 normative subjects served as controls. All patients and controls were acquired using a 3T MR scanner with routine sequences. The image analysis applies to high-resolution T1-weighted image data (MPRAGE). Slice thickness was 1mm and pixel volume 1mm³. All data were normalized to a stereotactical space, followed by an algorithm to segment GM, WM and CSF. A voxel wise two sample T-Test was applied to check for deviations of each patient and the normative database. Single subjects were analysed for any significant WM and GM atrophy. Atropy findings were allocated in a standard 3d brain map.

Results: Regional brain atrophy was detected in 41% (7/17) of our study cohort. Regional WM atrophy was revealed in 24% and GM atrophy also in 24%. One patient had WM combined with GM atrophies. In our cohort with early disease stages regional atrophies were allocated to hippocampus, temporal GM, temporal WM, frontal WM and corpus callosum.

Conclusions: The presented single subject cross sectional regional brain atrophy analysis of early stage rrMS provides important data. Regional brain atrophy in early stages of the disease is common and spreads to WM and GM. Routine application of the method could contribute to MS staging and monitoring.

P514

Resting state fMRI probed as predictor of fatigue symptoms in multiple sclerosis

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Background: Structural MRI, particularly lesion and atrophy mapping, has not revealed a conclusive topographical model of MS-related fatigue with its different aspects, but has still highlighted a role of progressive brain atrophy, anterior corpus callosum atrophy, basal ganglia involvement, frontal cortex atrophy and generally, involvement of attention-related areas. Task-fMRI suggest disturbed cortical-subcortical interaction with decompensation under prolonged demands. Here, we employ functional connectivity (FC) analysis of resting state fMRI (rs-fMRI) time series to probe if disconnection under basal (task-free) conditions predicts fatigue symptoms.

Objectives: To explore if different aspects of fatigue in MS across disease stage can be predicted from FC analysis of rs-fMRI, following a default mode network (DMN) hypothesis and exploring whole brain FC analysis without regional hypotheses.

Methods: Eyes-closed wakeful rs-fMRI (1.5 T, EPI sequence, TR 2 s, 180 images) and sMRI were obtained from 53 subsequent patients with a definite diagnosis of MS. Behavioral assessment included self-rated fatigue (mainly the modified fatigue impact scale [MFIS], fatigue severity scale [FSS]), MS-specific FSS), the MS functional composite score, EDSS and depression severity ratings. After exclusion of 4 patients (incomplete ratings [3], ventriculomegaly [1]). Image processing comprised slice time correction, spatial normalization (iterative DARTEL technique) to compensate for enlarged CSF spaces, spatial filtering (gaussian 6×6×6 mm³) and removal of motion effects and global WM and CSF signal. 8 lateralized DMN regions and 4 anticorrelated nodes were defined by PCC-seed analysis (N=26); further, the AAL parcellation was employed for hypothesis-free exploration of correlations with fatigue scores.

Results:

DMN-hypothesis: FC within the DMN/ACN system was weakly and inconclusively correlated with the MFIS and its subscores.

AAL-system: Across-group FC correlations with the MFIS at p< 0.001 were mostly negative with an emphasis on fronto-striatal and SMA/cingulate-connections. Notably, anatomical patterns were different between *physical* (frontostriatal, SMA/cingulate), *cognitive* (frontotemporal, less frontostriatal) and *psychosocial MFIS subscores* (hippococampus, parahippocampus, amygdala). Minor result changes were observed with regional GM- instead of CSF-based atrophy correction.

Conclusions: Analysis of resting state fMRI time series may allow for dissecting functional networks that underlie different aspects of fatigue.

P515

SLF: a MS white matter lesion filling toolbox for the SPM software

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Background: Several works have investigated the effects that white matter (WM) lesions have on gray matter (GM) and WM tissue volume estimations, proposing to improve volume measurements by filling WM lesions with intensities similar to WM before segmentation. Filling methods can be divided into local techniques, which use the intensities from the surrounding neighboring voxels of the lesions to fill them, and global techniques, which use WM intensities from the whole brain.

Objectives: To propose a new approach (SLF) to fill multiple sclerosis (MS) lesions in T1-w images that overcomes some of the existing limitations of the global and local filling techniques.

Methods: For each T1-w slice composing the whole brain image, WM lesion voxel intensities are replaced by random intensities of a normal distribution generated from the mean WM intensity of the current slice. Compared to global methods based on the mean signal intensity of all slices, our method re-computes the mean signal intensity of the WM at each two-dimensional slice with the aim of reproducing more precisely the signal variability between slices, especially in low resolution images.

Results: We computed the deviation in GM and WM tissue volume between a set of healthy images and the same images where artificial WM lesions similar to the mean GM/WM interface were filled with the proposed technique. Manual annotations done by experts of WM lesion masks from MS patients were registered into two sets of 30 1.5T and 3T T1-w images of healthy subjects, respectively. Tissue volume was computed using FAST and SPM8 segmentation methods. The results were compared with three state of the art filling methods (Chard et al., Battaglini et al., Magon et al.). The results on 1.5T showed that SLF reduced the deviation in WM between original and filled images, independently of the segmentation method used. SLF also provided the lowest differences in GM when FAST was used, and a similar performance to Chard et al., when SPM8 was employed. On 3T data, SLF again provided the lowest differences in GM and WM tissue when FAST was used. When SPM8 was used, SLF presented also a similar performance to Chard et al. Furthermore, volume estimations of lesion filled images using SLF appeared to be not affected by the segmentation method.

Conclusions: The proposed method obtained satisfactory results in both 1.5T and 3T, improving especially the results in low resolution images. SLF will be available to researchers as a SPM library extension.

P516

Magnetic resonance perfusion weighted imaging - a marker of inflammatory activity in multiple sclerosis?

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Background: Multiple sclerosis (MS) varies both in terms of severity and response to treatment. There is a need for new biomarkers of disease activity to optimize treatment and possibly improve the clinical outcome. Magnetic resonance imaging (MRI) is a central tool in MS follow-up and improved MRI measurements of MS disease activity are warranted. Dynamic susceptibility contrast MRI (DSC MRI) is currently the best established MRI-based perfusion method providing several hemodynamic parameters. Cerebral blood volume (CBV), cerebral blood flow (CBF) and mean transit time (MTT) can be potentially useful for characterizing disease activity in MS.

Objectives: The objective of the study was to explore the potential usefulness of DSC MRI in characterizing affected brain tissue in MS and to establish its possible association with disease activity.

Methods: Newly diagnosed patients with relapsing-remitting MS were included in the study (n=68, age between 22 and 49 years, 50 females and 18 males, mean disease duration 26 months, mean EDSS score 2.0). MRI was performed at 1,5 T (Avanto, Siemens Medical, Erlangen, Germany) using a DSC MRI sequence with 19 slices and voxel size 1.8 x 1.8 x 5 mm^3. Binary masks of white matter lesions (WML), normal appearing white matter (NAWM) and grey matter (GM) created from volumetric fluid attenuated inversion recovery (FLAIR) and T1 series were co-registered to the resulting perfusion maps. Perfusion analysis was performed using NordicICE software (www.nordicneurolab.com). Coregistration and WM/GM segmentation was performed in SPM8 (http://www.fil.ion.ucl.ac.uk/spm). WML masks were automatically generated using Cascade software (Karolinska Institute, Stockholm, Sweden). Perfusion parameters (CBV, CBF and MTT) were extracted from the whole compartments of WML, NAWM and GM. Differences in perfusion metrics between WML and NAWM were tested using paired samples t-test and non-parametric Wilcoxon signed rank tests. Statistical analysis was performed in SPSS (version 21).

Results: CBV in WML (mean 14.01; SD 4.51; CI [12.88; 15.15]) was significantly increased ($p < 0.001$) compared to NAWM (mean 11.99, SD 3.43, CI [11.14; 12.83]). Also MTT in WML (mean 3.64; SD 0.65; CI [3.48; 3.81]) was significantly increased ($p < 0.001$) relative to NAWM (mean 3.17; SD 0.49; CI [3.07; 3.32]). CBF did not differ in WML and NAWM.

Conclusions: CBV and MTT are significantly increased in WML compared to NAWM in newly diagnosed MS patients, indicating that DSC MRI may be used to evaluate inflammatory activity in MS.

P517

Cellular and microstructural changes due to iron deposition in multiple sclerosis lesions

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Background: Susceptibility-weighted imaging (SWI) has been emerged as a useful clinical tool in detecting iron deposition in many neurological diseases including multiple sclerosis (MS). Although several studies reported increased iron deposition in some MS lesions, its impact on brain tissues' microstructure has been poorly understood.

Objectives: This study is to evaluate the microstructural changes measured with diffusion tensor imaging (DTI) in MS lesions with and without iron deposition on SWI.

Methods: Seventeen patients with relapsing remitting MS (mean age: 35.8±9.8 years, mean EDSS: 2.9±1.8) were recruited for this study. MRI was performed on a 3T machine with conventional T1- and T2-weighted imaging, SWI and DTI. The imaging parameters of SWI include TR/TE=55/18ms, FA=20°, voxel size=0.49x0.49x2mm^3. The SWI was processed using both magnitude and phase information with 2-slice mIP (4mm thick) and phase multiplication factor of 4. DTI data were acquired using 30 directions with a single-shot spin-echo, echo-planar sequence with voxel size=1.8×1.8×3mm^3 and b=1000 s/mm^2. The data were processed offline using DTI studio. The corrected raw tensor images were combined to construct mean diffusivity (MD) and fractional anisotropy (FA) maps. The MS lesions with iron deposition were defined as hypointense on both phase (i.e., higher susceptibility) and SWI minimal intensity projection (mIP) images. MD and FA were measured and co-registered in corresponding lesions with and without hypointensity on SWI due to iron component.

Results: A total of 127 lesions were identified in these patients. Among them, 48 lesions had hypointense component (i.e., due to iron deposition) on phase and SWI, while 79 lesions did not show hypointensities. The averaged FA was found to be significantly different ($p< 0.0001$) between lesions with iron deposition (0.33±0.09) and lesions without (0.26±0.09), significant reduction ($p=0.02$) of MD was also observed in lesions with iron content (1.01±0.21mm^2/s) versus lesions without (0.99±0.18mm^2/s).

Conclusions: The significant difference of FA and MD between MS lesions with and without iron deposition may suggest a role of iron on microstructural tissue injury and recovery. It is worth noting that lesions with iron deposition in this patient group showed less tissue destructive indicated by higher FA and lower MD as compared to lesions without iron deposition, and such findings may be associated with initial blood products evolution and warrant further investigation.

P518

Imaging of brain perfusion in multiple sclerosis and neurodegenerative disorders: association with endothelial factors. An interim analysis

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Background: Among the factors contributing to brain damage in Multiple Sclerosis (MS), scientific evidences indicate ischemic changes, venous outflow abnormalities and accumulation of pro-inflammatory and neurotoxic substances.

Objectives: This project aims at acquiring new knowledge about the association of determinants of brain perfusion in patients with MS and neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (ALS), combining Magnetic Resonance (MR) and Ultrasound (US) imaging of the brain and intracranial and extracranial circulation, and endothelial factors.

Methods: 300 MS patients, 50 ALS patients, and 300 healthy subjects (HS) will be recruited over 3 years. To assess the vascular genetic susceptibility, serum levels of homocystein and endothelial factors will be assayed, as well as the association between the C677T polymorphism of methylen-tetrahydrofolate reductase (MTHFR), informative SNPs in VEGF-A, Endothelin 1 and HIF1A genes and micro and macro vascular abnormalities in MS and ALS. With MR, we will assess the arterial inflow and mean transit time using brain Perfusion Weighted Imaging (PWI), the state of the deep intracranial veins using Susceptibility Weighted Imaging (SWI), and measure iron deposits in the basal ganglia using dedicated software. Extracranial and transcranial color-Doppler US with time intensity curve analysis was used to quantitatively evaluate cerebral inflow and outflow parameters and cerebral perfusion.

Results: In the first year we recruited 228 MS patients, 33 ALS patients and 67 HS. Homocystein levels (HL) were assessed in 132 patients, VEGF-A in 50 patients and in 25 HS. Median HL were similar in MS and ALS patients (13.2 vs 16.0, μmol/L, $p=0.2$). Higher-than-normal HL were found in 32.2% of MS (CI 24-41%) and 66.7 % of ALS patients (CI 30-92%), but not significantly different in the two groups ($p= 0.08$). Instead, median HL were significantly higher in males with MS compared to females (17.3 vs 13.9 μmol/L, $p< 0.001$) .VEGF-A values tended to be higher in MS patients vs HS (251.7 vs 145.7 U/mL, p=0,4). 101 patients (78 RR, 2 PP, 11 SP and 10 ALS) underwent contrast-enhanced brain MR, and 68 patients (54 RR, 1 PP, 10 SP and 3 ALS) underwent US evaluation.

Conclusions: Combining different imaging modalities and laboratory analysis may provide new insights into the vascular aspects of MS pathogenesis. These preliminary results favor an altered vascular profile in MS and ALS patients. Definitive results will be available at project termination in 2016.

P519

MR frequency shift imaging of MS lesions and comparison to myelin water and magnetization transfer imaging

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Background: Multiple sclerosis (MS) is characterized by a variable degree of episodic inflammation in focal lesions resulting in demyelination of axons, neurodegeneration and axonal loss. Conventional magnetic resonance (MR) outcomes correlate poorly with the patient's clinical status, which might reflect the failure to quantify heterogeneity of MS lesions.
Objectives: MR frequency shift (FS) images are sensitive to myelin and axonal loss. This could improve the discrimination of the severity of MS lesion pathology. We compared MR FS in different lesion types to myelin-related MR metrics: magnetization transfer ratio (MTR) and myelin water fraction (MWF).
Methods: 25 subjects with relapsing-remitting MS (age range: 21-54 years; median EDSS=2) participating in a phase III clinical trial of Ocrelizumab (OPERA) were scanned before treatment initiation. MR FS maps were calculated from a gradient echo scan (TR/TE/ΔTE =38/4/4.5ms, reconstructed voxel size: 0.5x0.5x1mm^3). MTR and MWF were acquired in addition to FLAIR and gadolinium(Gd)-enhanced T_1 images. All scans were registered to the 22ms echo FS images. MS lesions were defined manually on FLAIR scans. The average FS, MTR and MWF values were computed for each lesion. Kruskal-Wallis and Wilcoxon Rank sum tests evaluated group differences.
Results: 16 Gd-enhancing, 583 T_2-hyperintense/T_1-isointense and 139 T_2-hyperintense/T_1-hypointense lesions were identified. T_1-hypointense lesions differed significantly ($p< 0.01$) from T_1-isointense lesions for all three techniques, but FS showed a much larger mean difference (121%) between lesion types than MTR (6%) or MWF (10%). FS varied widely between individual enhancing lesions, but only MTR differentiated enhancing and isointense lesions ($p=0.001$). Our observations agree with theoretical predictions and experimental findings of FS at different stages of demyelination and axonal loss. Apart from being markers for myelin, MTR is known to be influenced by inflammation and edema, while MWF is noisier than FS images. None of these measures distinguished enhancing from non-enhancing T_1-hypointense lesions.
Conclusions: FS images show greater heterogeneity between individual lesions compared to MTR and MWF suggesting that MR FS provides information of lesion variability within subtypes, referring to changes of the myelin state and axonal loss. Monitoring lesions for changes in FS, MTR and MWF could distinguish patterns that indicate success or failure of remyelination in MS lesions.

P520

MR frequency shift imaging demonstrates that iron accumulation is rare in multiple sclerosis lesions

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Background: One of the main radiological signs of multiple sclerosis (MS) is the appearance of focal lesions due to inflammation and blood brain barrier breakdown. Focal lesions on magnetic resonance (MR) frequency images demonstrate variable contrast, which has been attributed to iron accumulation. Histopathology studies, however, report no or low iron content only in the rim of MS lesions, which is in good agreement with recent findings that changes in tissue architecture are also a source of frequency shifts (Wiggermann et al., Neurology 81, 2013).
Objectives: Focal, nearly spherical lesions that contain iron should create a dipolar modification of MR frequency, similar to microbleeds, where hemosiderin is responsible for the dipolar pattern. If frequency shifts are caused by microstructural changes, no such dipole features will be observed, indicating a lack of iron in MS lesions. We investigated focal MS lesions for the presence and absence of dipole patterns in frequency maps.
Methods: 17 patients with relapsing-remitting MS completed serial monthly scanning over 6 months (mean age=39.5yrs (range 28-57yrs), median EDSS=2.5 (range 1-6), mean disease duration=9.4yrs (range 1-27yrs)). Gradient echo frequency shift images were acquired (FOV=240x166x64mm^3, reconstructed voxel size=0.43x0.43x1mm^3, TR/TE=40/20ms) along with FLAIR (Fluid Attenuated Inversion Recovery) and T_1 Gd-contrast enhanced scans, which were used to identify enhancing lesions and spherical T_2-hyperintensities. All MR images were registered to the baseline frequency image. Lesions were classified as dipolar if they showed a ring of reduced frequency in the equatorial plane and areas of increased frequency above and below the lesion. Field distortions due to deoxyhemoglobin in veins were excluded.
Results: In 9/17 subjects, 37 focal enhancing and 90 focal non-enhancing lesions were detected. Only 1/37 enhancing lesions and 4/90 of the non- enhancing lesions showed dipole characteristics as described above. We did not observe a prevalence of recently enhancing lesions or non-enhancing lesions to show dipole characteristics (3% vs. 4%).
Conclusions: Our findings agree with previous histopathological studies and theoretical suggestions that the frequency contrast is due to microstructural changes rather than changes of magnetic susceptibility due to iron (Yablonskiy et al., PNAS 109, 2012). The lack of dipolar modifications of the magnetic field indicates that iron accumulation is a rare phenomenon in focal MS lesions.

P521

Characteristics of patients with MRI-only conversion to multiple sclerosis after a clinically isolated syndrome

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Background: Up to a quarter of patients with a clinically isolated syndrome (CIS) who have abnormalities on a baseline MRI do not experience a second clinical attack, even with long term follow

up. A proportion of these patients satisfy McDonald criteria for a diagnosis of multiple sclerosis (MS) with MRI, but not clinical evidence of dissemination in time and space.
Objectives: Based on follow-up of a large, prospectively recruited CIS cohort, to describe the frequency of MRI-only conversion to MS, and the demographic, clinical and MRI characteristics of such patients.
Methods: Data were reviewed from a CIS cohort with serial clinical and MRI assessment over the first 6 years after onset. MS was defined according to the 2010 McDonald criteria when there was MRI evidence of dissemination in time and space or a second clinical attack.
Results: From a cohort of 157 CIS patients followed for a mean of 5.8 years (range 3.0 - 11.9 years), 107 (68%) patients satisfied 2010 McDonald criteria for a diagnosis of MS, of whom 71 had a second clinical attack and 36 had MRI-only evidence of dissemination in time and space. Patients with MRI-only MS were older (mean age 34.3 vs 32.0 years) and more often male (50% vs 25%), but these differences were not statistically significant. Baseline MRI findings (including T2 lesion volume, gadolinium-enhancing lesion number, spinal cord lesion number) were similar in patients with clinical and MRI-only conversion to MS, however, the mean T2 lesion volume 6 years after CIS onset was greater in patients who experienced a second clinical attack (9.82 vs 3.26 ml, $p < 0.01$). At the last follow up physical disability was greater in those patients with a second clinical attack (mean EDSS 2.5 vs 1.0, $p< 0.01$).
Conclusions: A substantial number of CIS patients (23%) have a form of MS that remains subclinical, at least over the first 6 years after CIS onset. There is a need to identify robust early predictors of the subsequent disease course, in order to effectively stratify patients with CIS. Further follow up is required to determine whether patients with MRI-only MS are at risk of physical disability and cognitive impairment in the long term.

P522
Discriminative radiological features of multiple sclerosis and neuromyelitis optica in Chinese patients
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Background: Multiple sclerosis (MS) and neuromyelitis optica (NMO) are recognized to be clinically distinct entities which management differs. Western studies have shown that certain radiological features may be helpful in discriminating them from one other. However, such data is scarce in the Chinese population.
Objectives: To identify the discriminative radiological features of MS and NMO in Chinese patients.
Methods: All patients who have been diagnosed with MS or NMO in a single regional hospital from 1996 to 2014 were recruited. They were retrospectively analyzed based on their most updated diagnosis as of March 2014, according to the 2010 McDonald criteria and the 2006 Wingerchuk criteria. All of the brain and spine magnetic resonance images ever performed for each subject were reviewed and compared. Univariate analysis was done by the Fisher's exact test and multivariate analysis was done by logistic regression with forward stepwise model to identify the discriminative value of each radiological feature.
Results: A total of 92 patients were recruited. 3 were excluded due to incomplete data. 62 patients had MS, and 27 had either NMO or NMO spectrum disease. The mean disease duration was 9.2 years. All MS patients had abnormal brain signals during the first attack (100%), and the lesions more commonly involved U-fibres (OR 10.2, p=0.002) and had Dawson's finger appearance (OR 45.4, $p< 0.001$). Spinal cord lesions in MS patients were more likely to be multiple short segmented (OR 13.2, $p< 0.001$), and had lateral cord involvement (OR 5.43, p=0.004). In contrast, only 9 NMO patients had abnormal baseline MRI brain (36%, $p< 0.001$). 2 of the remaining 18 subjects developed brain lesions in subsequent attacks. These brain lesions were more prevalent at the area postrema (OR 13.7, p=0.001) and had cloud-like/tumefactive appearance (OR 13.7, p=0.001). Cord lesions in NMO patients were more prone to be longitudinally extended (LETM, i.e. equal or more than three segments) (OR 28.4, $p< 0.001$), central (OR 5.43, p=0.004), and associated with cord swelling (OR 13.0, $p< 0.001$). Cervical cord involvement and periventricular brain lesions were common to both MS and NMO. Multivariate analysis showed that Dawson's finger is the most typical MS feature, while LETM is the strongest discriminative feature favouring the diagnosis of NMO.
Conclusions: Our review suggests that radiological features that discriminate NMO from MS in the western population are also applicable in Chinese patients.

P523
Central vein detection in MS lesions using FLAIR* at 7T
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Background: FLAIR* has been proposed at 3T for visualization of both veins and lesions [Sati et al., Radiology 2012]. This technique can be utilized for characterizing the presence of central veins within white matter (WM) lesions, a potentially highly predictive marker of MS diagnosis [Quinn MP et al., Front Neurol 2013]. By applying FLAIR* at 7T, the increased signal-to-noise and contrast-to-noise ratios as well as spatial resolution should allow for a more robust detection of these small central veins [Kilsdonk et al., Eur Radiol 2014]. However, there are problems inherent to 7T imaging, such as a strong image bias caused by field inhomogeneity, which must be addressed in order to allow clinicians to read the images reliably.
Objectives: This preliminary study aimed to establish an adapted image processing for 7T FLAIR* and quantify WM lesions with a central vein in MS patients scanned using 7T FLAIR* without contrast agent.
Methods: Four patients with RRMS (mean age 40 ± 5, EDSS 1.5-6.5) provided informed consent and underwent 7T MR imaging. Image acquisition included the following sequences: 3D T2-weighted FLAIR [0.8 mm isotropic resolution, acquisition time (AT) = 6 min], and a custom-built 3D T2*-weighted segmented echo-planar imaging (EPI) [0.5 mm isotropic resolution,

AT = 3 min 40 s]. The input images (FLAIR and T2*-weighted) were processed using a multi-step, in-house registration and bias-field correction. The FLAIR* images were then created by multiplying the coregistered outputs. An experienced neurologist then counted lesions within the final FLAIR* volumes, marking each lesion as either containing or not containing a central vein.

Results: A total of 141 white matter lesions were identified in the cerebrum of the four MS patients (mean 35.3 ± 23.1). Of those lesions, 136 (96.4% of total lesions) were marked as having a central vein on FLAIR*. The lesions without visible veins were very small (median size 12mm^3) and primarily located in the upper cerebrum.

Conclusions: This pilot study indicates that FLAIR* images can be collected at 7T and processed to allow clinicians to detect reliably central veins inside MS lesions. Here, almost all of the white matter MS lesions appeared venocentric. Further study will include a larger cohort of MS patients.

P524

Exploring the link between resting-state functional connectivity in the default mode network and subpial pathology in MS using multimodal 7 Tesla MRI

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Background: Changes in resting state functional connectivity (rsFC) in the default-mode network (DMN) represent a potential biomarker for clinical disability in multiple sclerosis (MS). Cortical areas part of the DMN are also frequently affected by subpial demyelination. Previous studies suggested that cortical atrophy may influence DMN rsFC in MS. However, given that cortical atrophy may result from pathology in both gray and white matter (WM), the link between subpial pathology and rsFC in MS remains elusive.

Objectives: We investigated rsFC in the DMN at 7T MRI using a seed-based approach from the posterior cingulate cortex (PCC), the most affected DMN area in MS, to determine the relationship between rsFC and regional subpial pathology as a function of cortical depth, as measured by T2* relaxation rates from ultra high resolution 7 Tesla (T) MRI.

Methods: Fifteen patients (2 Early MS, 8 RRMS, 5 SPMS) and 7 age-matched controls underwent 7T multi-echo T2* imaging (0.33×0.33×1 mm^3) for T2* maps; 7T resting state BOLD functional MRI (1.2×1.2×1.2 mm^3, TR=5s, 90 frames); and 3T MRI for acquisition of T1-weighted images optimized for cortical surface reconstruction using FreeSurfer. T2* maps were registered to cortical surfaces, and sampled along the cortex at 25%, 50%, and 75% depth from pial surface. The PCC seed was defined according to Yeo's cortical functional parcellation atlas in FreeSurfer, and rsFC z maps were computed using FSFAST pipeline. We explored in the 34 cortical regions from Desikian atlas 1) differences in rsFC between patients and controls using a Wilcoxon test, 2) the correlation between FC and mean T2* at different cortical depths in MS using Spearman correlation.

Results: Patients showed relative to controls decreased rsFC in several cortical regions including left caudal anterior cingulate ($p=8.10^{-4}$), left superior frontal gyrus, right precuneus, right pars opercularis and right frontal pole ($p< 0.05$). Increased rsFC in patients versus controls was also found in the right cuneus and right superior parietal gyrus ($p< 0.05$). In the left precuneus, rsFC decrease was associated with T2* increase (underlying cortical demyelination and decrease of iron content) at 25%, 50% and 75% depth from pial surface ($p< 0.05$).

Conclusions: Although local subpial pathology correlated with rsFC in one region part of the DMN, different pathological processes other than subpial demyelination might contribute to DMN rsFC changes in MS. These include compensatory mechanisms and the contribution of WM injury.

P525

Brain volume loss during the first year of interferon-beta treatment: baseline inflammation and regional brain volume dynamics

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Background: Some of the available treatments for multiple sclerosis (MS) have demonstrated to improve the curve of brain atrophy compared to placebo. These results may sometimes be difficult to interpret due to the pseudoatrophy effect. We have recently studied brain volume changes on natalizumab treatment assessing the effect of baseline gadolinium-enhancing (Gd+) lesions on its evolution. We found that brain volume loss occurred at a different rate in patients with and without baseline Gd+ lesions, mainly due to white matter changes.

Objectives: We aim to assess brain volume changes occurring under interferon-beta (IFNβ) treatment, and to confirm our previous findings on early white matter changes in an independent cohort.

Methods: From a prospective, ongoing cohort of all MS patients starting IFNβ treatment as a first line therapy in the MS Centre of Catalonia, 105 patients were included. A brain MRI was performed at baseline (not more than 3 months prior) and 12 months after therapy onset in all patients. Statistical Parametric Mapping 8 software was used for volumetric analysis. Brain parenchymal volume (BP), grey and white matter volumes (GM and WM) at baseline and follow-up were obtained, allowing calculation of percentage changes (BPPC, GMPC and WMPC). Descriptive statistics and lineal regression models were performed.

Results: Nineteen patients were excluded due to missegmentation and two patients were excluded due to outlier values in gadolinium counts, leading to a final cohort of 84 patients. Demographic and clinical characteristics at therapy onset are as follows: mean age was 33.6 years (SD 8.7), median disease duration was 2.8 years (0.3-14), and median annualized relapse rate was 1.5 (1-3.5), and median EDSS was 1.5 (0-6). Forty-nine patients (58.3%) had baseline Gd+ lesions with a median number of 1 (0-18). Mean BPPC, GMPC and WMPC during the first year were -0.52 (SD

1.39), -0.79 (SD 2.05), and -0.24 (2.71) respectively. The regression analysis showed (adjusted by number of Gd+ at follow-up MRI, age, disease duration and baseline EDSS) that baseline number of Gd+ lesions significantly predicted BPPC and WMPC (p=0.013 and p=0.003, respectively) but not GMPC (p=0.777).
Conclusions: Baseline inflammation affects brain volume dynamics during the first year of IFNβ therapy mostly due to white matter loss. Baseline inflammation has to be taken into account when interpreting early brain volume changes on therapy.

P526
Statistical estimation of quantitative T1 maps using standard clinical modalities
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Background: Quantitative T1-mapping measures longitudinal relaxation times (T1) and can be used to study differences within normal appearing brain tissue. Many studies have documented increased T1 associated with multiple sclerosis (MS) disease progression in normal appearing white and gray matter. T1-maps hence provide valuable information for studying the progression and treatment of MS. However, T1-maps are often more time-consuming and difficult to obtain than standard clinical images and are thus not included in standard clinical protocols. A method for estimating T1-maps from commonly available MR modalities would greatly, and retroactively, increase the availability of T1-maps available for research.
Objectives: We aim to design a normalization procedure and statistical model to estimate T1-maps using only T1-weighted (T1w), T2-weighted (T2w), proton density-weighted (PDw), and fluid attenuated inversion recovery (FLAIR) volumes. Our estimated maps should accurately reconstruct observed group-level differences in T1 between MS patients and controls and between MS subtypes.
Methods: Two datasets were used to evaluate the performance of our T1-map estimation model. Both datasets included MRI studies from subjects with relapsing-remitting MS (RRMS), primary progressive MS (PPMS), and secondary progressive MS (SPMS). All studies included a T1-map, T1w, T2w, PDw and FLAIR volumes. Model training and cross-validation was performed on Dataset 1, and model validation was performed on Dataset 2. Each modality (excluding T1-map) was intensity normalized using z-score normalization (Shinohara et al. 2011) with respect to extra-cerebral soft tissue. This procedure extracts quantitative information from the unitless clinical modalities. Within each tissue class, a voxel-wise spline regression model was fit relating the normalized T1w, T2w, PDw and FLAIR intensities to the T1-map intensity.
Results: Both the acquired and estimated T1-maps showed SPMS patients having statistically significant differences in T1 compared with PPMS and SPMS patients in lesions. The group difference in median T1 was 155.1 ms using the acquired T1-maps and 75.3 ms using the estimated T1-maps.
Conclusions: We have proposed and validated a method for estimating quantitative T1-maps using commonly available scans. Following further validation, this method can be used to greatly increase the availability of T1-maps for research of MS and other neurodegenerative diseases.

P527
1-H MRSI in patients with relapsing multiple sclerosis at 7 Tesla
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Background: Ultra high-field MR spectroscopic imaging has the potential to provide mechanistic insights in multiple sclerosis (MS). With improved detection of myo-Inositol (mI), glutamate (Glu), glutathione (GSH), GABA, glutamine (Gln), and glycine (Gly) at 7T, it is possible to investigate these molecular markers of inflammation, axonal injury and repair in MS.
Objectives: To compare brain metabolite levels in patients with relapsing MS (RMS) and healthy controls.
Methods: 639 1cc-voxels were studied in 14 subjects (7 RMS patients enrolled in a phase III clinical trial of ocrelizumab (Roche ORCHESTRA programs) and 7 healthy controls). 3D H-1 MRSI was obtained at 7T. Quantification with LCModel provided valid estimates for mI, Glu, tCho, GSH, NAA, Glx (Glu+Gln), mI-Gly, GSH in > 90% of voxels. The regions included 361 white matter (WM) voxels from controls, 187 normal-appearing white matter (NAWM) and 91 T2 lesion (T2L) voxels from patients. These results were computed based on ratios to Creatine (Cr). Wilcoxon rank sum tests were used to compare regions and spearman correlation tests were used to compare metabolites.
Results: Compared to WM, NAWM had (< NAA/Cr***, >mI/Cr**, >GSH/Cr**, Glu/Cr and tCho/Cr not significant) and T2L had (< NAA/Cr***, > mI/Cr***, >tCho/Cr ***, < GSH/Cr*, < Glu/Cr*) where ***:p< 0.0001; **: p< 0.001; *: p< 0.05. Within WM, NAMW, and T2L respectively, NAA/Cr correlation coefficients were -0.26, -0.25, -0.61 vs mI/Cr and -0.32, -0.38, NS vs GSH/Cr. No significance was found on Gln/tCr, GABA/tCr and Glx/tCr between regions. Additional correlation were found in control WM for NAA/tCr with GABA, r=0.43 and Gln/tCr, r=0.38.
Conclusions: This study demonstrated the application of 7T H-1 MRSI to patients with RMS and healthy volunteers. We found the expected decrease in NAA and increase in mI - but also show that these metabolites are inversely correlated in MS. GSH was elevated in NAWM while decreased in lesions, although inversely correlated with NAA in both lesions and NAWM. When conditioned on NAA, we found a strong (p< 0.0001) decrease in GSH in T2L compared to both NAWM and WM. This observation

suggests that oxidative stress may be better understood in functioning axons. We reported this preliminary data in relative concentration to tCr; further analyses will study absolute concentrations. The patients in this study are being followed longitudinally for changes in metabolite levels and lesion evolution.

P528

Utilization of routine magnetic resonance imaging in multiple sclerosis patient management

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Background: Magnetic Resonance Imaging (MRI) is an important tool in establishing MS diagnosis, as well as monitoring the course of disease and response to therapy. However, little is known about its impact on therapeutic decision- making in clinically stable patients who are receiving disease modifying therapy (DMT).
Objectives: To determine the utility of MRI in therapeutic decision-making in clinically stable patients with relapsing multiple sclerosis (RMS), treated with first-line DMT.
Methods: A retrospective chart review was performed, by a trained RN, of all MRIs obtained between January 1, 2009 and December 31, 2012 at Providence MS Center. Inclusion criteria were patients with RMS who had been treated with Interferon beta (IFN) or glatiramer acetate (GA) for 12 months or longer. Types, indications, and results of MRI, and whether change of DMT occurred as a result of the scan were documented and analyzed in the study.
Results: The mean age and disease duration of the 518 patients included were 51.2±11.7 and 10.7±7.4 years, respectively. The majority were female (82.2%), had RRMS (94%), and used IFN (79.2%). On average 0.85 MRI were done per patient per year. A total of 1,357 scans were obtained: 64.2% brain, 24.8% cervical spine and 10.5% thoracic-lumbar spine. Of those, 886 met the definition of "routine" MRI, performed in patients who have been clinically stable. Changes were observed on 84 (9.4%) of these scans: 82.3% had new, 22.3% had enhancing, and 14% had enlarged lesions. But only 19 of the 84 changed scans (22.6%), or 2.3% of all routine scans, led to a change in the DMT used.
Conclusions: It is evident from the results obtained in this patient cohort that one should question the practice of obtaining routine MRI scanning in clinically statle patients who have been receiving DMT. The use of routine MRI has, at least in part, been supported by radiologic evidences of ongoing disease activity in patients who appeared to be clinically stable. However clinical trials have shown that most approved DMTs have a robust effect on suppressing MRI disease activity and thus the low yield on MRI in DMT treated patients should not be surprising. Given the relatively minor clinical impact and the high monetary costs of MS care, the savings that could be achieved by reduction of "routine" MRI is substantial and important in this era of diminishing healthcare resources.

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Basal ganglia iron in multiple sclerosis patients measured with 7T quantitative susceptibility mapping (QSM) correlates with inhibitory control task

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Background: Iron increase in the basal ganglia (BG) of MS patients measured by T2 hypointensity has been associated with physical and cognitive disability and correlates with EDSS and brain atrophy. BG are implicated in action selection by influencing/modulating activity of the motor cortex and descending motor pathway through inhibitory control.
Objectives: The objective was to examine the association between BG iron and performance on measures of inhibitory control. Given the functional significance of BG to engage in task-relevant information, while ignoring task-irrelevant information, we hypothesized a negative association between iron deposition and measures of inhibitory control.
Methods: With IRB approval, 22 RRMS patients age 30-60y had 7T MRI (TR/TE/flip= 23ms/4-20ms/5, 0.5mm isotropic). Quantitative Susceptibility Maps (QSM) were computed using a novel Wiener filter. Globus pallidus, putamen and caudate were manually traced. All patients underwent comprehensive neuropsychological testing involving assessment of working memory, episodic memory, selective attention, and inhibitory control. Specifically, we examined associations between iron and interference scores on the Stroop and Flanker tasks. The Stroop task involves presenting list of colored words, and requires responding to the color of the word, while ignoring the identity of the word (congruent, incongruent and neutral conditions). The Flanker task presents five arrows, and participants are asked to respond to the direction of the central arrow, while ignoring the flanking arrows (congruent, incongruent conditions). The dependent variables are reaction time (RT) and accuracy (Acc) interference scores measuring greater conflict in the incongruent, relative to the congruent condition for both tasks. QSM and measures of inhibitory control were correlated using Spearman's rho (one-tailed).
Results: Our novel QSM processing retained anatomic detail with only minimal streaking and blurring artifact. Small vessels and regional variability were seen in BG. Mean susceptibility values for the ROIs had 5-19% error and correlated with age and EDSS. Higher iron deposition in globus pallidus and caudate was associated with RT interference score on the Flanker, but not the Stroop task.
Conclusions: We have preliminary support for an association between BG iron and performance measures of inhibitory control. Future directions of the ongoing study include a more parsimonious examination of iron deposition with nuanced measures of inhibitory control.

P530

Predictor status of disease modifying therapy time-exposure to brain atrophy using NeuroQuant

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Background: Brain atrophy in multiple sclerosis (MS) occurs at a faster rate than the normal population. Disease modifying therapies (DMTs) have demonstrated the ability to alter the clinical and radiographic course of MS. However, access to objective volumetric imaging has been limited to research labs until the advent of NeuroQuant—a fully automated volumetry platform for conventional MRI.

Objectives:

1) Describe the clinical characteristics, DMT exposure, and magnetic resonance volumetry (vMRI) data in our heterogeneous MS cohort.
2) Assess the relationship of brain volume to physical disability.
3) Evaluate the independent predictor status of DMT exposure, stratified by first- and second-line agents, to brain volume.

Methods: Retrospective chart review was performed on 484 patients at our MS center. NeuroQuant vMRI metrics were obtained from T1 3D MPRAGE sequences on a 1.5 T Siemens scanner. Expanded Disability Status Scale (EDSS) was extracted to quantify physical disability. Potential predictor variables of brain volume screened: age, gender, intracranial volume (ICV), disease duration, progressive disease status, and patient-year DMT exposure. Cross-sectional analysis using multilinear regression was applied on candidate predictors to total brain volume. SAS v9.4 was used for all analyses.

Results: Cohort characteristics (n=484): women, 80%; age, 46.9+/-11.2 years; progressive form of MS, 19%; EDSS, 2.2+/-1.9; and disease duration, 12.6+/-9 years. Mean brain volume is 1668+/-178 cm^3. Cumulative first- and second-line DMT exposure: 2582 and 324 patient-years, respectively. Significant correlation of brain volume to EDSS, rho= -.29 (p-value < .001). Adjusted predictors of brain volume [beta coefficient (p-value)]: ICV 1.15 (< .001), progressive MS status -50 (< .001), first-line -2.2 (.46), and second-line DMT exposure -7.4 (.11).

Conclusions: NeuroQuant vMRI metrics are easy to obtain in routine clinical practice and correlate brain atrophy with physical disability. Independent predictors of brain volume include ICV and progressive disease status which suggests that NeuroQuant may help identify relevant neurodegenerative tissue changes of the brain in routine clinical practice. Exposure-time on DMT is not an independent predictor of brain volume and may suggest a beneficial effect of DMT in mitigating the rate of whole brain atrophy in MS. Longitudinal evaluation of vMRI metrics is needed for further validation of NeuroQuant in MS.

P531

Depressive symptoms in MS patients are associated with abnormal hippocampal resting state functional connectivity

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Background: Previous studies have shown an association between depression and hippocampal atrophy in patients with multiple sclerosis (MS).

Objectives: Aim of this study was to investigate the interaction between depressive symptoms and hippocampal resting state (RS) functional connectivity (FC) in MS.

Methods: RS fMRI, dual-echo and 3D T1-weighted scans were obtained from 36 MS patients and 42 matched healthy controls (HC). The severity of depressive symptoms was assessed with the Montgomery-Asberg Depression Rating Scale (MADRS) and used to stratify patients into depressed (D, MADRS $\geq$ 9) and non-depressed (nD, MADRS < 9). RS FC analysis was performed by computing the cross-correlation between left (L) and right (R) hippocampi (derived from FIRST segmentation) and any other area of the brain. Between-group differences of hippocampal RS FC, and correlations between RS FC and MADRS scores were evaluated using SPM8 and analyses of variance and linear regression models.

Results: Eighteen (50%) patients were D. The main clinical and demographic characteristics did not differ between D and nD MS patients. Compared to HC, MS patients had significant atrophy of the whole brain and bilateral hippocampi, without significant differences between D and nD MS patients. Compared to HC, MS patients experienced a distributed reduction of hippocampal RS FC, both for the right and the left hippocampus. Compared to nD, D MS patients showed reduced RS FC between both hippocampi and the R inferior temporal gyrus. They also experienced increased RS FC between the L hippocampus and the L inferior parietal lobule. Significant correlations were found between RS FC modifications and MADRS scores.

Conclusions: Depressive symptoms in MS are related to RS FC alterations within limbic-neocortical networks independently from the presence of hippocampal atrophy.

P532

New insights on the pathophysiology of fatigue in MS: a fMRI study of the motor network

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Background: Functional imaging studies have shown an abnormal recruitment of the network connecting the basal ganglia to the frontal lobes in MS patients with fatigue.

Objectives: Using a motor task during functional MRI (fMRI), we wished to provide some additional insights into the pathophysiology of fatigue in MS by investigating its functional correlates according to the presence of cognitive or motor fatigue and the

influence of disease duration on these findings. Adaptation of motor network recruitment during prolonged effort were also assessed.

Methods: fMRI scans during a simplex motor task were acquired from 79 MS patients (50 with fatigue [F] and 29 without fatigue [nF]) and 26 matched healthy controls (HC). In all patients, the severity of cognitive and physical fatigue was rated using the Modified Fatigue Impact Scale (MFIS). Full factorial and multiple regression analyses were performed with SPM8. A time modulation analysis was also performed.

Results: Compared with HC, both nF and F-MS patients had increased activations of the right precentral gyrus, right inferior temporal gyrus (ITG) and bilateral cerebellum. Compared to HC and nF patients, F-MS patients had reduced activations of the left middle temporal gyrus, left supplementary motor area (SMA), bilateral superior frontal gyrus, left postcentral gyrus, left putamen and bilateral caudate nucleus. They also showed selective increased activation of the right middle frontal gyrus (BA46). This latter finding was mainly driven from F MS patients with disease duration longer than 5 years. In HC, the time modulation analysis showed a reduced activity of the SMA and right precentral gyrus and increased activity of the basal ganglia over time. Such a behaviour was significantly impaired in F MS patients. In MS patients, higher activity of the right MFG was related to cognitive ($r=0.39$; $p< 0.001$) and physical MFIS score ($r=0.36$; $p< 0.001$). Physical MFIS score was also related to reduced activity within the right thalamus and SMA.

Conclusions: Distributed abnormalities of activation as well impaired timing of communication between different areas of the motor network occur in MS patients with fatigue. The involvement of specific GM regions, only partially related to disease duration, may contribute to explain differences between cognitive and motor fatigue in these patients.

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Correlation analysis of *in vivo* MRI and post-mortem quantitative immunohistochemistry data in a mouse model of inflammatory cerebral demyelination

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Background: Diffusion tensor imaging (DTI) and magnetization transfer imaging (MTI) are increasingly utilized in multiple sclerosis (MS) clinical trials. The relationship between these MRI measures and underlying pathology, however, remains obscure. In order to maximize the utility of these imaging biomarkers and most appropriately guide the clinical development of novel therapeutics, we have performed a rigorous correlation analysis between *in vivo* MRI measures and post-mortem quantitative immunohistochemistry (qIHC) data in mice with focal inflammatory/demyelinating cerebral lesions.

Objectives: The objective of this study was to determine the neuropathological correlates of DTI and MTI measures in a mouse model of inflammatory cerebral demyelination.

Methods: Experimental autoimmune encephalomyelitis (EAE) was induced in C57BL/6 mice (n=12) using MOG_{35-55} peptide emulsified in CFA containing *M. tuberculosis*. Seven days post-EAE induction, mice underwent unilateral stereotaxic injections of TNF-α and IFN-γ into the left corpus callosum. Two weeks post-EAE induction, 3D DTI and MTI images were acquired using a 7T MRI scanner. MR images were processed using fully-automated software (NIGHTWING™, Biospective Inc.) to generate DTI fractional anisotropy/diffusivity and MTR maps. Following euthanasia, brains were serially-sectioned and stained to assess myelin density, microglia/macrophages inflammation, and vasogenic edema. The IHC sections were digitized, reconstructed into 3D qIHC volumes, and spatially normalized to the MRI data (PERMITS™, Biospective Inc.). Lesion-based correlation analyses were performed between MRI and qIHC measures.

Results: All mice developed MRI-visible, focal inflammatory/demyelinating lesions in the vicinity of the corpus callosum. Using linear model analysis of the MRI measures within the demyelinated corpus callosum, there was significant contribution of both demyelination and the presence of microglia/macrophages on the MTR signal ($P_{MBP} = 0.0062$, $P_{IBA1} = 0.042$), whereas only demyelination contributed significantly to the DTI-FA signal ($P_{MBP} = 0.0081$, $P_{IBA1} = 0.253$).

Conclusions: The seamless combination of *in vivo* MRI and post-mortem 3D qIHC data is a valuable strategy for interrogating the relationship between quantitative neuroimaging and gold-standard quantitative neuropathology measures. This unique approach is expected to provide improved interpretation of MRI data and guide the rational use of imaging biomarkers in MS clinical development.

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Automatic multiple sclerosis brain lesion localisation and volumetry

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Background: The presence and location of white matter lesions on magnetic resonance imaging (MRI) are important criteria for diagnosis, follow-up and prognosis of multiple sclerosis. Quantitative values such as lesion volumetry are expected to have high impact in clinical practice. However, manual lesion quantification is time-consuming, costly, and suffers from observer variability.

Objectives: We propose an accurate, automatic method for lesion quantification based on MRI, which is independent of the scanner and acquisition protocol and does not require a validated patient population.

Methods: 3D T1-weighted and FLAIR MR images are used simultaneously in a probabilistic model to segment the brain tissue into grey matter, white matter and cerebrospinal fluid using expectation-maximization, allowing for an outlier class in order to accommodate the lesions. Lesion segmentation is performed based on prior knowledge that lesions appear hyperintense on FLAIR images.

20 multiple sclerosis patients participated in a study at VU University Medical Center, Amsterdam, the Netherlands. They

were scanned on a 3T whole body scanner (GE Signa HDxt, Milwaukee, WI, USA). Expert lesion identification and manual segmentation was performed based on the FLAIR images by a highly trained neuroradiological team.

The total volume of the lesions in the brain is computed for both the automated method and the expert lesion segmentation. We report the mean, standard deviation and range of the total lesion volume over all patients, as well as the overlap between both segmentations. The consistency between the two methods is assessed by the intraclass correlation coefficient (ICC).

Results: The automatic method provides lesion volumes that are in agreement with those obtained by manual tracing. The total lesion volume obtained from the automatic segmentation is 10.02 ± 6.91 mL (range: 1.32 to 26.04 mL), while that of the manual segmentation equals 13.78 ± 8.31 mL (range: 1.88 to 28.99 mL). The overlap between the automated and manual lesion segmentation is 62.96 ± 14.61%. The ICC equals 68.42% indicating a good consistency between the automated and expert labelling.

Conclusions: We propose a fully automatic method that is generally applicable and allows consistent and reliable volumetry of lesions in the whole brain or in different brain regions. Complementary information is thus provided to the MS clinicians, supporting early diagnosis. Moreover, segmentation of new lesions and volume changes of old lesions over time is also possible.

P535

Optimal detection of infratentorial lesions with a combined dual-echo sequence: ´P2T´

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Background: Multiple sclerosis (MS) lesions typically develop in the periventricular, juxtacortical, and infratentorial white matter of the brain. Magnetic resonance imaging (MRI) sensitively detects white matter lesions (WML). Fluid-attenuated inversion recovery (FLAIR) allows easy discrimination of supratentorial WML from cerebrospinal fluid, but traditional FLAIR scans are less sensitive to infratentorial lesions. Dual-echo, T2-weighted (T2w) and proton density (PD) sequences are more sensitive than FLAIR for visualizing infratentorial WML, but image quality and conspicuity of infratentorial lesions still need to be improved.

Objectives: To combine the contrast of PD and T2w sequences for improvement of infratentorial image quality and better assessment of infratentorial WML.

Methods: Participants with clinically defined MS underwent a comprehensive 3T-MRI protocol. Whole brain, 2D dual-echo T2-weighted fast spin echo scans were acquired (slice thickness, 3mm; acquisition time, 1 minute 57 sec; repetition time, 5320 msec; echo time 1, 23 msec; echo time 2, 116 msec). T2-w and PD sequences were averaged, resulting in a new image, "P2T". Each of two trained raters evaluated the 3 sequences separately. Qualitative assessment was performed for supratentorial and infratentorial image quality and lesion conspicuity using a grading system.

Results: MRI data from 16 patients (8 women; mean age, 42 years) were analyzed. All lesions visible on PD and T2w were also seen on P2T. Furthermore, P2T received high grades for infratentorial and supratentorial image quality, superior to T2w ($P < 0.001$) and PD ($P < 0.001$). P2T received higher grades than T2w for infratentorial ($P < 0.001$) and supratentorial ($P < 0.001$) lesion conspicuity. P2T also had better grades than PD for infratentorial ($P < 0.001$) and supratentorial ($P < 0.001$) lesion conspicuity.

Conclusions: This preliminary study showed that "P2T" imaging, which combines both acquired contrasts in the dual-echo sequence, provides better image quality and higher lesion conspicuity than PD and T2w alone in both infratentorial and supratentorial brain. As the infratentorial brain is one of the cardinal compartments for diagnosis of MS, use of this post-processing technique, which requires no additional acquisition time, could improve our ability to diagnose and monitor the disease and is feasible in the clinical setting.

P536

Disruption of brain functional reorganization leads to disability progression in multiple sclerosis

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Background: Functional MRI studies have demonstrated consistent reorganization of brain networks in patients with multiple sclerosis (MS), with complex relationships with disability.

Objectives: We aimed to assess in this longitudinal resting-state functional MRI (rs-fMRI) study the potential relationships between disability progression and alteration of brain functional topology using graph theoretical approach.

Methods: In the first step of the study, we assessed the stability over time of graph metrics derived from rs-fMRI data performed on 6 healthy subjects at baseline and at year 2. The three main functional connectivity metrics characterizing the brain network topology namely the long-range connectivity (nodal efficiency, Enod), the short-range connectivity (local efficiency, Eloc) and the density of connections (degree, k) were assessed. Connectivity metrics in healthy controls remained stable over time. In the second step of the study, 43 patients with relapsing-remitting MS (mean age 37±5.1 years, mean disease duration 10±3 years) were included. They underwent rs-fMRI at baseline and at two years.

Results: At baseline, significant increase of global brain connectivity metrics were evidenced in patients (for Enod, p=0.040, Eloc, p=< 0.001, and k, p=0.030). During the follow-up period, Enod and k of patients decreased to reach normal values at year 2. Between baseline and year 2, Eloc decreased significantly but remained higher than normal values (p< 0.001). At the regional level, long-range functional connectivity and density of connections reflected by Enod and k respectively were increased mainly in hubs at baseline, whereas high short-range connectivity reflected by Eloc was equally distributed throughout the brain. Finally, the decrease in the reorganization of brain functional topology during the follow-up period was associated to the progression of disability in patients (rho=0.30, p=0.02).

Conclusions: This longitudinal rs-fMRI study evidenced that brain functional reorganization characterized by widespread higher functional connectivity, involving mainly the hubs,

dramatically decreased with disease progression. The disruption of reorganization in brain functional topology was significantly associated with disability progression suggesting that compensatory mechanisms progressively failed with the course of MS.

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Combined DIR and PSIR images improve detection and classification of cortical lesions in multiple sclerosis and clinically isolated syndromes

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Background: Cortical gray matter lesions (CL) constitute a relevant aspect of multiple sclerosis (MS) pathology. Studies based on double inversion recovery (DIR) images have demonstrated an association between CL load and both cognitive and physical disability in MS patients. However, DIR detects only 20% of pathologically confirmed CL. Recently, phase-sensitive inversion recovery (PSIR) was demonstrated to be more sensitive and accurate that DIR in detecting CL.
Objectives: We combined DIR and PSIR images to analyse the frequency and the type of CL in MS patients having different disease duration (DD) and degree of disability, including patients with clinically isolated syndromes suggestive of MS (CIS).
Methods: 30 patients (20 MS, 10 CIS) were included in the study (F/M=2.0; DD range: 0.2-22 years; age range: 21-50 years; EDSS range: 1-6.0). DIR and PSIR images were acquired by a 3T MRI (Philips) and inspected by 3 experts, with identification of CL by consensus. PSIR and DIR images were jointly used to classify lesions as purely intracortical, mixed gray-white matter (leukocortical), and juxtacortical. The difference in the number of lesions detected by PSIR and DIR in each category was analyzed.
Results: Combined DIR/PSIR images allowed: 1) the identification of CL in 100% of the patients, independently of DD and EDSS score, 2) the detection of CL in all the CIS suggestive of MS (with dissemination in space of lesions), 3) a significant increase (>60%) in the number of detected CL (intracortical+leukocortical) compared to DIR alone, 4) the re-classification of about 15% of CL detected by DIR. Moreover, PSIR allowed the identification of 233% more leukocortical lesions, 30% intracortical and 53% juxtacortical compared to DIR.
Conclusions: Our data further strengthens previous observation and shows that the combination of DIR and PSIR images allows the detection of a significantly greater number of CL than DIR alone. Moreover, we confirm the higher accuracy and sensitivity of PSIR in classifying the types of lesions. The presence of at least one intracortical lesion in all CIS patient further points out the relevance of cortical pathology in MS. DIR/PSIR images may be useful when a diagnosis of MS is suspected but not confirmed and may allow a better stratification of the patients for clinical studies.

P538

Compensatory remapping of fMRI functional connectivity during resting state in multiple sclerosis

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Background: Altered functional connectivity appears to be a robust feature in multiple sclerosis (MS). Compensatory mechanisms, whereby functional connectivity between brain regions is enhanced to compensate for disrupted connectivity elsewhere may represent an independent therapeutic target to minimize overall disability.
Objectives: To determine if there are robust alterations in fMRI connectivity patterns in patients with relapsing multiple sclerosis (RMS) compared to age and gender matched healthy controls.
Methods: Twenty-five RMS patients enrolled in a Phase III clinical trial of ocrelizumab (OPERA) and 29 age- and gender-matched controls were scanned at baseline. Resting state fMRI scans were acquired with resolution 80x80 mm, 36 slices, 240 volumes and TR 2000 ms (8 min). Cortical parcellation was performed in Freesurfer and 38 ROIs, including cognitive regions, were chosen for connectivity analysis. Data preprocessing was performed with in-house scripts which partially included SPM and FSL (FMRIB) functions. Connectivity analysis was derived using a PCfdr-initialized Bayesian Network (BN) learning approach. The group-wise false-discovery rate was set at 5%. In addition, simple correlation results were calculated to compare to the BN approach.
Results: RMS subjects had increased overall connectivity compared to controls ($p < 0.001$) using correlation approach. However, the strength of overall connectivity from the BN approach was comparable between groups (connection strength of MS/normal: $0.26 \pm 0.22/0.24 \pm 0.23$). These results suggest a fundamental remapping of connectivity involving inferior prefrontal regions: in controls this area was connected to superior frontal and medial orbitofrontal regions, whereas in RMS subjects it was connected to anterior cingulate and posterior parietal regions. In posterior cingulate regions, connections to/from orbitofrontal and precuneus regions in controls were connected to supramarginal regions in RMS.
Conclusions: The results emphasize the importance of distinguishing direct *vs* indirect (co-activation) connectivity in MS, which is possible with the BN approach but not with correlation analysis. Our observations suggest that the increased overall co-activation in MS is a result of de-differentiation of normally focal connectivity patterns between discrete regions. The co-activation changes in RMS likely represent compensatory, broadly-enhanced connectivity of still intact pathways.

P539

Permeability of the blood-brain barrier predicts conversion from optic neuritis to multiple sclerosis

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Background: We have previously reported increased permeability of the blood-brain barrier (BBB) in normal appearing white matter (NAWM) in patients with multiple sclerosis (Cramer et al.

2013). 50% of patients with Optic Neuritis (ON) later develop MS (Marques et al. 2013).

Objectives: We aimed to investigate if BBB permeability can predict later conversion to MS according to the McDonald 2010 criteria. Furthermore, we aimed to validate our MRI measure of BBB permeability by correlating with inflammatory markers from the cerebrospinal-fluid (CSF); leukocyte count, IgG-index and biomarkers for inflammation and cellular trafficking, namely matrix-metallo-protease-9 (MMP-9), C-X-C chemokine ligand 10 (CXCL-10) and CXCL-13 in ON patients and healthy controls (HC).

Methods: Dynamic contrast-enhanced MRI at 3T (Larsson et al. 2009) was used to measure BBB permeability in 31 ON patients, all referred for MRI as part of diagnostic workup at time of diagnosis. Measurements were compared with 17 HCs matched for age and gender. Patients had MRI and lumbar puncture performed within 2 weeks of onset of ON symptoms.

Results: We found a significantly higher (p=0.005) permeability of the BBB in periventricular NAWM in ON patients compared to HCs. In normal appearing grey matter and thalamic tissue, no such difference was found. Pooling ON patients and HCs we found that BBB permeability correlated (linear regression) with the following CSF markers: Leukocyte count (log10), R^2=0.30; p=0.0002 and CXCL10 (log10), R^2=0.15; p=0.01. In ON patients with conversion to McDonald MS within 2 years after initial diagnosis of ON we found significantly higher BBB permeability in periventricular NAWM (2-tailed T test; p=0.015).

Conclusions: Our results indicate a higher degree of leakiness of the BBB in ON patients who later develop MS, emphasizing the importance of very early BBB pathology in NAWM. However, ON patients who did not convert to MS also had significantly higher permeability when compared to HCs, indicating that BBB permeability is also a marker of non-specific CNS inflammation, although it must be kept in mind that the observation period was only two years. We find a significant correlation between BBB permeability in NAWM and several CSF markers of inflammation and cellular trafficking, which seems to validate BBB permeability as a marker of CNS inflammation. Measuring BBB permeability could be an important supplementary prognostic marker for determining later conversion from ON to MS.

P540

Comparison of visual conspicuity between two contrast-enhanced T1-weighted sequences in the detection of multiple sclerosis lesions with 3.0T MRI

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Background: It is not well established whether gradient-echo (GE) or spin-echo (SE) is the sequence of choice to detect enhancing multiple sclerosis (MS) lesions with 3.0T MRI.

Objectives: The aim of this study was to compare the ability of these two sequences to detect active MS lesions.

Methods: 100 MS patients (73 women; mean age, 35.8 years; age range [23, 50] years, median EDSS, 3; EDSS range, [0, 8]), underwent 3.0 T brain MRI (Trio, Siemens) including enhanced GE and SE T1-weighted sequences. GE images were acquired 15 minutes after injection of a double dose (0.2 mmol/kg) of gadobutrol. In half of the patients, SE images were acquired just before, and in the other half just after, acquisition of the GE images. To define the gold standard, an experienced neuroradiologist identified and marked contrast-enhanced MS lesions in these sequences using Jim 5.0 software. Each of the two sets of contrast-enhanced T1W scans was then evaluated in a random order by three experienced neuroradiologists. The results were compared with the gold standard reference to obtain the number of true-positive (TP), false-negative (FN), and false-positive (FP) evaluations. Quantitative assessment of lesion conspicuity and the effect of spatial location were based on image contrast and the contrast-to-noise ratio, and division of the intracranial region into four quadrants in each slice.

Results: We found 607 MS lesions (105 periventricular, 274 subcortical, 165 juxtacortical, and 63 infratentorial). Most lesions showed a nodular pattern of contrast uptake (nodular, 527; ring, 13; open-ring, 34; heterogeneous, 33). We found a better sensitivity to detect lesions with GE images (0.828) than with SE (0.767), and a similar mean number of FPs (GE, 16.33; SE, 16.67). SE images showed a higher image contrast (TP, 0.37; FN, 0.20; FP, 0.25) than GE images (TP, 0.23; FN, 0.11; FP, 0.16), whereas the contrast-to-noise ratio was higher for GE (TP, 37.76; FN, 17.02; FP, 20.71) than for SE (TP, 27.26; FN, 13.69; FP, 14.85). Both comparisons presented statistically significant differences ($p< 0.05$). Finally, for both sequences, most misclassifications occurred in the right posterior quadrant.

Conclusions: Obtained/measured sensitivity to detect MS lesions suggests a better visual conspicuity of lesions in images acquired at 3.0T in GE sequences than in SE. Selection of the best sequence requires the use of image indices that incorporate surrounding complexity of lesions rather than only the image contrast ratio.

P541

Altered resting state homologous inter-hemispheric functional connectivity is related to clinical measures of disabilityin multiple sclerosis

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Background: Homologous regions, symmetric areas of the right and left hemisphere, have high inter-hemispheric resting state functional connectivity (RSFC) in somatomotor and visual cortices in healthy controls (HC). In multiple sclerosis (MS), studies have largely examined inter-hemispheric connectivity of a single seed in the motor area to its homologous point. Establishing whole-brain homologous connectivity provides unique information of functional homotopy between hemispheres and can potentially be a useful tool for detecting inter-hemispheric impairment in MS.

Objectives: To map alternations in whole-brain homologous interhemispheric functional connectivity (HIFC) in relapse-remitting (RR) MS compared to HCs and to relate this functional marker to clinical outcomes in MS.

Methods: Anatomical and RSFC data were collected for 20 RRMS (mean disease duration 8.48 years; median EDSS=2.0, range 1-6.5) and 35 age- and gender-matched HC subjects at 3T. A novel surface-based registration technique aligning cortical folding patterns across both hemispheres was performed on the anatomical images. Preprocessed RSFC was mapped onto the homologous atlas to allow for vertex to homologous vertex inter-hemispheric connectivity over the whole brain using a Pearson's correlation. Data were Fisher Z-transformed to facilitate group comparison. Network HIFC was extracted using a commonly used 7-network parcellation scheme. HIFC was compared between MS and HC using the Mann-Whitney U test. HIFC was correlated with EDSS using Kendall's tau-b.
Results: Mean whole-brain HIFC was decreased in MS compared to controls (0.32 vs. 0.28, p=0.001). HIFC was decreased within regions attributed to the default mode network (p=0.001), dorsal attention network (p< 0.001), frontoparietal control (p=0.005) and visual network (p=0.017). Whole-brain HIFC trended toward correlation with EDSS (tau=-0.32, p=0.06). Combined pyramidal and sensory EDSS functional system scores correlated with somatomotor HIFC (tau=-0.38, p=0.029).
Conclusions: Whole brain HIFC is decreased in MS, particularly in areas represented by the default mode, dorsal attention, frontoparietal control, and visual networks, suggesting widespread inter-hemispheric dysfunction. Alterations of HIFC may have implications for cognitive and motor dysfunction in MS and may be sites for targeted interventions to improve connectivity.

P542

Central and peripheral alterations in glucose uptake in patients with multiple sclerosis during treadmill walking

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Background: Multiple Sclerosis (MS) is an auto-immune like disease characterized by the infiltration of T-cells into the central nervous system (CNS) resulting in demyelination of white matter. The most common motor side effects due to MS are reductions in muscle strength, balance perturbations, and difficulties in ambulation.
Objectives: Identify alterations in CNS and skeletal muscle glucose uptake (GU) in patients with MS during walking using positron emission tomography (PET)/computed tomography (CT).
Methods: Eight patients with MS (4 women) and 8 healthy age/sex matched controls performed 15 min of walking on a treadmill at a self-selected speed. Two min after the start of walking each participant was injected with ≈ 8 mCi of the PET glucose analog tracer [^{18}F]-Fluorodeoxyglucose. Regions of interest where identified on corresponding CT images to quantify skeletal muscle GU and statistical parametric mapping (SPM) was used to quantify GU within regions of the brain. GU is reported in mean standard uptake values.
Results: Global brain GU was lower in patients with MS compared to healthy controls (2.29 ± 2.11 and 2.55 ± 2.38, $P < 0.01$) specifically in the cerebellar (3.91 ± 0.58 and 4.47 ± 0.83, $P = 0.04$) and cerebral (5.18 ± 0.81 and 5.71 ± 1.15, $P < 0.01$) cortices, the thalamus (5.05 ± 0.63 and 5.69 ± 0.93, $P = 0.03$) and the temporal lobe of the cerebrum (4.82 ± 0.74 and 5.32 ± 0.96, $P = 0.02$). Although patients with MS walked at a slower self-selected speed (m/sec) (1.12 ± 0.22 and 1.37 ± 0.13, $P = 0.01$) they demonstrated greater GU of the accessory muscles than the control subjects: knee extensors/flexors (0.83 ± 0.11 and 0.72 ± 0.06, $P < 0.01$ / 1.07 ± 032 and 0.78 ± 0.22, $P < 0.01$), hip flexors (1.33 ± 0.48 and 0.98 ± 0.21, $P = 0.01$) and sartorius (0.97 ± 0.22 and 0.76 ± 0.07, $P < 0.01$).
Conclusions: Increased GU in the skeletal muscles of the legs could lead to an increased cost of walking in patients with MS which may play a role in the reduced GU in certain brain regions responsible for continuous integration of sensory and motor drive during walking. Additionally, alterations in corticospinal tract activation could contribute to the increased difficulty in ambulation seen in many patients with MS. Larger future studies are needed to expand the generalizability of these findings.

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Treatment effect on brain atrophy differs between first and second-line therapies for relapsing-remitting MS: a meta-analytic approach

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Background: Quantification of brain atrophy in relapsing-remitting MS (RRMS) could be a marker of clinical state, disease progression and treatment response but no one study compared effect of first-line and second-line disease-modifying drugs (DMD) on cerebral volume.
Objectives: The aim of this study is to compare effect of first-line and second-line DMD on brain atrophy progression up to 48 months of follow-up through a meta-analytic approach.
Methods: We reviewed all reports in Medline, Embase and Cochrane database about trials in RRMS assessing effect of DMD, including all phase II-III drug trials and reporting data about cerebral atrophy. For each study, number of patients, percentage of brain volume change (PBVC) to baseline, technique of atrophy measurement and DMD were collected. Glatiramer acetate, interferon, teriflunomide, dimethyl fumarate and laquinimod were classified as first-line DMD and natalizumab, fingolimod, daclizumab and alemtuzumab classified as second-line ones. To compare effect of DMD, we used a Generalized Estimated Equation, adjusting for time-trend and group, in the SaS 9.4 software between PBVC and time for first-line, second-line and placebo groups. 2 periods were analyzed, the total one up to 48 months (A) and between month 12 and 48 (B) to correct pseudo-atrophy effect. Secondary analysis kept only randomized trials using SIENA and BPF methods, and health authority's recommendation doses.
Results: We identified 251 studies between January 1, 1995 and August 31, 2013. 111 were analyzed and 35 included representing 17,770 patients. For period A, there was a statistical difference for annual slope of atrophy between second-line DMD and placebo (0.23%/y vs 0.50%/y, p< 0.05), but not between first-line DMD (0.42%/y), and placebo (p=0.13) or between first and second-line

therapies (p=0.41). For period B, annual slope of atrophy was reduced for second-line DMD compared to placebo (0.10%/y vs 0.55%/y, p=0.0001) and first-line DMD (0.46%/y, p=0.005). Secondary analysis, keeping only randomized trials using BPF and SIENA, confirm better atrophy reduction under second-line DMD versus first-line ones and placebo, ($p< 0.005$ and $p< 0.0001$ respectively).

Conclusions: This meta-analytic approach suggests that second-line DMD reduced brain atrophy slope in RRMS whatever the period considered and methods of atrophy measurement. These results suggests that long term atrophy measurement may be a major surrogate marker for DMD effect and offers new insights about mechanisms underlying atrophy process.

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MS subtype classification using lesion geometry and texture

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Background: The assessment of multiple sclerosis (MS) is largely based on clinical scores and only a few quantitative measures derived from magnetic resonance imaging (MRI), such as lesion count and total lesion load.

Objectives: We exploit a large set of quantitative features about individual lesions to perform 5-way classification into one of the five subcategories of MS (relapsing-remitting, primary progressive, secondary progressive, progressive relapsing and clinically isolated syndrome) by using support vector machines (SVMs). In addition to producing an accurate objective classifier, we sought to determine which types of features are most important for successful classification.

Methods: In addition to basic clinical measures (EDSS, PASAT, disease duration) and demographic variables (age, gender), our classifier uses a wide range of lesion-specific measures. Using lesion masks defined on T1w, T2w and T1w-Gad-enhanced images, individual lesions are identified with FSL's cluster program. Lesion geometry is measured with Minkowski functionals, i.e. lesion volume, surface area, mean breadth and Euler-Poincare characteristic (EPC). Lesion "texture" is based on intra-lesion intensities in each respective image type. Lesion-specific values are aggregated into whole-brain or white-matter track-specific summaries (incl. sum, min, max, mean, median and standard deviation). Lesion segmentation was done for all image types and 250 subjects using a strict semiautomatic protocol with two independent raters and review by a third.

Results: Good classification could be obtained with clinical and demographic features alone (average accuracy 39%; chance = 20%). However, even better accuracy was found with the addition of geometric and intensity measures (47.8% accuracy). SVM weights showed that the significance of various features largely depends on the subtypes involved during classification: e.g. median T2w lesion volume has a positive weight for relapsing-remitting MS and a negative weight for primary progressive MS. For most classifiers, T2w features are more important than their T1w counterparts. Additionally, EPC showed higher weights than lesion count; median lesion volume, area and mean breadth were more useful than the respective means; finally, better prediction accuracy was obtained by decomposing scores by ROI tracks than using whole brain summaries.

Conclusions: We found that prediction of MS subtypes can be made more accurate by using different aspects of lesion geometry and intra-lesion intensity measures.

P545

Thoracic spinal cord lesions are influenced by the degree of cervical spine involvement in multiple sclerosis

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Background: Multiple sclerosis (MS) is a demyelinating disease affecting both the brain and spinal cord. Advances in imaging techniques have improved the ability to detect spinal cord lesions, facilitating the diagnosis of MS and the identification of important disability correlates. As the cervical spinal cord is more frequently involved, little is known about its relationship to the presence of lesions within the thoracic spine.

Objectives: To determine if the presence of cervical spine lesions predicts the presence of thoracic spinal cord lesions in patients with MS.

Methods: All MS patients, with MRI studies of the brain, cervical, and thoracic spine obtained during a single scanning session were consecutively acquired during a 1-year period from a single MS clinic. The presence of cervical spine demyelinating lesions was analyzed as a predictor of thoracic spinal cord involvement. Clinical, demographic and imaging covariates were used in a multivariate regression model to refine predictors of thoracic spine involvement.

Results: A total of 687 patients were consecutively evaluated over a 1-year period. The study cohort was comprised of 126 patients (94 women, median age = 39 years (y), interquartile range 32-49 y). Excluded were 561 patients due to a diagnosis of other neuroinflammatory or neurological disorders, or not meeting 2010 McDonalds criteria for MS (n=222) or incomplete neuraxis imaging (n=339). The presence of at least 1 demyelinating lesion within the cervical and thoracic spinal cord was identified in 105 (83.3%) and 86 (68.3%) patients, respectively. There was an increase in the odds of thoracic spine involvement when any cervical spine lesion was present (odds ratio (OR) = 6.08, 95% confidence interval [2.21-16.68], $p< 0.001$). The multivariate logistic regression model demonstrated a substantial increase in the odds of thoracic spine involvement with each increasing number of cervical spine lesions: for 2 lesions (OR 4.44, [0.914-21.60], p=0.06), 3 lesions (OR 19.76, [3.51-111.17], p=0.001), 4 or more lesions (OR 20.49, [1.97-213.23], p=0.012), and diffuse cervical spine lesions (OR 71.94, [5.2-979.88], p=0.001) when adjusting for significant covariates including brain lesions, disease duration, and treatment exposure.

Conclusions: Our findings suggest that the presence of thoracic spinal cord lesions is predicated on the presence and degree of

cervical spine involvement in patients with MS, a risk that appears to be independent of brain findings or clinical features.

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Glutamate as a marker for disease progression in patients with relapsing-remitting multiple sclerosis using magnetic resonance spectroscopic imaging

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Background: Magnetic resonance spectroscopic imaging (MRSI) in patients with multiple sclerosis (MS) offers the possibility to detect metabolic alterations in combination with morphological changes.
Objectives: In our study, we aimed to differentiate RRMS patients with mild and fulminant disease progression by identifying reliable metabolic markers of disease severity. Clinical classification was based on the current treatment level of the MS patients.
Methods: MRSI data were acquired from 44 RRMS patients (female: 32, mean age: 36.1). Fourteen patients were receiving no medical therapy (NT), 17 immunomodulatory baseline therapy (BT) and 13 escalation therapy (ET). The latter were treated with Natalizumab, recently reported not to change metabolite concentrations pre- to post-treatment in MRSI. These 44 RRMS patients as well as 20 healthy controls (HC) were examined using a 2D-PRESS sequence with water saturation at 3 T.

The volume of interest was localized above the corpus callosum in all subjects. MRSI data were analyzed using LCModel to estimate white-matter metabolite levels (N-acetylaspartate [NAA], glutamate and glutamine [Glx] and myo-inositol [Ins]). One-way ANOVA tests were used to compare the concentrations in 4 central white-matter voxels between the four groups.
Results: Ins concentrations were increased in all patient groups, increasing continuously from the HC to ET group. Ins was significantly higher in BT patients compared to HC ($p < 0.001$) and in ET patients compared to HC ($p < 0.0001$).

Glx showed significantly higher levels in ET patients compared to HC ($p < 0.002$) and also to those receiving BT ($p < 0.01$). NAA exhibited no significant differences between groups.
Conclusions: All RRMS patients showed elevations in a marker for (neuro)inflammation, namely myo-inositol. However, our study demonstrated that metabolites of neuronal excitotoxicity (Glx) were specifically upregulated in the patient group with a more aggressive disease course, indicating surrogate factors for progression. Interestingly, as other groups have reported, we found no reduction in NAA in our groups. Thus, if one does not question NAA as a parameter for neuronal integrity, it must be concluded that neuronal excitotoxicity precedes neuronal loss, indicated by the higher Glx but stable NAA values in all our patient groups.

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Grey-matter atrophy rate is not related to disease activity or MRI lesion accumulation in relapsing-remitting MS patients: a longitudinal study

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Background: For a long time MS has been regarded as a demyelinating autoimmune disorder of the central nervous system related to T lymphocytes autoreactive for myelin. However, there is also an axonal degeneration that is present from the early stages of the disease and seems to correlate better with the progressive neurological disability. The relation between inflammation and neurodegeneration is currently intensively debated and, at later stages of MS, brain atrophy often occurs in the absence of new inflammatory lesions. However, in the earlier stages of the disease where inflammation is more pronounced, there is little data addressing the contribution of disease activity to GM atrophy.
Objectives: To evaluate the influence of clinical features and MRI lesion accumulation in GM atrophy rates in RRMS patients.
Methods: Forty-two RRMS patients and 30 controls were followed up for 24 months. All individuals underwent MRI (3T) including FLAIR, DIR and a volumetric T1 at baseline, 12- and 24-months. Clinical features evaluated were: relapses during follow-up and in the previous 2 years, disease duration and gender. MRI metrics included accumulation of cortical and WM lesions, and gadolinium-enhancing lesions during follow-up. GM atrophy rates (cortical and subcortical) were calculated. Group comparisons and correlations were performed with age as covariate. Multivariate analyses were performed to assess the contribution of clinical and MRI metrics to GM atrophy rates.
Results: In the patients group, atrophy rates were significantly higher than controls for cortical (2.57 vs. 0.11%, $p = 0.023$) and subcortical GM volumes (3.80 vs. 1.33%, $p = 0.004$). GM atrophy rates (cortical and subcortical) were neither related to clinical features ($p > 0.1$) nor to MRI metrics ($p > 0.1$). On multivariate analyses no clinical or MRI factor remained in the model as predictor of GM atrophy rate.
Conclusions: GM atrophy is significantly higher in RRMS patients when compared to healthy subjects but atrophy rate is not influenced by clinical factors, disease activity or accumulation of demyelinating lesions. Mechanisms other than inflammatory lesions may contribute to GM degeneration.

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Multiple sclerosis lesion geometry in quantitative susceptibility mapping and phase imaging

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Background: Recent pathological studies suggest that geometrical distributions (e.g. solid, shell) of magnetic susceptibility sources (e.g. iron, myelin) in MS lesions may help to identify stages of inflammation and neurodegeneration. MRI sequences sensitive to susceptibility may therefore have the potential to serve as markers of disease activity in MS patients. According to Maxwell's equations, both solid and shell distributions of susceptibility can lead to shell-like patterns on phase imaging, whereas quantitative susceptibility mapping (QSM), by deconvolving the phase images, directly depicts the underlying susceptibility sources. This has been demonstrated in numerical simulations and MRI phantoms experiments. Here we investigate whether the same patterns are present in *in vivo* MS patient imaging data.

Objectives: To demonstrate geometrical patterns created by MS lesions on phase and QSM and to explore whether shell-appearing lesions on phase may correspond to either solid or shell lesions on QSM.
Methods: T2-weighted (T2w), high-pass-filtered phase, and QSM images for twenty consecutive MS patients were reviewed in accordance with the NYPH Institutional Review Board. For each MS patient, a neuroradiologist identified the largest lesions on the T2w images. The morphology of each lesion was then characterized as "solid", "shell" or "not seen" on the phase and QSM images.
Results: Of the 60 lesions identified in T2w images of the 20 MS patients, 42 lesions were detected in QSM with 35 (83%) solid and 7 (17%) shell, 33 lesions were detected in phase with 10 (30%) solid and 23 (70%) shell. Of the 23 shell-like lesions detected on phase, 16 (70%) appeared solid on QSM, 5 (22%) appeared shell on QSM, and 2 (8%) were not visible on QSM. Of the 5 shell-like lesions detected on QSM, all 5 (100%) lesions were also shell-like on phase.
Conclusions: Given previous work validating that QSM accurately depicts solid and shell patterns of magnetic susceptibility, here we show that phase imaging may depict *in vivo* MS lesions with solid susceptibility distributions as shell-like. QSM also detects more lesions than phase imaging. In light of the potential importance of susceptibility geometry in characterizing clinically relevant characteristics of MS lesions, QSM should be included in future MRI studies of susceptibility in MS.

P549

Angle resolved R2* is sensitive to tissue changes: study in MS patients and controls

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Background: Focal lesions in brain´s white matter (WM) are a radiological hallmark of Multiple Sclerosis (MS). In addition, WM tissue outside focal lesions, referred to as diffusely abnormal and normal appearing WM, is increasingly recognized as being affected by the disease.
Objectives: Here, we investigated the transverse magnetic resonance relaxation rate R2* in the normal appearing and diffusely abnormal WM of MS patients, their healthy siblings and unrelated healthy controls.
Methods: We investigated 39 subjects with MS, 31 healthy siblings and 30 age and gender matched controls. Expanded Disability Status Scale (EDSS): [0 - 6.5], disease duration: [3 - 41] yr. Data acquisition parameters: SWI - acquisition voxel: 0.9x1x1.6mm^3 reconstructed to 0.8x0.8x0.8mm^3, TR/TE/ΔTE=28/5/5ms, 5 echoes, α=17°. WM was segmented from a combined T1 and T2 image using FSL´s FAST (FMRIB´s Automated Segmentation Tool). R2* maps were calculated from the multi-echo SWI data by fitting a monoexponential function. As R2* in WM depends also on the microstructural tissue orientation relative to the main magnetic field, the WM fibre orientation from the eigenvectors of a diffusion tensor imaging scan (TR/TE=7465/75 ms, b-value=1000, 16 directions) was determined and the R2* of WM was analyzed in angle intervals of five degrees. The average for all subjects in each group was calculated. At each angle interval, differences among the three groups were evaluated using a Kruskal-Wallis test and in pairwise using a Tukey HSD test.
Results: While the orientation dependency of R2* was similar in all three cohorts, there was a significant difference at all angles between the MS group and the siblings group and between MS group and the controls ($p < 0.05$), with R2* being lowest in the MS group, followed by the controls and the siblings. We found also significant differences between the siblings and controls ($p< 0.05$).
Conclusions: The decrease in R2* in MS patients compared to controls and siblings may be related to increased water content due to edema. Compared to whole-brain histograms of R2*, which overlap due to the fibre orientation dependency of R2*, the proposed approach results in well separated curves for the three cohorts and is able to show subtle differences that are masked otherwise.

P550

Vitamin D levels are associated with low cortical thickness in secondary progressive MS

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Background: Vitamin D deficiency is a risk factor for multiple sclerosis (MS). Low vitamin D levels are associated with increased risk of brain lesions, relapses and early progression of disability. Vitamin D levels have also been correlated with grey matter fraction; however the relation between vitamin D and cortical thickness is unknown.
Objectives: To examine associations between vitamin D and cortical thickness and other MRI measures in relapsing remitting (RR) and secondary progressive (SP) MS.
Methods: 8 subjects with RRMS and 14 subjects with SPMS were enrolled into a phase I trial of mesenchymal stem cell transplantation. Baseline MRI studies and serum 25 OH vitamin D levels were obtained along with details regarding MS disease history. Cortical Longitudinal Atrophy Detection Algorithm (CLADA) was used to determine cortical thickness and probabilistic tractography of the corticospinal tract was used to analyze diffusion tensor imaging data. Pearson's product-moment correlations between cortical thickness and vitamin D levels were examined along with 95% bootstrap percentile confidence intervals (CI) based on 500 replicates. The correlation between vitamin D and other MRI measures was also examined.
Results: Vitamin D levels correlated significantly with cortical thickness in SPMS subjects (correlation coefficient 0.41, 95% CI: 0.03-0.83), but not in RR subjects. Vitamin D levels were also significantly correlated with brain parenchymal fraction (BPF) in SPMS subjects (correlation coefficient 0.46, 95% CI: 0.08-0.84). Similar results were found for the relation between vitamin D and T2 lesion load in SPMS subjects (correlation coefficient -0.61, 95% CI: -0.11-0.82). No relation was found between vitamin D

and T2 lesions or BPF in RRMS subjects. Diffusion tensor imaging data will be available at the time of presentation.
Conclusions: These findings suggest vitamin D might play a role in grey matter atrophy and neurodegeneration in MS. The lack of correlations in the RRMS group may be related to the differential role of vitamin D deficiency based on disease course with inflammatory effects in RRMS and neurodegenerative effects in SPMS. Treatment with vitamin D, particularly in SPMS may limit cortical atrophy and this observation is further rationale for clinical trials of vitamin D in SPMS.

P551

A prospective, case-control, longitudinal MRI study of the effect of glatiramer acetate on iron deposition in RRMS

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Background: No longitudinal studies examined evolution of iron deposition on susceptibility-weighted imaging (SWI) in multiple sclerosis (MS) patients.
Objectives: To explore whether treatment with glatiramer acetate (GA) may alter iron deposition and atrophy accumulation in the subcortical deep gray matter (SDGM) of relapsing-remitting MS patients followed over 31 months.
Methods: This study included 40 MS patients treated with GA and 20 age- and sex-matched healthy controls. All subjects were assessed with 3T MRI and clinical outcomes at baseline and at the follow-up. The accumulation of iron deposits over the follow-up was assessed by determining change in mean phase of low phase voxels (MP-LPV) of the SDGM on SWI. Regional SDGM brain volume changes were determined also over the follow-up. Within and between subject statistical analyses were performed.
Results: Over the follow-up, significant within subject MP-LPV change of putamen (+3.9%, $p=0.002$) was detected in MS patients, while no significant changes were found in controls. MS patients developed significant brain volume loss of the total SDGM, caudate, putamen, globus pallidus and thalamus (all $p< 0.001$) over the follow-up, while controls showed significant volume loss of the putamen ($p< 0.05$). No significant MP-LPV differences between the MS and control groups were detected over the follow-up. However, MS patients showed more advanced volume loss in the total SDGM, caudate, globus pallidus and thalamus ($p< 0.05$), compared to controls.
Conclusions: This study showed that MS patients treated with GA showed similar rates of iron deposition in the SDGM structures compared to controls, except for in the putamen, while the atrophy development of these structures was more advanced in MS patients. The temporal relationship between the iron deposition and brain atrophy accumulation should be further investigated in relation to disease-modifying treatment in MS patients.

P552

Cortical and subcortical volume dynamics in active relapsing-remitting multiple sclerosis patients treated with in Fingolimod (Gilenya)

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Background: Morphometric brain changes in relapsing-remitting multiple sclerosis (RRMS) include alterations in the volume of subcortical regions, as well as in the thickness of the cortex. These changes signify the on-going pathological processes that characterize the disease. Accurate measurements of structural thickness provide important information about the integrity of the cerebral cortex and white matter tracts. These may become non-invasive markers of disease progression and may assess in establishing treatment response.
Objectives: To investigate the effects of Fingolimod (Gilenya) treatment on cortical thickness and sub-cortical structures volumes in clinically active RRMS patients.
Methods: An observational, single-center, open-label, 1-year prospective study. Thirty RRMS patients were enrolled and 29 completed the one-year follow-up assessment, mean±SD age was 40.0±8.7 years, disease duration 12.8±6.9 years and EDSS 3.2±1.5. Patients were clinically active in the year prior to enrollment as evidenced by mean progression in the EDSS of 0.4 and a mean number of 1.2 relapses. Patients underwent high-field 3.0T MRI, three-dimensional T1-FSPGR (voxel size 1×1×1 mm) at baseline (prior to initiation of Gilenya treatment) and at 1 year. Cortical thickness and subcortical structures volume was measured using FreeSurfer (http://surfer.nmr.mgh.harvard.edu/fswiki) in 116 brain regions.
Results: After one year of treatment with Gilenya the majority of brain regions measured (97/116, 84%) were unchanged. In the 19 brain regions in which a significant decrease was detected, of interest are the following: central and mid-posterior corpus callosum and the left and right parahippocampal gyri. No correlations were found between changes in areas of interest and age, disease duration or EDSS.
Conclusions: In parallel to conferring effective clinical response, Gilenya treatment demonstrated preservation of structural thickness in the majority of brain areas examined over one year of treatment.

P553

Perfusion and diffusion changes in multiple sclerosis lesions and correlation with brain atrophy and clinical disability

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Background: Although the pathogenesis of the multiple sclerosis (MS) is not yet fully understood, it has been known that hemodynamic abnormality and disruption of white matter (WM) integrity are associated with the pathological processes of MS lesions.

Magnetic resonance imaging (MRI) plays an important role in the diagnosis and monitoring of the disease by non-invasively visualizing the focal lesions. There is considerable interest in revealing the degrees of hemodynamic and structural injury of tissue within the MS lesions. Meanwhile, the clinical relevance of these stratified lesions needs to be established.

Objectives: To test the hypothesis that hemodynamic and structural impairment, as assessed by cerebral blood volume (CBV) and fractional anisotropy (FA), characterizes the extent of tissue injury, and that the load of lesion with substantial tissue destruction would reflect the disease status related to clinical disability.

Methods: A total of 1135 MS lesions were evaluated. 7 relapsing-remitting MS patients and 7 sex- and age-matched controls underwent perfusion, diffusion and conventional MRI scans. CBV and FA were measured in three types of MS lesions: T1-enhanced, T1-hypointense and T1-isointense lesion and compared with values obtained in WM from controls. Brain volumetric measurements and their correlations with lesion volumes and scores of clinical disability were also performed. Two-sample t-test and linear regression (with Pearson correlation coefficient) were used for statistical analysis

Results: Compared with normal WM, CBV and FA were significantly reduced in the T1-hypointense lesion ($p< 0.05$), while insignificant changes in both parameters were exhibited in the T1-isointense lesion. Increased CBV but significantly decreased FA ($p< 0.05$) was detected in the active lesion. Meanwhile, a close spatial relationship between active and T1-hypointense lesion was observed. Lesion load represented by T1-hypointense plus active lesion volume significantly correlated with brain atrophy ($R=0.87, p< 0.05$), which, in turn, significantly correlated with the severity of clinical disability ($R=0.77$, $p< 0.05$).

Conclusions: A unique combination of CBV and FA characterizes the status of a specific lesion type in MS. A severe structural impairment does not solely occur in the T1-hypointense lesion, but is also associated with the active lesion. A burden of the lesion with extensive structural damage provides an image index, indicative of disease status.

P554

Impaired homologous interhemispheric functional connectivity is related to atrophy of the corpus callosum in multiple sclerosis

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Background: Atrophy of the corpus callosum (CC) is a hallmark feature of white matter pathology in multiple sclerosis (MS). Degeneration of the CC is hypothesized to impair communication between homologous regions, symmetric areas of the right and left hemispheres, resulting in abnormal intrinsic functional connectivity profiles. Homologous inter-hemispheric functional connectivity (HIFC) could provide a sensitive biomarker for callosal pathology in MS by mapping inter-hemispheric network integrity.

Objectives: To explore the relationship between CC atrophy and HIFC in relapsing-remitting (RR) MS compared to healthy controls (HC) using a novel surface-based registration technique for establishing homologous regions.

Methods: Anatomical and resting state functional connectivity (RSFC) data were acquired in 20 RRMS (mean disease duration 8.48 years; median EDSS=2.0, range 1-6.5) and 35 age- and gender-matched HC subjects at 3T. Mid-sagittal CC area was calculated following a semi-automated segmentation technique. Atrophy measures were compared between groups with a Mann-Whitney U test. Following registration of preprocessed RSFC data to a precise left-right registered atlas of the reconstructed cortical surfaces, HIFC was calculated by correlating the time course of each vertex to its homologous counterpart using a Pearson's correlation. Data were Fisher´s Z-transformed to facilitate between group comparison with a Mann-Whitney U test. Mean whole-brain HIFC and was extracted and compared with CC atrophy measures using a Spearman's correlation.

Results: Mid-sagittal CC area was significantly decreased in RRMS compared to HC ($p< 0.001$). Mean HIFC was also significantly decreased in RRMS ($p=0.001$). Mean HIFC correlated with mid-sagittal CC area ($rho=0.397$, $p=0.002$).

Conclusions: The results of this study validate and extend previous work on HIFC in RRMS. HIFC calculated using a novel technique based upon the reconstructed cortical surface revealed a significant relationship between deceased HIFC and CC atrophy in RRMS. This suggested that commonly observed CC pathology in RRMS has a significant effect on inter-hemispheric network connectivity. Surface-based HIFC may represent a sensitive early biomarker for increasing CC disease burden in RRMS.

P555

Imaging myelin pathology in the gray matter in multiple sclerosis

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Background: Clinical disability in multiple sclerosis (MS) is often associated with distinct myelin pathology in the central nervous system. Yet, much of the tissue damages, particularly in the cortical regions, often appears normal on conventional MRI. For this reason, we studied positron emission tomography (PET) as a promising modality for imaging of cortical myelin.

Objectives: To establish an imaging marker of myelin pathology in the gray matter with a strong correlation between myelin content and PET signals.

Methods: Develop a myelin-specific radiotracer for PET imaging in a rat model of cortical demyelination.

Results: A rat model of cortical demyelination was prepared. Subsequent longitudinal PET imaging was conducted using a novel radiotracer, termed MeDAS. Radioactivity concentration in the gray matter was quantified as a function of time and the pharmacokinetic profile was correlated with cortical myelin pathology.

Conclusions: PET is a promising imaging modality for imaging myelin pathology in the gray matter.

P556

Sex differences in brain glucose uptake in patients with multiple sclerosis during walking

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Background: Multiple Sclerosis (MS) is a chronic disease of the central nervous system, leading to progressive disability. Although MS is disproportionately diagnosed in women, men possess greater advancement of disability including walking and cognitive dysfunction. The reason for this sex difference is not well understood, with no previous studies having investigated brain metabolism sex differences during a physical task.
Objectives: Determine if differences in glucose uptake in brain structures important for independent walking are present between mildly disabled men and women with MS.
Methods: Eight participants with MS (4 men) were injected with the positron emission tomography (PET) glucose analogue tracer, [^{18}F]-fluorodeoxyglucose (^{18}F-FDG) and then performed an unassisted, self-determined speed, 15-min walk on a treadmill. After walking, participants underwent PET/Computed Tomography imaging. Tissue activity was quantified as standardized glucose uptake values (SUV).
Results: SUVs were significantly less for men compared to women: thalamus (4.50 ± 0.30 and 5.60 ± 0.25, $P = 0.001$); cerebellum (3.44 ± 0.30 and 4.39 ± 0.35, $P = 0.006$); pre-frontal cortex (4.63 ± 0.53 and 5.54 ± 0.40, $P = 0.034$); frontal cortex (4.73 ± 0.54 and 5.64 ± 0.41, $P = 0.036$); motor cortex (5.00 ± 0.59 and 5.95 ± 0.45, $P = 0.043$).
Conclusions: Sex differences in brain glucose uptake of patients with MS, with otherwise comparable disease characteristics, during physical activity may provide subclinical reasoning for disability acceleration in males. Larger studies are required to confirm these preliminary findings, compare findings to healthy controls and determine if differences exist at rest.

Immunology

P557

Epigenetic changes in CD8$^+$ T cells and CD19$^+$ B cells isolated from relapsing/remitting multiple sclerosis patients

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Background: Multiple sclerosis (MS) is an immune mediated disease of the central nervous system, with much attention focused on the T cells contribution to the disease with the B cells now playing a more appreciated and important role. The pathogenesis of MS is influenced by environmental exposures that include smoking, Epstein-Barr virus infection and vitamin D deficiency. These factors have the potential to influence gene expression via epigenetic modification.
Objectives: To explore the epigenetic risk due to CpG island methylation in CD8$^+$ T cells and CD19$^+$ B cells.
Methods: 30 RRMS and 24 healthy blood donors were recruited; CD8$^+$ T cells and CD19$^+$ B cells were isolated from peripheral blood, DNA extracted. A genome-wide DNA methylation analysis of each lymphocyte DNA was performed using the Illumina 450K methylation arrays. A step-wise prioritisation process was used to identify a panel of CpG's associated with MS
Results: Of the CpGs that were detectable in this survey we found that CD8$^+$ T cells had an eight fold increase in the number of differentially methylated CpGs compared to CD19$^+$ B cells. Neither CD8$^+$ T cells nor CD19$^+$ B cells had differentially methylated CpGs neither in the HLA-DRB1 locus nor in MHC region on chromosome 6. Outside of the MHC region, CD8$^+$ T cells and CD19$^+$ B cells did not contain differentially methylated CpGs in genes that were in common with the International Multiple Sclerosis Genetic Consortium (IMSGC) MS risk genes. Using the WEBGesalt engine, we performed a Gene Set Enrichment Analysis (GSEA) analysis with CD8$^+$ T cells which showed a significant alignment with metabolic pathways. GSEA analysis of CD19.$^+$ B cells revealed no significant alignment with any putative pathways and the alignment analysis against disease association databases revealed no associated diseases
Conclusions: Previously, we have shown that CD4$^+$ T cells have a strong HLA-DRB1 differential methylation in MS cases compared to controls. Here we investigated other lymphocyte cell types for differentially methylated genes which could contribute to the pathogenesis of MS. From our cohort, CD8$^+$ T cells and CD19$^+$ B cells did not contain differential methylated CpGs in the HLA complex nor IMSGC risk genes . The methylation profiles of the major lymphocytes subsets in MS patients are different to each other and with CD4$^+$ T cells, which have a strong *HLA-DRB1* focus.

P558

Abnormal functional phenotypes of effector and regulatory T-cell subsets in pediatric-onset MS

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Background: While studies in adult MS have implicated several abnormalities in T cell effector (Teff) and regulatory T cell (Treg) measures, these studies are typically done years after biological disease onset. Results may therefore be encumbered by epiphenomena of chronic immune dysregulation, such that abnormalities that contribute to pathogenesis become indistinguishable from those that may emerge as responses to the injury process. In contrast, children with MS offer a unique window into the early disease spectrum, though relatively little is known about their disease-related immune profiles.
Objectives: To examine the profile of Teff and Treg subsets at the earliest possible time in the MS disease process.
Methods: We designed multiparametric flow cytometry (FACS) panels capturing both phenotypic and functional response-profiles of the implicated immune cell subsets. Assay development

included miniaturization of FACS panels and validation for use with cryopreserved PBMC obtained using our strict standard operating procedures. Cryopreserved PBMC were obtained from children at presentation with an incident clinical episode of acquired demyelinating syndromes (ADS), as part of our prospective Canadian Pediatric Demyelinating Disease Study. Results were compared between children who, in prospective follow-up, were ascertained to have either MS ('ADS-MS') or monophasic ADS ('ADS-Mono'). PBMC from age- and sex-matched healthy controls ('HC') were analysed in parallel.

Results: A sequential cohort of 33 children examined to date demonstrates that, compared to HC and ADS-Mono, ADS-MS harboured higher proportions of circulating $CD4^+CCR2^+CCR5^+$ T cells ($p = 0.0341$) and $CD8^+CD161^{high}$ mucosal associated invariant T (MAIT) cells ($p = 0.0211$). These two effector T-cell subsets were also the highest producers of IL-17 ($p = 0.0186$) and IFNγ ($p = 0.0043$). While frequencies of $CD25^{hi}CD127^{low}$ Tregs did not differ across groups, the $CD25^{hi}CD127^{low}$ Tregs of children with ADS-MS expressed lower levels of FOXP3 compared to the controls.

Conclusions: Our findings selectively implicate both $CD4^+CCR2^+CCR5^+$ and $CD8^+CD161^{high}$ MAIT effector cell subsets in early pediatric-onset MS pathophysiology, but not in pediatric monophasic CNS inflammatory conditions. In addition, although frequencies of circulating $CD25^{hi}CD127^{low}$ Tregs appear similar across groups, their expression of FOXP3 is lower in the MS group, which could be consistent with a deficient Treg suppressive capacity in these children.

P559

Unique immune profile in benign MS identified with antigen arrays

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Background: Using antigen arrays we have found serum antibody signatures associated to different stages of MS, pathology and MRI. In this study we used antigen arrays to analyze the immune response in benign MS.

Objectives: To analyze the immune response associated with benign MS.

Methods: Antigen microarrays consist of 420 antigens including CNS-related autoantigens, lipids, virus-derived antigens and other autoantigens. We analyzed serum samples from benign MS patients based on strict clinical criteria and compared it to that of disease duration (DD) and *Expanded Disability Status Scale* (EDSS) matched patients from the CLIMB longitudinal MS study at the Partners MS Center. We analyzed: 1) Samples taken from benign MS patients (n=21); 2) Samples obtained from DD-matched MS patients (n=13); 3) Samples obtained from EDSS-matched MS patients (n=15). Benign patients were defined as those having an EDSS=< 1 at 10 or an EDSS=< 2 at 15 years after diagnosis. Patients were never treated with a disease-modifying treatment. Samples from DD-or EDSS-matched controls were taken at an untreated timepoint.

Results: We found unique IgG antibody patterns associated with benign MS. Specifically, benign MS patients showed decreased antibody reactivity to myelin when compared to EDSS- and DD-matched groups. In addition, we have previously described that increased antibody reactivity to heat shock proteins (HSPs) characterizes relapsing-remitting MS patients and we found a significant decrease in HSP-specific antibodies in benign patients.

Conclusions: Since HSP-specific immunity is linked to ongoing inflammation, our data suggests a decreased inflammatory response in benign MS. Moreover, since IgG antibodies are T-cell dependent, decreased myelin-specific IgG antibodies suggest a reduced antigen specific T-cell response in benign MS. Our findings identify a potential biomarker for benign forms of MS and identification of the mechanisms responsible for decreased immune responses in benign MS might open new therapeutic avenues.

P560

Oxysterols impair type 1 regulatory T-cell differentiation and promote autoimmunity

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Background: Oxysterols, oxidised forms of cholesterol, have been assigned with novel functions in modulating the immune response. More specifically, the enzyme cholesterol 25 hydroxylase (Ch25h), the rate-limiting step to synthetize 25-hydroxycholesterol (25-OHC) from cholesterol, contributes to antiviral response and Immunoglobulin secretion. However the function of oxysterols in $CD4^+$ T lymphocytes has not been assessed. The development and progression of multiple sclerosis result in part from the balance between effector and regulatory $CD4^+$ T cell. Since the original classification of $CD4^+$ T lymphocytes into T_H1 and T_H2 subsets, the repertoire of $CD4^+$ T cell has expanded to include additional effector T cell like T_H17 cell and regulatory T cell, such as $Foxp3^+$ regulatory T-cell or type 1 regulatory T (T_R1) cells.

Objectives: We proposed to assess the expression of oxysterols in subsets of $CD4^+$ Thelper and to examine their function during autoimmunity.

Methods: Subsets of $CD4^+$ Thelper cells were generated *in vitro* and expression of oxysterol converting enzymes was examined by real-time PCR. Secretion of oxysterols was measured by mass-spectrometry on T cell supernatant in culture. We observed that Ch25h was specifically expressed on T_R1 cells and therefore further differentiated T_R1 cell *in vitro* and *in vivo* from $Ch25h^{-/-}$ and wild-type mice.

Results: We showed that Ch25h and 25-OHC were specifically induced by Interleukin-27 (IL-27) during $CD4^+$ T cell differentiation *in vitro*. IL-27 is a critical factor for T_R1 cell differentiation and is instrumental in prevention of autoimmune diseases and multiple sclerosis. We further demonstrated that 25-OHC prevented T_R1 cell development both *in vitro* and *in vivo* and dissected the underlying signaling pathways.

Conclusions: Together, our findings show that 25-OHC acts as a negative regulator for T_R1 cell differentiation both *in vitro* and *in vivo*. Not only these findings unravel novel molecular mechanisms

accounting for the generation of T_R1 cells, but they also provide oxysterols as critical players to regulate differentiation of $CD4^+$ T cells and to promote autoimmunity. Understanding the complex interactions between metabolic and immune systems may lead to substantial therapeutic promise for autoimmune disorders.

P561

Role of melatonin in MS pathogenesis

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Background: Several reports show seasonal variations in MS disease activity, but no clear pattern has emerged, as shifts in monthly and seasonal relapses may respond to environmental triggers whose nature and pathophysiological mechanisms remain unknown. In the local population, fewer relapses were found during fall and winter, with inverse correlation to melatonin levels assayed. We aim to determine whether melatonin exerts a protective effect on MS, and if so, what the underlying mechanism of this effect might be.
Objectives: Characterize the role of melatonin in MS pathogenesis.
Methods: One hundred and nineteen patients recruited in November 2010 were followed until November 2013 and the number of clinical relapses recorded. Blood samples were taken every three months during one year and melatonin metabolite levels determined in urine by ELISA. EAE was induced in C57/B6 mice and clinical scores registered. Additionally, proliferation assays were performed in spleen and lymph nodes extracted on day 7, and percentages of T_H17 and Treg cells assessed by FACS. For *in vitro* experiments, naïve T cells were differentiated in T_H17 or Treg, with or without the addition of melatonin.
Results: We found an inverse correlation between exacerbation rates and melatonin levels for each season (Spearman's rho -0.73, P=0.006). Higher melatonin levels observed during fall and winter correlated with lower disease activity. We then tested whether melatonin had an effect on EAE. Decreased disease incidence (65% vs 87.5%, P=0.001) and severity (mean maximum score of 1.89±0.8 vs 2.65±1.4, P=0.049) were observed in mice treated with melatonin compared to vehicle-treated mice. This effect was related to decreased numbers of T_H17 cells in lymph nodes (1.12±0.27 vs 2.06±0.39, P=0.05) and increased number of $CD4^+Foxp3^+$ T_{reg} (4.45±0.4 vs 6.55±0.4, P=0.009). Finally, melatonin affected *in vitro* differentiation of naïve $CD4^+$ T cells to T_H17 cells, with decreased expression of both RORγt and IL-17 as well as increased secretion of IL-10 (472.5±8.5 pg/ml vs. 763.6±15.5 pg/ml, P>0.001).
Conclusions: Melatonin ameliorates EAE and may be a potential environmental factor involved in MS pathogenesis and seasonality.

P562

Identification of a transcriptional regulator of pathogenicity of Th17-cells

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Background: Experimental autoimmune encephalomyelitis (EAE) induced by myelin protein peptides serves as the most commonly used animal model of Multiple Sclerosis (MS). The interleukin (IL)-17 producing (Th17) T helper cell subset induces EAE and plays an important role in different autoimmune diseases including MS. The differentiation of Th17 cells is regulated by the cytokines transforming growth factor (TGF)-β and IL-6, while IL-23 is essential for Th17 cell stabilization. In the context of autoimmune disorders IL-23 receptor (IL-23R) signaling controls the pathogenicity of Th17 cells *in vivo* and polymorphisms of IL-23R have been associated with different human autoimmune diseases. Despite its relevance for human autoimmune diseases the transcriptional regulation of IL-23R is unknown.
Objectives: The objective of the study was to identify pathways involved in regulating pathogenicity of Th17 cells.
Methods: We used EAE as an animal model of MS in genetically modified mice. Th17 cell differentiation was assessed in cell culture. Promoter binding and activation studies were performed using chromatin immunoprecipitation PCR and luciferase assays.
Results: We identified a transcription factor (TF) which induces IL-23R in Th17 cells and is required for its maintained expression. It binds and transactivates the IL-23R promoter in cooperation with other TF known to be relevant for the Th17 lineage. In accordance, its Th17-cell specific deficiency ameliorates EAE and reduces the pathogenicity of Th17 cells *in vivo*.
Conclusions: Our findings extend our understanding of the transcriptional control of pathogenicity of Th17 cells and identify a potential new target for therapeutic manipulation of Th17 cells.

P563

Identification of a key role for complement in neurodegeneration in multiple sclerosis

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Background: Loss of neurons and axons is a main cause of clinical disability in multiple sclerosis (MS) but the mechanisms responsible for neurodegeneration are largely unknown. A number of studies suggest that it occurs independently of inflammation. Our previous findings that activation of the complement system, part of innate immunity, accelerates neuroaxonal degeneration after nerve or brain trauma and the recent identification of the association between complement genetic variants and neurodegeneration in classical neurodegenerative diseases, led to our hypothesis that complement may also contribute to neuroaxonal pathology in MS.
Objectives: To study localization and function of complement components and activation products in relation to neuropathology in MS.

Methods: We analysed the localization of key complement components and regulators in post-mortem brainstem tissue from progressive multiple sclerosis donors, in relation to neuropathological changes, and compared the findings to brainstem tissue of donors with other neurological diseases or non-neurological controls. Complement function was tested *in vitro* using primary human astrocytes, and *in vivo* using mice with genetic or pharmacological inhibition of key complement components.
Results: We detected substantial deposition of complement activation products on neurons, axons, synapses and astrocytes in human brainstem. Neurons up-regulated stress markers; axons showed disturbed fast transport and lost synapses. Astrocytes were positive for pro-inflammatory cytokines. C1q and C3d were detected on neurons, axons and synapses, whereas the terminal activation product, membrane attack complex (MAC), co-localized on astrocytes positive for interleukin 1 beta (IL-1β) in lesions and induced pro-inflammatory cytokines in human primary astrocytes. Neurons were strongly positive for the complement regulatory protein CD59 but lost expression of CD55 within lesions. Further, using mice genetically deficient in CD55 or CD59a, we found that regulation of the terminal complement pathway is crucial to protect from MAC deposition on neurons, subsequent clinical disability and neuropathology in the experimental autoimmune encephalomyelitis (EAE) model of MS. In addition, pharmacological inhibition of the MAC in wild-type mice prevented astrocyte activation and neuronal damage, suppressing clinical disease and pathology in EAE.
Conclusions: Our findings indicate that complement activation occurs in MS grey matter and plays a key role in neurodegeneration in models and man.

P564
Intrathecal Epstein-Barr virus specific CD8 T-cell responses in multiple sclerosis patients are directed to lytic viral antigens
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Background: Epstein-Barr virus (EBV) infection is a causative risk factor for MS. Overt EBV-specific immune responses in MS patients are potentially pathogenic either directed to intra-cerebral EBV infection or cross-reactive autoantigens.
Objectives: We determined the frequency, phenotype and antigens recognized by T-cells in cerebrospinal fluid (CSF) of patients with clinically isolated syndrome (CIS), established MS and other neurological disease (OND).
Methods: CSF-derived T-cell lines (CSF-TCL) were generated by mitogenic stimulation of surplus CSF from patients with CIS (n=17), MS (n=12) and OND (n=13). Autologous EBV-transformed B-cells (autoBLCL) were used as antigen presenting cell (APC) to assay the reactivity (IFNγ expression), phenotype (CD4/CD8) and clonality (TCRVß usage) of CSF-TCL towards EBV. The patients' EBV-restricting HLA-I allele was identified using partially HLA-matched allogenic BLCL. Finally, Cos-7 cells were co-transfected with the patient's EBV CD8 T-cell response restricting HLA-I allele combined with (1) individual plasmids encoding 59 of the total of 86 (69%) known EBV proteins or (2) MS-associated human oligodendrocyte- (MAG, MBP, MOG and PLP1), glia- (CRYAB, KIR4.1 and S100B) and neuron-specific proteins (CNTN2 and NF155) and subsequently used as APC in functional T-cell assays.
Results: Frequencies of autoBLCL reactive CSF-TCL, particularly CD8 T-cells, were significantly increased in MS and CIS patients compared to OND. Detailed analyses of CSF-TCL of 5 patients with high (>10%) autoBLCL-specific CD8 T-cells demonstrated

(1) an oligoclonal CD8 T-cell responses and
(2) selective CD8 T-cell reactivity to lytic (BaRF1 BRLF1, BCRF1, BBRF3 and BXLF1), but not latency-associated EBV proteins.

Finally, neither high autoBLCL CD8 T-cell reactive CSF-TCL responded towards the 9 MS-associated autoantigens assayed.
Conclusions: AutoBLCL-specific CD4 and particularly CD8 T-cell responses were significantly increased in CSF-TCL of CIS and MS patients compared to OND. Intra-CSF autoBLCL reactive CD8 T-cells were selectively directed to lytic EBV proteins and did not cross-react with MS-associated human autoantigens. The elevated and selective recognition of lytic EBV proteins by CSF-derived CD8 T-cells implicate a local EBV infection in a subgroup of CIS and MS patients.

P565
MicroRNA miR-21 is induced by interferon-beta and inhibits IL-12 expression: a novel immunomodulatory mechanism in multiple sclerosis
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Background: Monocytes are a major source of IL-12 which promotes generation of Th1 cells and secretion of IFN-gamma, a cytokine that triggers Multiple Sclerosis (MS) relapses. IFN-beta treatment inhibits IL-12 production by antigen-presenting cells and decreases the frequency of MS relapses. The mechanism of IL-12 inhibition by IFN-beta is not completely understood.
Objectives: To elucidate whether microRNAs (miRNAs) contribute to the immunomodulatory effect of interferon (IFN)-beta in patients with MS.
Methods: Monocytes were separated from 8 healthy donors, 8 untreated patients with relapsing forms of MS, and 8 IFN-beta 1a (30mcg IM weekly) treated MS patients. miRNA expression in cells was tested by miRNA arrays followed by validation of selected miRNAs using individual RT-qPCR assays. *In vitro* experiments were conducted to validate the induction of miR-21 by recombinant IFN-beta in human monocytes. Transfection experiments with miRNA mimics were performed to study the effect of miR-21 over-expression on cytokine expression.
Results: MiR-21 expression was increased in monocytes of IFN-beta treated patients (Mean ± SEM = 3.592 ± 0.778 relative units) compared to healthy donors (1.090 ± 0.325, p=0.012) and untreated patients (1.248 ± 0.302, p=0.0139). The expression of IL-12a (p35), the specific target for miR-21, was significantly decreased in IFN-beta treated patients compared to healthy donors

and untreated patients. In-vitro experiments demonstrated that recombinant IFN-beta induced miR-21 expression in monocytes in a dose-dependent manner. Transfection of monocytes with miR-21 mimic led to decreased expression of IL-12a (p35) by 49.1% ± 6.1%, $p < 0.005$, but did not affect expression of control cytokines, such as IL-6.

Conclusions: We report a novel immunomodulatory mechanism for IFN-beta in MS: IFN-beta induces miRNA miR-21 which leads to decreased IL-12 expression. We also propose that miR-21 is a potential biomarker and therapeutic target in MS. Our findings suggest that miRNA-mediated regulation may be among other mechanisms mediating the therapeutic effect of disease-modifying treatments in MS.

P566

Transcriptional analysis of proinflammatory capacity of human Th17 subsets in healthy subjects and patients with multiple sclerosis

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Background: Multiple Sclerosis (MS) is a chronic central nervous system (CNS) autoimmune disease in which proinflammatory Th17 cells are increased. Recent studies indicate Th17 cells are not a uniform population and can be sub-divided into pathogenic and nonpathogenic Th17 cells in terms of EAE induction. Molecular signatures that differentiate pathogenic and nonpathogenic Th17 cells have been reported in a murine EAE model (Lee et al., 2012). In humans, IFN-g$^+$IL-10$^-$ and IFN-g$^-$IL-10$^+$ Th17 cells are induced in response to *Candida albicans* and *Staphlococcus aureus* infection, respectively, which possess different inflammatory capacity as well as factors that modulate their effector function (Zielinski et al., 2012). In MS, IFN-g$^+$ Th17 cells are preferentially recruited to the brain (Kebir et al, 2009).

Objectives: To transcriptionally compare the pathogenic and nonpathogenic molecular signatures of murine Th17 cells with various human Th17 cells.

Methods: Live IFN-g$^+$ Th17 (Th1Th17) and IFN-g$^-$ Th17 (Th17) cells were isolated from the peripheral blood of healthy donors and MS patients. Cell lysates of Th1Th17 and Th17 cells and RNA isolated from human IFN-g$^+$IL-10$^-$ and IFN-g$^-$IL-10$^+$ Th17 clones were analyzed with the nCounter Analysis System for gene expression profiling (nanoString Technologies) using CodeSet HuTh17 that encompasses a 419-gene expression detection panel specific for human T cell activation and differentiation. Enrichment analyses of gene signatures were conducted among isolated human Th17 cells (nanoString data), human Th17 clones (nanoString data) and mouse Th17 cells (microarray data).

Results: Th1Th17 cells (relative to Th17 cells) from healthy donors displayed gene signatures with high degree of similarities to (TGF-b3 and IL-6) or (IL-1b, IL-6 and IL-23)-induced mouse pathogenic Th17 cells (relative to (TGF-b1 and IL-6)-induced mouse nonpathogenic Th17 cells), as well as to the pro-inflammatory human IFN-g$^+$IL-10$^-$ Th17 clones (relative to IFN-g$^-$IL-10$^+$ Th17 clones). In healthy donors, the proinflammatory Th1Th17 cells expressed higher levels of three major Th17 specific molecules including IL-23R, IL22 and RORC when compared to Th17 cells. In contrast, in untreated MS patients Th17 cells had acquired this proinflammatory module, which is normally very prominent only in Th1Th17 cells.

Conclusions: These results provide new insights in defining the molecular signature of Th17 cells in MS that may modulate the pathogenicity of the cells.

P567

The immunological architecture of multiple sclerosis and treatment

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Background: Genetic studies and animal models highlight the importance of the adaptive immune system in the development of multiple sclerosis. However in-depth analysis of the role of the adaptive immune system in the development and resolution of autoimmune disease in human patients has proven to be difficult due to trade-offs between scale and level of detail, and the confounding effects of treatment.

Objectives: We have applied a systems immunology approach to the study of multiple sclerosis (MS) and for comparison another autoimmune disorder, autoimmune thyroid disease (AITD), to identify unique immunological signatures of disease and treatment.

Methods: We have performed a detailed assessment of 38 variables covering the adaptive immune system in 219 autoimmune disease patient samples (MS: N=164, AITD: N=55) and 36 healthy controls. MS patients include untreated patients as well as patients on four common immunomodulatory treatments (interferon-beta, glatiramer acetate, natalizumab, FTY720) applied in a routine tertiary outpatient clinic.

Results: In an unbiased analysis, we find a unique immunological signature in untreated MS patients, consisting of activation of CD8 T cell populations. This systems immunology approach applied to MS patients receiving four different immunomodulatory treatments revealed the immunological signatures of MS treatment, identifying both unique and unexpectedly shared effects of the different treatment regimes, such as the B cell pathway in interferon-beta and FTY720 MS treatment.

Conclusions: Together, these results shed light on the immunological architecture of MS and its treatment, as applied in a routine treatment setting.

P568

Induction of the glucocorticoid-induced leucine zipper protein in dendritic cells by hepatocyte growth factor limits autoimmune neuroinflammation

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Background: We previously observed that selective overexpression of neuron-derived hepatocyte growth factor (HGF), a potent neuroprotective factor, reduced central nervous system (CNS) leukocyte infiltration and demyelination, as well as promoted CNS T cell immune modulation in the multiple sclerosis (MS) model, experimental autoimmune encephalomyelitis (EAE) (Benkhoucha M. et al., *PNAS*, 2010). We showed that HGF-treated dendritic cells (DCs) displayed a tolerogenic phenotype and promoted the differentiation of Tregs *in vitro*, conferring a possible mechanism by which HGF could limit EAE immunopathogenesis. We postulated here that expression of glucocorticoid-induced leucine zipper (GILZ), a transcriptional regulator reported to correlate with the regulatory activity of tolerogenic DCs and to mediate the immunosuppressive effects of glucocorticoids (Cohen N. et al., *Blood*, 2005), could be one of the modalities by HGF could exert regulatory activities on DCs.
Objectives: To unravel the mode of actions of hepatocyte growth factor (HGF) on T cell immune modulation in EAE.
Methods: Systemic HGF treatment was applied s.c. two days before mouse EAE (myelin oligodendrocyte glycoprotein p35-55) induction. DC functions were evaluated *ex vivo* by using HGF-treated DCs as antigen-presenting cells for myelin-specific T cells and as donor cells by transferring HGF-treated DCs into mice with established EAE.
Results: Using biodegradable microspheres for the controlled release of HGF, systemic HGF treatment reduced clinical and histopathological signs of EAE, and was associated with decreased Th1 and Th17 responses, and an increase in regulatory T cells (Tregs) within the brains and the spleens. HGF-treated DCs were characterized by increased levels of GILZ and ability to promote the development of Tregs. RNA-interference-directed inhibition of GILZ expression by DCs suppressed the induction of tolerance caused by HGF. In adoptive transfer studies, HGF treatment of WT DCs, but not GILZ gene-deficient DCs, potently mediated functional recovery in mice with established EAE.
Conclusions: Our results identify GILZ as a critical factor for effective suppression of T-cell-mediated CNS inflammation by HGF via the induction of tolerogenic DCs, a mechanism that may be exploited for therapeutic benefit in MS.

P569

The protection of A2aR on BBB permeability from Th1 cytokines in MS

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Background: Multiple sclerosis (MS) is an autoimmune disease of the CNS characterized by a CD4+ Th1 lymphocyte-mediated autoimmune response, inflammatory cell infiltration and demyelination in the CNS, and progressive and recurrent impairment. Th1 cytokines are key factors in the regulation of inflammatory responses correlated with the loss of BBB integrity. We investigated the effects of A2aR manipulation on Th1 cytokines.
Objectives: To address the effects of A2a receptor (A2aR) on the brain endothelium and BBB integrity in MS.
Methods: We examined the distribution of F-actin and tight junction proteins in primary cultured endothelial cells treated by IFN-gamma or IFN-gamma plus specific A2aR agonist (CGS21680) by immune-fluorescence. Western-blot was used to detect the expression of tight junction proteins in primary cultured endothelial cells treated by IFN-gamma or IFN-gamma plus CGS21680. C57BL/6 mice were immunized by myelin oligodendrocyte glycoprotein (MOG_{35-55}) to induce experimental autoimmune encephalomyelitis (EAE) and then given CGS21680 by i.p. daily. Neurological impairment was evaluated using standardized scores. Blood-brain barrier (BBB) permeability was assessed with quantitative measurement for sodium fluorescin (Na-F) content and fluorescent tracer, FITC-Dextran.
Results: IFN-gamma decreased the expression of A2aR in brain endothelial cells. IFN-gamma caused the disturbance of cytoskeleton and a decrease of tight junction proteins. A2aR specific agonist could prevent endothelial cells from the injury caused by IFN-gamma *in vitro*. A2aR specific agonist could decrease the BBB permeability, inhibit neuroinflammation and EAE pathology and ameliorate neuro-behavioral deficits in vivo.
Conclusions: Activation of the A2aR exerts a strong protection on BBB from increased permeability caused by Th1 cytokines. Our results indicated that A2aR agonists could represent a novel therapeutic tool for MS treatment.

P570

Characterization of naïve, memory and effector $CD4^+$ T-cell subsets in progressive MS

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Background: Autoreactive $CD4^+$T cells are important in the pathogenesis of multiple sclerosis (MS). $CD4^+$T cells can be classified into six major subsets of naïve, central memory (CM), effector memory (EM) $CD28^{+/-}$ and terminally differentiated effector memory (TEMRA) $CD28^{+/-}$ by three surface markers CCR7, CD45RA and CD28. This allows a comprehensive classification of all $CD4^+$T cells according to antigen experience, homing potential, effector functions and proliferative capacity. CD26 is a marker of T cell activation and the expression of CD26 on $CD4^+$T cells correlates with clinical and MRI disease activity in MS patients. CD49d is expressed on lymphocytes and facilitates their entry to the CNS, which is blocked by natalizumab.
Objectives: To characterize naïve, CM, EM and TEMRA $CD4^+$T cells and their expression of CD26 and CD49d in patients with progressive MS; to investigate the effect of natalizumab on these cell subsets; and to examine if these subsets correlate with disease progression.
Methods: Flow cytometry was performed on blood drawn from relapsing-remitting (RR), secondary progressive (SP) and primary

progressive (PP) MS patients (n=87) and healthy controls (HC) (n=31). Nine SPMS and PPMS patients received natalizumab for 60 weeks. Patients were scored on expanded disability status scale (EDSS) at baseline, and disease progression was assessed retrospectively as the change in EDSS in the 2 years prior to blood sampling.

Results: We found a significant increase in $CD28^+$ TEMRA $CD4^+$T cells in MS patients compared with HC. CD26 was expressed on nearly all subsets, except $CD28^-$ effector cells. The frequency of $CD26^+$ $CD28^+$ TEMRA $CD4^+$T cells in MS patients was significantly increased compared with HC. All 6 subsets expressed CD49, and we did not find significant differences in the expression of CD49d on the subsets between HC and MS patients. There was a significant negative correlation between $CD28^-$ EM and $CD28^-$ TEMRA $CD4^+$T cells and disease progression. Natalizumab treatment induced a significant decrease in the frequency of $CD4^+$T cells expressing CD26 on all subsets, except TEMRA $CD28^-$, and, as expected, the expression of CD49d decreased.

Conclusions: This study suggests that $CD28^+$ TEMRA $CD4^+$T cells expressing CD26 could be involved in systemic inflammation of progressive MS patients and decreases during natalizumab treatment. Furthermore, we hypothesize that their negative correlation with disease progression could indicate an immunoregulatory function of CD28- EM and TEMRA $CD4^+$T cells in progressive MS.

P571

Impact of glucocorticoid treatment on the migration and polarization of human monocytes

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Background: Glucocorticoids (GCs) are the standard first-line therapy for Multiple Sclerosis (MS) patients suffering an acute relapse. Although we have previously identified T cells as central targets of GCs in neuroinflammatory diseases, new data suggest that the modulation of myeloid cells such as monocytes and macrophages also contributes to treatment success. Potentially relevant GC effects on monocytes include an altered migratory behavior and the polarization to the anti-inflammatory M2 phenotype.

Objectives: The activities of GCs are multifaceted and concern various cell-types. In this study we aimed to identify how GCs alter functional characteristics of monocytes, a cell-type which is believed to play an important role for the pathogenesis of MS.

Methods: We have isolated monocytes from the peripheral blood of healthy human volunteers and performed Boyden chamber assays in the presence of various chemokines to determine the impact of GC treatment on their migratory behavior. In addition, we determined changes in mRNA levels and surface expression of proteins related to migration and polarization of monocytes in response to GCs.

Results: GC treatment alters the migratory behavior of human monocytes along chemokine gradients and impacts chemokine receptor expression. It also results in the increased expression of genes such as CD163 which are characteristic for a M2 polarization of monocytes. Since we have previously shown that T cell behavior is significantly altered in MS patients undergoing methylprednisolone pulse therapy (Acta Neuropathologica 127, 713-29), we are currently analyzing whether the same applies to monocytes in these patients.

Conclusions: GC treatment induces functional changes in human monocytes which could provide another explanation for the therapeutic effects of high-dose GC therapy in MS patients.

P572

Molecular dynamics and intracellular signalling of the TNF-R1 carrying the R92Q mutation

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Background: The tumor necrosis factor receptor superfamily, member 1A (TNFRSF1A) gene codes for TNF-R1, one of the main TNF receptors that mediates its inflammatory actions. In a recent study, serum levels of the soluble form of TNF-R1 (sTNF-R1) and cell surface mRNA expression of the full length receptor were significantly increased in patients carrying the R92Q mutation. Furthermore, R92Q carriers were younger at disease onset and progressed slower as compared to non-carriers.

Objectives: We aimed to investigate the functional changes associated with the R92Q-mutated receptor by using molecular dynamics modelling approaches and by measuring the expression of key TNF-R1 downstream intracellular mediators signalling for apoptosis and proliferation.

Methods: mRNA expression levels for TRAF2 (TNF receptor-associated factor 2) and CASP3 (caspase 3, apoptosis-related cysteine peptidase) were determined by real time PCR in peripheral blood mononuclear cells (PBMC) from 61 untreated MS patients, 9 patients carrying the R92Q mutation (CT genotype for rs4149584) and 52 R92Q non-carriers (CC genotype for rs4149584). Models of the extracellular domains of human TNF-R1 and human TNF-R1 carrying the R92Q mutation, alone or bound to TNF, were constructed using Modeller and the pdb structures 1TNF and 1TNR as templates. Structures were submitted to 50 ns of molecular dynamics (NAMD) at CESCA supercomputing facilities. The effect of R92Q mutation during protein dynamics was analysed with VMD and CMA programs.

Results: Gene expression levels for intracellular mediators of the TNF-R1 pathway were increased in R92Q carriers compared to non-carriers, and differences reached statistical significance for CASP3 ($p=0.01$), whereas a trend was observed for TRAF2 ($p=0.07$). Molecular dynamic studies revealed that the R92Q mutation increased the contact area between receptor and TNF (1070 and 1388 Å2 for native and mutated receptor) and decreased the distance between them (28.7 to 27.9 Å), whereas Van der Waals (-72 and 94 Kcal/mol) and electrostatic (-314 and -375 Kcal/mol) interaction energies increased. *In vitro* and *in silico* experiments suggest that the R92Q mutation gives rise to a stronger interaction between the receptor and ligand, which results in the potentiation of the TNF-R1 signalling pathway.

Conclusions: These functional changes associated with the mutated TNF-R1 receptor may be related with the modulation in disease course reported in MS patients carrying the R92Q mutation.

P573

Plasmocytoid dendritic cells deficit of early response to toll-like receptor 7 agonist stimulation in multiple sclerosis patients

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Background: Plasmacytoid dendritic cells (pDCs) were originally discovered in humans as a small subset of blood leukocytes specialized in the secretion of high amounts of type I interferons (IFNs) in response to viruses. In contrast to conventional DCs (cDCs), pDCs uniquely express toll-like receptor (TLR) 7 and 9, intracellular receptors that recognize single-stranded RNA or unmethylated CpG DNA within endosomal compartments. pDCs have been postulated as an important immunoregulatory population but their role in autoimmune conditions is still largely unknown.
Objectives: To analyze the role and function of pDCs during autoimmune demyelination.
Methods: We studied function of pDCs in relapsing-remitting multiple sclerosis (RRMS; n=32) patients and controls (n= 33) by analysis of TLR7 responses. We assessed a pDCs secretion pattern of cytokines in the short term PBMC cultures stimulated with TLR7 agonist. pDCs sorted from PBMCs of both RRMS patients and controls were used to assess TLR7 expression profile. TLR7 induced signaling in pDCs has been analyzed with intracellular flow cytometry.
Results: We have identified a clinically correlated significant decrease of the TLR7-induced IFN-alfa (IFNa) secretion by pDCs from RRMS patients. This deficit has been accompanied by insufficient intracellular phosphorylation of protein kinase Akt and a decrease of the TLR7 gene expression in RRMS pDCs.
Conclusions: Our results demonstrated a selective pDCs deficit in RRMS supporting a relationship between pDCs and mechanisms of MS.

P574

Neuroprotective effects of calcitriol in autoimmune optic neuritis

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Background: Optic neuritis can result in persistent visual impairment due to apoptosis of retinal ganglion cells (RGCs) and degeneration of optic nerve (ON) axons. Therefore, efficient protection of retinal ganglion cells is a major challenge.
Objectives: We assessed the effects of calcitrol, the hormonally active form of vitamin D_3, in a rat model of optic neuritis, using myelin oligodendrocyte glycoprotein (MOG) induced experimental autoimmune encephalomyelitis (EAE).
Methods: RGCs were retrogradly labelled by stereotactical fluorogold injection in the superior colliculus. After MOG immunization, brown Norway rats were randomly assigned to be treated with either vehicle or calcitriol 1 mg/kg i.p.. On day 8 of clinical manifest EAE, animals were sacrificed and ON histopathology was assessed by Luxol-fast blue (LFB) staining, immunohistochemistry to detect β-amyloid precursor protein (ß-APP), Bielschowsky's silver impregnation and hematoxylin-eosin (HE) staining. Furthermore RGC density was compared using fluorescence microscopy on flat mounted retinas.
Results: We did not detect significant differences in clinical disease severity, demyelination (LFB: vehicle 72.2% ± 9.582%; calcitriol 79.72% ± 7.334%; $p< 0.5421$), acute axonal damage (β-APP: vehicle 5.40 ± 1.516; calcitriol 3.939 ± 2.927 positive axons per mm^2; $p< 0.712$), chronic axonal damage (Bielschowsky: vehicle 9.517% ± 1.472%; calcitriol 9.204% ± 1.625%; $p< 0.8949$), and infiltrating cells (HE: vehicle 2.391 ± 0.2154; calcitriol 2.667 ± 0.2462 cells per mm^2; $p< 0.4342$) in the ON. However, there was a significant lower densitiy of RGCs in vehicle- versus calcitiriol-treated animals. (vehicle 651.6 ± 56.21; calcitriol 926 ± 87.72 cells per mm2; $p< 0.0101$).
Conclusions: Despite calcitriol did not show any anti-inflammatory effects, we found a significant influence on RGC survival. This suggests a direct neuroprotective property of this substance which could be of value in combination with anti-inflammatory drugs.

P575

Netrin-1 regulates blood-brain barrier function and CNS inflammation

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Background: Netrins are laminin-related proteins that promote cell adhesion during development and are known to regulate endothelial cell (EC) and immune cell function.
Objectives: Here, we investigated the expression and the role of netrins in blood brain barrier (BBB) homeostasis and dysfunction during neuroinflammation.
Methods: mRNA (qPCR) and protein (WB, lipid raft fractionation, FACS and immunostaining) analysis of netrins was performed in primary cultures of human BBB-ECs, post-mortem MS samples and netrin-1 deficient mice. The *in vivo* role of netrin-1 was study in experimental autoimmune encephalomyelitis(EAE) mice by determining the extent of demyelination, BBB disruption and immune cell infiltration.
Results: Herein, we report that human brain ECs express netrins *in vitro* and *in situ.* We also found that netrin-1 promotes BBB integrity by up-regulating endothelial junctional protein expression, while netrin-1 knockout mice display disorganized tight junction protein expression and BBB breakdown. Upon inflammatory conditions, BBB-ECs significantly up-regulate netrin-1 levels *in vitro* and *in situ*. Finally, netrin-1 treatment during EAE significantly reduced BBB disruption and decreased clinical and pathological indices of disease severity.
Conclusions: We conclude that netrin-1 is an important regulator of BBB maintenance and protects the CNS against inflammatory conditions like MS and EAE.

P576

Immune cells in the diffusely abnormal white matter of multiple sclerosis

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Background: Magnetic resonance imaging (MRI) is useful for demonstrating areas of damage which can occur in multiple sclerosis (MS) brain. Diffusely-abnormal white matter (DAWM) exhibits a signal intensity intermediate between lesion and normal-appearing white matter (NAWM) and has an ill-defined boundary. Histology studies report reduced myelin and axons in DAWM; however, it is unknown whether the pathological process leading to DAWM is similar to lesions with an immunopathogenic etiology, or if it is due to a neurodegenerative process.
Objectives: In this study we examined immune cells to determine whether the degree of microglia activation or the presence of B and T-cells differs between DAWM and NAWM.
Methods: Twelve slices of formalin-fixed brain from 8 MS cases were imaged. Slices were stained immunohistochemically for CD3 (T-cells), CD20 (B-cells) and Class II MHC (CR3/43 antibody, activated microglia/macrophages). Regions of interest (ROIs) of lesion, DAWM and NAWM were mapped from MRI onto histology and 5 high-resolution (40x) fields were photographed per ROI. CD3 and CD20 cells were counted, CR3/43 stained area was quantified and activated microglia were counted/classified (ameboid macrophage, intermediate, ramified) using Image-Pro Plus.
Results: On average, no significant differences between DAWM and NAWM were observed for CD3 or CD20 count, CR3/43 positively stained area, total activated microglia count and total non-ramified activated microglia count. Some individual variation was observed: 3 samples showed significantly more CR3/43 staining in DAWM and several cases demonstrated morphological differences in microglia shape between DAWM and NAWM.
Conclusions: The presence of T-cells, B-cells and activated microglia doesn't generally discriminate between DAWM and NAWM, suggesting that the immune response does not play a more significant role in DAWM than it does in NAWM. Consequently, a neurodegenerative process may be responsible for the previously reported myelin and axonal pathology found in DAWM.

P577

Interleukin-1β activates the apoptotic protein p53 to cause excitotoxic neurodegeneration and disease progression in multiple sclerosis

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Background: Proinflammatory cytokines can increase excitatory synaptic transmission, and are involved in the enhancement of apoptotic pathways. Apoptosis and excitotoxcicity are proposed as determinants of neurodegeneration in many neurological diseases and could be the link between neuroinflammation and neurodegeneration in multiple sclerosis (MS).
Objectives: To investigate the role of the apoptotic cascade in the synaptic abnormalities, neuronal loss, and disease progression caused by inflammation in relapsing-remitting MS (RRMS) patients.
Methods: the effect of interleukin-1β (IL-1β) and of tumor necrosis factor (TNF-α) on glutamate-mediated synaptic transmission and excitotoxic neuronal damage was investigated *in vitro*, and following pharmacological inhibition of p53 by mean of pifithrin-α (PFT). The involvement of p53 in IL-1β-driven neuronal damage and disease progression was investigated in 170 RRMS patients carrying genetic variants of p53 associated with altered apoptotic function, who underwent cerebrospinal fluid (CSF) determination of IL-1β.
Results: The synaptotoxic effect of IL-1β, consisting in enhanced frequency of glutamate-mediated spontaneous excitatory post-synaptic currents (sEPSC) (122±4%, $p< 0.05$ respect to pre-drug values), was blocked by PFT (102±3%). PFT also blocked IL-1β-induced neuronal swelling ($p< 0.05$), whereas it failed to affect the post-synaptic enhancement of sEPSC decay time and half-width mediated by TNFa ($p>0.05$). In RRMS patients, Multiple Sclerosis Severity Scale (MSSS) was significantly lower among subjects with undetectable IL-1β (IL-1β-) than subjects with detectable IL-1β (IL-1β+) in the CSF (2.26±2.1 vs 3.41±2.6, $p< 0.01$). Retinal nerve fiber layer (RNFL) thickness was preserved in IL-1β- group. The Pro/Pro genotype of p53, associated with low efficiency of transcription of p53-regulated genes, abrogated the association between central IL-1β and disability progression or neuronal damage. Pro/Pro IL-1β+ subjects showed lower MSSS and higher levels of RNFL thickness respect to Arg/Arg and Arg/Pro carriers (MSSS: 0.91±0.88 vs 4.26±2.58 vs 3.28±2.58, $p=0.02$; RNFL: 107.53±12.9 vs 93.38±9.07 vs 100.09±7.2, $p=0.005$), showing that IL-1β-driven neurodegenerative damage was modulated by p53 genotype.
Conclusions: Inflammatory synaptopathy and neurodegeneration caused by IL-1β in RRMS patients involve the apoptotic cascade. Targeting IL-1β-p53 interaction might result in neuroprotection.

P578

EBV nuclear antigen-1 epitope reactive to intrathecal antibodies in the cerebrospinal fluid of patients with multiple sclerosis

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Background: Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of unknown etiology. The most common laboratory abnormality associated with MS is increased intrathecal IgG synthesis and the presence of oligoclonal bands (OCBs) in the brain and cerebrospinal fluid (CSF). However, the major antigenic targets of the antibody response are unknown. The risk of

MS is increased after infectious mononucleosis, and MS patients have higher serum titers of EBV antibodies than control populations.
Objectives: To identify disease-relevant epitopes of MS IgG.
Methods: We screened a phage-displayed random peptide libraries (12-mer) with total IgG purified from an acute MS brain. We characterized phage peptide binding specificity by ELISA and phage-mediated Immuno-PCR.
Results: Two phage-displayed peptides were identified that share linear sequence homologies with EBV nuclear antigens 1 and 2 (EBNA-1 and EBNA-2), respectively. The specificity of the EBV epitopes to panning MS brain IgG was confirmed by ELISA and competitive inhibition assays. Using a highly sensitive phage mediated immuno-PCR assay, we determined specific bindings of the two EBV epitopes to CSF from 50 MS and 5 inflammatory control (IC) patients. Antibody binding to EBNA-1 epitope, but not to EBNA-2 epitope, was found in 25 of the 50 MS patients and 1 of the 5 IC patients. Furthermore, EBNA-1 epitope was recognized by OCBs in multiple MS CSF by isoelectric focusing.
Conclusions: Our data suggest that EBNA-1 epitope is reactive to MS intrathecal antibodies corresponding to oligoclonal bands.

P579

Heat shock protein 40 family promotes autoimmune demyelination

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Background: Heat shock proteins 40 (HSP40) are family of small proteins which regulate molecular chaperone HSP70 function by stimulating ATPase activity. HSP40 has been implicated in processes like stress responses, cell differentiation and cell death regulation. We have identified two transcripts of HSP40, Dnaja2 and Dnajb1 to be regulated by microRNA-155 during development of myelin autoreactive T helper (Th) responses.
Objectives: To analyze the role of Dnaja2 and Dnajb1 in Th cells during autoimmune demyelination.
Methods: To identify the gene expression changes in the absence of miR-155 we profiled Th cells from miR-155 knockout using small density arrays. Functional significance of Dnaja2 and Dnajb1 has been assayed by Th cells transfection with overexpression plasmids or with siRNA.
Results: We have found that most upregulated genes in miR-155 deficient were two genes of heat shock protein 40 chaperone family, Dnaja2 and Dnajb1. These two genes were also upregulated in Th following a miR-155 antagomir treatment. Dnaja2 and Dnajb1 inhibition led to increase of Th17 marker genes expression, conversely Dnaja2 and Dnajb1 overexpression had diminished production of IL17A. Finally MOG TCR transgenic T helper cell overexpressing either Dnaja2 or Dnajb1 were less encephalitogenic in an EAE transfer model.
Conclusions: Our results highlight the HSP40 genes expression changes as previously unknown mechanism of Th cell function regulation and suggest miR-155 - Dnaja2 and Dnajb1 - Th17 axis to control development of autoimmune demyelination.

P580

Ephrin B1 and B2 are essential for the pathogenicity and migration capacity of TH17 cells in EAE and MS

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Background: In multiple sclerosis (MS), $CD4^+$ T helper (TH) cells are of great importance to the pathogenesis of the disease. Especially TH17 cells exhibit a strong proinflammatory phenotype, and have been shown to disrupt the blood-brain barrier (BBB). Using a proteomic approach, we previously revealed that TH17 cells express several members of the ephrin/Eph receptor tyrosine kinase system. Ephrins (EFNs) interact with their complementary receptors (Ephs) via cell-cell contact, and these complexes are involved in a variety of biological processes including neural development, vasculogenesis/angiogenesis and T cell regulation.
Objectives: In this study, we aimed at eludicating the role of the ephrin/Eph system in MS and its animal model, experimental autoimmune encephalomyelitis (EAE), specifically in the pathogenicity and migration potential of TH17 cells.
Methods: To identify expression of ephrins and Ephs, we used qPCR and WB on RNA and protein fractions of differentiated TH1, TH2 and TH17 cells. TH17 cells were also allowed to migrate through a monolayer of human BBB endothelial cells (BBB-ECs), after stimulation of the EFNB2-EPHB4 interaction. Furthermore, RNA from *ex vivo* CD4 and CD8 cells was isolated from healthy donors and MS patients to identify differential expression of ephrins/Ephs. Immunohistofluorescence was used to determine expression of EFNB1 and EFNB2 in MS lesions. A conditional double knockout (dKO) of EFNB1 and EFNB2 in TH cells was used to induce EAE, after which clinical scores, weight, immune infiltration and demyelination was studied.
Results: We demonstrate that EFNB1 and EFNB2 are preferentially expressed by TH17 cells and that they are upregulated on T cells of MS patients when compared to healthy donors. Furthermore, stimulation of the EFNB2-EPHB4 interaction on human TH17 cells increases their migratory capacity through a monolayer of human BBB-ECs. We found EFNB1- and EFNB2-expressing infiltrating T cells in MS brain lesions, along with a striking upregulation of EFNB1 on astrocytes surrounding the infiltrate. Using a conditional dKO of EFNB1 and EFNB2 in TH cells, we found that clinical symptoms of EAE are significantly reduced. Further experiments revealed that this effect is due to decreased migration of TH cells (both TH1 and TH17) to the CNS, a reduced activation status of these cells and decreased demyelination.
Conclusions: Taken together, these results elucidate a crucial role for the EFN/EPH system in EAE and MS.

P581

Heterogeneous biological effect of AQP4-IgG on astrocyte

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Background: Neuromyelitis optica (NMO) is associated with a specific serum auto-antibody targeting aquaporin 4 (AQP4), a water channel expressed on astrocyte endfoot. AQP4 belongs to a membrane protein complex that includes potassium channel Kir4.1 and glutamate transporter EAAT2/GLT1. AQP4 auto-antibodies (AQP4-IgG) have a pathogenic role through complement-dependent toxicity leading to astrocyte death. We hypothesized that AQP4-IgG could also differentially modulate antigen density on astrocyte, triggering cell dysfunction, prior or in addition to the cytotoxic molecular cascade of complement.
Objectives: We aimed at identify the potential neuro-modulatory effect of AQP4-IgG on the behaviour of proteins involved in astrocyte function. We also evaluated the potential immuno-modulatory effect of AQP4-IgG by investigating the profile of chemokine-cytokine production by glial cells.
Methods: Mixed glial rat primary culture were treated for 24h with IgG purified from sera of 16 AQP4-IgG positive NMO patients and 8 healthy donors. Cell lysates were used for western blot and supernatant culture for cytokine secretion (Rat Cytokine Array Panel A, 29 molecules tested).
Results: AQP4-IgG induced a neuro- and an immuno-modulatory effect on glial cells. AQP4-IgG from different patients diversely modulated the expression level and cell localization of AQP4, GFAP, Kir4.1, GLT1 and Connexin 43. Thus, the neuro-modulatory effect of AQP4-IgG, on astrocyte proteins that are crucial for cell homeostasis and functions, was heterogeneous. By contrast, AQP4-IgG from 8 acute NMO induced a common profile of cytokine and chemokine production by glial cells. CCL5, CXCL3, sICAM-1, L-Selectin, IL-1ra, IL-2, MIP-1α and MIP-3α, that are implicated in the recruitment of effector immune cells, were specifically raised up, independently from the neuro-modulatory effect described overhead. This specific glial immune response suggested a molecular signature during attacks of NMO. The cytokine profile follow-up for two patients in remitting course did not show this specific signature.
Conclusions: AQP4-IgG isolated from different NMO patient's sera induce a heterogeneous neuro-modulatory effect on astrocyte despite a common effect on cytokine secretion.

P582

In vivo imaging of molecular mimicry of CD8+ T cells in healthy and inflamed brain

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Background: CD8+ T cells are frequently found in multiple sclerosis lesions, however their precise influence on disease pathology is not clear. We investigated here if foreign/self cross-recognizing CD8+ T cells induce central nervous system damage *in vivo*.
Objectives: We identified a model of molecular mimicry in which Ovalbumin $(OVA)_{257-264}$-specific transgenic T cells (OT1) cross-recognize a myelin antigen (MOG40-54) with overlapping steric homology motifs, as confirmed by the analysis of steric peptide homology and alignment in 3D reconstruction. We used this model to monitor myelin- recognizing CD8+ T cells in CNS tissue *ex vivo* and *in vivo*.
Methods: We monitored interactions of OT1 CD8 T cells with neurons in *ex vivo* and *in vivo* tissue by two photon laser scanning microscopy. For in vivo studies, we transferred OT1 CD8+ T cells in C57/Bl6 mice and induced active experimental autoimmune encephalomyelitis (EAE) by immunization with MOG_{33-55} after a reconstitution period of 4 weeks.
Results: Analyzing OT-1 T cells by intravital microscopy, we detected antigen recognition of OT-1 T cells within the CNS *in vivo* leading to a selective enrichment of OT-1 in experimental autoimmune encephalomyelitis lesions. However, the myelin-recognition of CD8+ T cells in the CNS did not lead to any clinical effects in EAE diesease course. These cross-reacting T cells were also not able to induce subclinical damage as shown by histological analysis of brain tissue.
Conclusions: Our findings indicate that (self-)antigenic peptides are recognized by CD8+T cells in the CNS *in vivo* and this recognition might be much more promiscuous than previously thought. However, it is on its own not sufficient to induce damage in a clinically significant manner.

P583

Mucosal associated invariant T-cells from multiple sclerosis patients are effector cells with increased activation and homing abilities

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Background: Mucosal Associated Invariant T (MAIT) cells are semi-invariant T cells (Vα7.2-Jα33 chain) implicated in the defense against pathogens. These cells are also found in demyelinating inflammatory lesions of the Central Nervous System (CNS) of Multiple Sclerosis (MS) patients. However, to date, little is known about their implication in the pathophysiology of the disease. Two studies showed divergent results on the frequency and function of MAIT cells in the blood of patients with MS as compared to Healthy Volunteers (HV).
Objectives: Deciphering the role of MAIT cells in the inflammatory process of MS.
Methods: MAIT cells frequency was studied *ex vivo* in the blood of MS patients (n=39) as compared to age- and sex- matched HV (n=27). Relapsing-remitting (RR, n=30), primary progressive (PP, n=4) and secondary progressive (SP, n=5) MS patients were included. The *ex vivo* phenotype and the transcriptional profile of MAIT cells were analyzed by flow cytometry and TaqMan low density array. Immunohistofluorescent staining of MAIT cells with anti-CD3/CD161/Va7.2 was also performed on active (n=10) and chronic active (n=10) CNS lesions.
Results: MAIT cells frequency in the blood of MS patients tended to decrease as compared to HV (4.70±0.46 and 5.53±0.57, respectively), especially in PP patients. Besides, surface expression of

PSGL-1 (MFI: 16150±705 in MS vs 12630±818 in HV, p< 0.01) and CD11a (MFI: 8534±566 in MS vs 7045±456 in HV, p< 0.05) was increased on MAIT cells from MS patients. Both markers are described as implicated in the migration into the CNS. Besides, CD95 expression was increased on MAIT cells from MS patients as compared to HV (92.01±2.56% vs 81.20±6.10%, p< 0.05), suggesting an increased activated state in MS. Interestingly, Lag-3 and CD244 were shown to be downregulated in qPCR experiments while tbet was upregulated in MAIT cells from patients. These results suggest an increased effector function associated with an increased "Th1" switch. Finally, MAIT cells could be identified in CNS lesions.

Conclusions: Although the frequency of MAIT cells in the blood of MS and HV seems to be similar, our results suggest that MAIT cells from MS patients have increased effector functions together with increased transmigration/homing abilities. *In vitro* functional experiments are in process to confirm these observations. Besides, detection of MAIT cells in CNS lesions will allow further characterization in MS lesions.

P584

Metabolic syndrome and multiple sclerosis. Metformin and thioazolinediones activate different immunomodulatory pathways

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Background: Nutritional status and metabolism affect immune responses. Leptin participates in a wide range of biological functions strengthening the link between immune function, metabolism and nutritional state. Moreover, several pharmacological compounds affecting glucose and cholesterol metabolism, also exert immunomodulatory activity.

Objectives: To evaluate effects of metformin and thioazolinediones on immune regulation in obese MS patients who developed metabolic syndrome

Methods: Twenty obese patients with diagnosis of relapsing remitting MS, who also developed metabolic syndrome, were studied. Fourteen patients received metformin and 6 patients were given pioglitazone as treatment. Patients underwent complete neurological examination every 3 months, and brain MRIs were performed every 6 months. Blood samples were collected before treatment and at two-month intervals thereafter. AMP-activated protein kinase (AMPK), and peroxisome-proliferator activating receptor-γ (PPAR-γ), a transcription factor, were assessed by RT-PCR. Serum leptin was measured by ELISA, and secretion of IL-4, IL-6, IL-10, IL-12, IL-17, IFN-g, and TNF-a by MBP-peptide specific-peripheral blood mononuclear cells was studied using ELISPOT. Numbers of CD4+CD25+FoxP3+ regulatory T cells were evaluated by flow cytometry.

Results: After metformin and pioglitazone treatment, MRIs showed significant decreased in number of new or enlarging T2 lesions (p< 0.001), as well as in number of Gd-enhancing lesions (p< 0.001), compared to the 2 preceding years. Metformin treatment resulted in robust increase in AMPK activity, which was associated with significant fall in leptin levels, and in number of IFN-γ and IL-17 producing cells, as well as robust increase in percentages of CD4+ CD25+ FoxP3+ regulatory T cells (p=0.01 to p< 0.0001). Meanwhile, pioglitazone induced significant activation of PPAR-g, and robust fall in serum leptin, as well as in IL-6 and TNF-a producing cell numbers (p= 0.01 to p< 0.001).

Conclusions: Nutritional status and metabolism can affect immune response. Strategies to treat obesity-related metabolic disturbances may contribute to down-regulate immune response. Therefore, knowledge of common pathways linking metabolism and immune tolerance could be harnessed to influence immune response in autoimmune diseases like MS.

P585

Amino acid catabolism is altered in immune cells from multiple sclerosis patients

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Background: Cells expressing enzymes that degrade amino acids (AA), modulate antigen presenting cells and lymphocyte function. Basal AA catabolism may contribute to immune homeostasis by preventing autoimmunity, whereas increased AA catalytic activity may reinforce immune suppression promoting pathogen persistence and chronic infection.

Objectives: To evaluate pathways sensing and catabolizing Tryptophan (Trp) and Arginine (Arg), and how these pathways modulate immune cell function in MS patients.

Methods: Twenty patients with diagnosis of relapsing remitting MS, 20 patients with other inflammatory neurological diseases (OIND) and 20 healthy control subjects were studied. Expression of enzymes catabolizing Trp (IDO1, IDO2, and TDO) and Arg (iNOS, ARG1 and ARG2) in peripheral blood mononuclear cells (PBMC) was assessed by RT-PCR. IDO activity was measured applying the Kynurenin:Trp ratio, whereas ARG activity was assessed by colorimetric assay. IL-2, IL-4, IL-6, IL-10, IL-12, IL-17, IFN-g, and TNF-a secretion by MBP- peptide specific-peripheral blood mononuclear cells in culture was studied using ELISA. CD4+CD25+FoxP3+ regulatory T cell numbers were evaluated by flow cytometry.

Results: In 16 MS patients (80%), significant reduction in expression and activity of IDO1 and ARG1 was observed. However, IDO2, TDO, iNOS, and ARG2 expression and activity were similar in MS patients, patients with OIND and healthy controls. Trp and Arg depletion are sensed in T cells through a serine/threonine kinase GCN2-dependent mechanism. TLR9 stimulation of PBMCs from MS patients resulted in lack of GNC2 expression, and elevated levels of mammalian target of rapamycin (mTOR), indicating high levels of Trp and Arg. mTOR is a crucial negative regulator of regulatory T cell de novo differentiation, and population expansion. A parallel and significant decrease in number and function of CD4+CD25+ FoxP3+ regulatory T cells was observed, as well as robust increase in IFN-g, TNF-α, IL-2 and IL-17 production

Conclusions: Increased Trp and Arg catabolism protects tissues from damage caused by dysregulated immunity. Therefore, failures in AA sensing or catabolism may contribute to autoimmune response development. These findings raise prospects for research on novel therapies based on manipulation of this pathway.

P586

The role of T regulatory cells in the therapeutic effect of glatiramer acetate in experimental autoimmune encephalomyelitis

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Background: Glatiramer acetate (GA), a therapeutic agent for the treatment of multiple sclerosis (MS), induces a broad immunomodulatory effect on various subsets of the immune system, resulting in a shift from pro-inflammatory towards anti-inflammatory responses. Several studies have demonstrated an effect of GA on T regulatory cells (Tregs), which are potent immunosuppressor cells pivotal in the maintenance of self-tolerance. However, the role of this cell population in the therapeutic effect of GA has not been clarified.
Objectives: To elucidate the requirement for Tregs in the therapeutic activity of GA in the animal model of multiple sclerosis - experimental autoimmune encephalomyelitis (EAE).
Methods: Tregs were identified immunohistochemically in the CNS of mice that have recovered from EAE following GA treatment. The ability of GA to ameliorate EAE was tested in Foxp3GFPLuciDTR-4 transgenic mice, in which Tregs express diphtheria toxin (DT) receptor, thus facilitating their selective depletion by exposure to this toxin. GA (1mg/mouse) or PBS were injected daily for 7 days following EAE induction by the MOG 35-55 peptide. DT was injected one and two days after conclusion of GA/PBS treatment. Flow cytometry was used to verify Tregs depletion.
Results: Immunohistological analysis revealed 2-fold elevation in the amount of CD3+Foxp3+ Tregs in the CNS of EAE-mice following GA treatment, compared to untreated EAE mice. GA treatment drastically reduced disease manifestation in wild type mice and its effect was not abrogated by DT injection (average maximal clinical score 0.5 in GA-treated compared to 2.9 in untreated mice). In mice carrying the Foxp3GFPLuciDTR-4 transgene and injected with DT, GA was less effective than in the wild type mice. Yet, even in these mice the clinical score of the GA-treated mice was lower than in the PBS-treated mice (1.6 and 3.0, respectively).
Conclusions: GA treatment induces elevation of T-regulatory cells. Selective depletion of these Tregs reduces but does not eliminate the ability of GA to ameliorate EAE. These findings support the role of Tregs, but not in an exclusive fashion, in the therapeutic effect of GA.

P587

Impact of minocycline and established MS medications on EMMPRIN, a new factor implicated in MS immunopathogenesis

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Background: Multiple sclerosis (MS) is an immune-mediated disease directed towards the central nervous system. EMMPRIN (CD147), known as a matrix metalloproteinase (MMP) inducer, is a novel factor in MS involved in multiple immunological functions in addition to trafficking of leukocytes across the blood-brain barrier. We reported EMMPRIN to be up-regulated in leukocytes in active lesions in MS and experimental autoimmune encephalomyelitis (EAE) (Brain 136:1760, 2013). Furthermore, anti-EMMPRIN antibodies reduced the severity of EAE (J Neurosci 31:669, 2011).
Objectives: We investigated whether emerging or established immunomodulators used in MS affect the expression of EMMPRIN on leukocytes. We focused on minocycline, an immunomodulator that we have reported to reduce the severity of EAE in mice (Brain 125:1297, 2002), and which decreased MRI activity in a pilot trial in relapsing-remitting MS (Ann Neurol 55:756, 2004; Can J Neurol Sci 35:185, 2008). Minocycline is currently in a Phase III trial in clinically isolated syndromes (ClinicalTrials.gov Identifier: NCT00666887).
Methods: Mouse splenocytes and human PBMCs were pre-incubated with or without specific immunomodulators before polyclonal T cell activation (anti-CD3, anti-CD28) in culture. Cells were analyzed for viability and EMMPRIN expression by flow cytometry, and for proliferation by tritiated thymidine incorporation.
Results: EMMPRIN expression is increased upon T cell activation. Minocycline attenuated the up-regulation of EMMPRIN on activated T cells at concentrations as low as 0.5 μg/mL ($p=0.009$ compared to no drug), without obvious effects on cell viability and proliferation. At concentrations (5-10 μg/mL) of minocycline that are encountered in MS subjects on this medication, T cell expression of EMMPRIN along with proliferation and viability were lowered. Minocycline was more robust in reducing the mean fluorescence intensity of EMMPRIN, compared to percent cells with EMMPRIN. For established MS immunomodulators, interferon-beta (10-1000 U/mL) reduced EMMPRIN expression, while fingolimod (100-300 ng/mL) was without effect.
Conclusions: An emerging (minocycline) and established (interferon-beta) MS medication attenuated the activation-induced elevation of EMMPRIN on T cells, highlighting the potential importance of EMMPRIN as a novel pathogenic factor in MS.

P588

The nuclear receptor Nur77 restricts T-cell responses and limits central nervous system autoimmunity

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Background: The nuclear receptor Nur77 is upregulated in T cells early after triggering of the T cell receptor and contributes to induction of T cell apoptosis, especially in the context of thymic negative-selection. However, it is not known if Nur77 is involved in modulation of autoreactive T cell responses during autoimmunity.
Objectives: We investigated the role of Nur77 during autoreactive T cell responses in the animal model of Multiple Sclerosis (MS), i.e. experimental autoimmune encephalomyelitis (EAE).
Methods: We evaluated the effects of genetic ablation of Nur77 on T cell proliferation and antigen-specific T cell activation. Moreover, EAE disease course and MOG-specific T cell responses were characterised in Nur77-deficient mice and control mice.

Results: Upon T cell receptor-mediated activation, Nur77-deficient T cells proliferated stronger than wildtype T cells and exhibited an increased potential to differentiate into pathogenic T_H1 and T_H17 cells. Also *in vivo*, antigen-specific T cell activation by Nur77-competent dendritic cells resulted in enhanced proliferation and cytokine production when T cells lacked Nur77. After induction of EAE, Nur77-deficient animals exhibited an earlier onset of disease and a significantly aggravated clinical score. This was accompanied by enhanced MOG_{35-55}-specific T_H1 and T_H17 cell responses both in the periphery and within the CNS. Importantly, also the transfer of MOG_{35-55}-specific Nur77-deficient T cells into healthy wildtype recipients induced a more aggravated disease course than transfer of MOG_{35-55}-specific wildtype T cells underlining the importance of Nur77 in restriction of pathogenic T cell responses. In human T cells, we observed dysregulated Nur77 Expression under inflammatory conditions, thus suggesting that Nur77 plays an important role also in restricting human autoreactive T cell responses.
Conclusions: Nur77 limits $CD4^+$ T cell responses in the context of CNS autoimmunity by restricting proliferation and differentiation into Th1 and Th17 effector cells. Hence, Nur77-dysregulation might contribute to enhanced T cell activation in T cell-mediated autoimmune diseases such as MS.

P589

CD8+CD161hi, Tc17 and mucosal-associated invariant T cells in treated and untreated multiple sclerosis patients

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Background: Th17 cells are important in the immunopathogenesis of multiple sclerosis (MS). However, IL-17 production is not limited to CD4+ cells. CD161 is expressed by IL-17 producing lymphocytes. It has been suggested that CD8+CD161hi cells participate in the pathogenesis of relapsing-remitting MS (RRMS). This population comprises Tc17 and mucosal-associated invariant T cells (MAIT) which can only be differentiated by the expression of the invariant T-cell receptor of MAIT cells. The role of these cell populations in patients with progressive MS and their modifications by disease-modifying drugs (DMD) in RRMS remains largely unknown.
Objectives: To compare the frequency of CD3+CD8+CD161hi (CD8+CD161hi), CD3+CD8+CD161hiValpha7.2- (Tc17), CD3+CD161hiValpha7.2+ (MAIT) cells and CD3+CD8+CD161hiValpha7.2+ (CD8+MAIT) in patients with different clinical subtypes of MS and healthy controls (HC); (ii) to investigate the effect of DMD in RRMS patients.
Methods: Cell surface staining for CD3, CD8, CD161, Valpha7.2 and subsequent flow cytometric analyses were performed in fresh whole blood samples from 17 HC, 39 untreated MS patients (20 patients with RRMS; 8 with secondary progressive MS - SPMS; 11 with primary-progressive MS - PPMS), and 43 RRMS patients treated with DMD (interferon-beta (IFNb; n=16); glatiramer acetate (GA; n=8); fingolimod (F; n=9); and natalizumab (NTZ; n=10)).
Results: No statistically significant differences were observed in the frequencies of CD8+CD161hi, Tc17 and MAIT cells among untreated RRMS, SPMS, PPMS patients and HC. However, DMD treatment was associated with significant reductions in the frequencies of CD8+CD161hi, Tc17 and CD8+MAIT cells when compared with the untreated RRMS group ($p< 0.05$). When the treated RRMS group was further stratified by DMD, NTZ-treated patients showed significantly reduced frequencies of CD8+CD161hi, and MAIT cells compared to untreated RRMS patients ($p< 0.05$), and a trend towards lower frequency of Tc17 cells. IFNb-treated patients exhibited lower frequency of Tc17 cells ($p< 0.05$) and trends towards lower frequencies of CD8+CD161hi and MAIT cells. Frequencies of these cell populations were similar between untreated and F- and GA-treated RRMS patients.
Conclusions: Pathogenic CD8+CD161hi, Tc17 and MAIT cell populations can be modulated by current DMD in RRMS patients. These results point to differential and previously unknown mechanisms of action of IFNb and NTZ which may further contribute to the beneficial effects of these therapies in MS.

P590

Altered glycosylation patterns during multiple sclerosis generate neo-autoantigens

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Background: The complex trait of MS pathogenesis may arise from epistatic and /or additive interaction between multiple seemingly unrelated genes, environmental and immunological factors that converge to dysregulate a critical final common physiological pathway. Recently, in different works, some authors have reported that different environmental factors converge with a subset of genetic variants to dysregulate the N-Glycosylation pathways. N-glycan branching has been shown to be directly influenced by (1) metabolism and nutriment, (2) Vitamin D, a well described factor associated with MS and (3) IL-2 and IL-7 as critical regulators of N-Glycan branching. Finally, in the past years, some authors have clearly demonstrate the importance of the auto-antibodies directed against the glycosylated epitope of MOG, while others have reported the diagnostic value of anti-N-glucosylated peptide or antiGlc-Glc disaccharide antibodies. All these observations have pointed out that glycosylation may be implicated in MS pathological processes and that the deficiencies in glycosylation pathways may lead to autoreactivity against specific epitope or generate neo-epitope.
Objectives: To test the global glycosylation pattern and its influence on autoreactivity
Methods: The glycosylation patterns were analyzed in MS but also in EAE using multiplexed proteomic approach with agglutinin labeling of glycoproteins. The agglutinins used allow us to analyse the N-glycosylation but also the O-glycosylation.
The analyses have been performed in human brain tissue and in a PLP induced EAE SJL/J mice brain.
Results: We demonstrated specific qualitative and quantitative changes in the glycosylation patterns in cerebral tissues associated with MS condition and EAE model. If we did not observe any

modification of the fucosylation status specifically associated with the MS condition. We report two major alterations with regard to the sialylation and the branching glycosylation process of brain glycoproteins. In our study we observed an increase of the sialylation process concerning a large panel of different molecular weight glycoproteins. This phenomenon concerned both altered area as well as apparently normal brain tissue. Finally, we show that glycans alterations dramatically impact the antigenic properties of CNS glycoproteins and could explain dynamic changes of autoantibody repertoire in MS patients.
Conclusions: The results provide evidences for the implication of glycosylation in the MS pathophysiology and has potential diagnostic value.

P591
Cholesterol is main autoantigen recognized by antibodies reacting with brain lipids in MS patients
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Background: Several autoantigens mainly proteins and peptides have been involved in autoimmune recognition in MS. Increasing data indicate that lipids might also induce autoimmune responses and determine the course of MS. The protein and lipid composition of central nervous system (CNS) is changing during MS course and thus might influence antigen recognition by autoantibodies.
Objectives: To assess serum antibody recognition of lipid fraction isolated from MS brains.
Methods: Lipid fractions isolated by Folch procedure from MS and non-CNS pathology brains were assessed by thin layer chromatography (TLC) and used as antigens in ELISA assays with serum of RR-MS patients (n=10) and control patients (n=10). In confirmatory set of experiments serum immunoreactivity with synthetic lipids: L-α-lysophophatidylocholiline, cholesterol, galactocerebrosides, β2-glycoprotein 1 (β2-GP1) and cardiolipin was assessed. The serum concentration of IgG and IgM were measured by nephelometry. To confirm antibody specific immunoglobulins serum binding, IgG and IgM were purified from serum by affinity chromatography (IgG) or dialysis and size - exclusion chromatography (IgM), digested with pepsin and papain and used for ELISA.
Results: As assessed by TLC, in MS brains dominated neutral lipids and all fractions contained cholesterol esters. In control brains dominated phospholipids and cholesterol esters were present only in a few fractions. MS serum IgG bound to almost all lipid fractions with higher efficacy than control serum IgG. Similarly serum IgM of MS patients bound significantly stronger to all lipid fractions than serum IgM of controls. To assess the specificity of lipid binding by MS serum, purified IgG and IgM were digested with papain and pepsin and generated $F(ab)_2$ and F(ab) fragments showed high binding to lipid fractions but not Fc fragments. To further characterize lipids recognized by MS serum cholesterol, β2GP1, cardiolipin, phosphatidylinositol and galactocerebrosides were used as antigen in ELISA assays. Only cholesterol was found to bind IgG and IgM with high efficacy. Preabsorbtion of MS serum with cholesterol significantly decreased subsequent IgG and IgM recognition of lipid fractions by up 70% confirming specific cholesterol recognition by serum of MS patients.
Conclusions: These results indicate that serum IgG and IgM from MS patients specifically recognize cholesterol and that anti-cholesterol antibodies are universally present in MS.

P592
Functional characterization of myeloid dendritic cells in peripheral blood of patients with multiple sclerosis
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Background: Naturally occurring regulatory T cells of $CD4^+CD25^+CD127^{low}Foxp3^+$ phenotype (Treg) are important players in controlling immune and autoimmune responses. Treg are functionally defective in patients with MS and this dysfunction is related to a dysbalanced composition of naïve and memory Treg subtypes in the systemic circulation. Several lines of evidence indicate that these abnormalities might result from premature decline in thymic dependent Treg neogenesis. Myeloid dendritic cells (mDCs) critically determine the differentiation of Treg in the thymus and the thymic stromal lymphopoietin receptor (TSLPR) expressed on the surface of mDC is a key component of signalling pathways involved in this process.
Objectives: We hypothesized that alterations of mDC contribute to the aberrant Treg homeostasis and function detectable in MS, and have therefore assessed phenotypes and functional activities of mDCs isolated from peripheral blood of patients with MS and age-matched healthy control donors. As previous studies documented normalization of both function and homeostasis of Treg under prolonged therapy with Interferon-beta (IFN-beta) we additionally tested treatment effects of IFN-beta on phenotype and immune function of mDCs.
Methods: We determined frequencies and phenotypes of $CD4^+$ T-cells, Treg, and mDCs in blood specimens from 45 MS patients (treatment-naïve: n=27; IFN-beta treated: n=18) and from 14 healthy control donors (HC). FACS-data were correlated with Interleukin-7 (IL-7) and TLSP plasma concentrations as determined by ELISA. Moreover, immune function of mDCs was analyzed *in vitro*.
Results: We found, that mDC frequencies in peripheral blood, TSLPR expression levels on mDCs, and TSLP-induced activation of mDCs *in vitro* were reduced in MS when compared to HC. Therapy with IFN-beta had only a moderate increasing effect on mDC frequencies, but led to significantly enhanced TSLPR expression levels on mDCs.
Conclusions: Our observations, in particular, the decreased TSLP-induced activation of MS-derived mDCs *in vitro*, further corroborate our hypothesis of myeloid dendritic cells being critical involved in the impaired development of regulatory T cells in patients with multiple sclerosis.

P593
c-Myc activity in T-cells is critical for autoimmune demyelination
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Background: c-Myc is a transcription factor that functions as a major mediator of cell development, differentiation and survival. c-Myc has been demonstrated to be a critical molecule for the development of T cell subpopulations such as NK T cells, intraepithelial lymphocytes or memory CD8 T cells. However any involvement of c-Myc in the major T helper (Th) cell populations has not been investigated so far.
Objectives: To analyze the role of c-Myc in Th cell development and function during autoimmune demyelination.
Methods: We used conditional knockout mice (cKO) that lack c-Myc expression in T cells. In order to analyze the role of c-Myc in thymic T cell development we have generated mice in which c-Myc is conditionally deleted by the lck-cre transgene at the DN3 stage. To investigate the role of c-Myc in later stages of thymic development or function in the periphery, we generated mice in which c-Myc is conditionally deleted by the CD4-cre transgene.
Results: In lck-cre c-Myc cKO mice, T cell development is partially arrested, and only a few pre-T cells pass through the DN3 and DN4 checkpoint, mostly in the absence of cell division. In contrast, once past this critical developmental checkpoint, in CD4-cre c-Myc cKO mice further intrathymic development of mature CD4 and CD8 T cells appears to be independent of c-Myc. Both CD4 and CD8 subsets of peripheral T cells developed normally in CD4-cre c-Myc cKO. Furthermore, activation of peripheral T cells by T cell receptor stimulation resulted in normal upregulation of CD69 and downregulation of CD62L in c-Myc deficient as well as control mice. In contrast, profound inhibition of proliferation of c-Myc deficient peripheral T cells, in particular naïve T helper cells, was observed. CD4-cre c-Myc cKO were also completely resistant, both clinically and histologically, to MS animal model, experimental autoimmune encephalomyelitis. This can be correlated with a specific loss of the Th17 population in c-Myc cKO mice.
Conclusions: Our results demonstrate a previously unrecognized role of c-Myc in the development and differentiation of peripheral T helper cells with a particular role in the generation of Th17 cells and development of autoimmune demyelination.

P594

Global metabolomic analysis of cuprizone toxicity

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Background: The cuprizone model of toxic demyelination is widely used to study oligodendrocyte pathology and test new remyelinating therapies. Administration of this compound results in selective oligodendrocyte injury and loss of myelin in the brain. Despite its extensive use in basic research and preclinical studies, the mechanism of action of cuprizone is not known.
Objectives: We sought to understand how cuprizone induces oligodendrocyte dysfunction by measuring global alterations in cellular biochemistry with mass spectrometry-based metabolomics.
Methods: The cuprizone-treated oligodendrocyte cell line, MO3.13, and tissue isolated from mice six weeks after cuprizone administration were examined with hydrophilic interaction liquid chromatography-mass spectrometry (HILIC-MS) and shotgun lipidomics. Relevance of dysregulated pathways were tested using the oligodendrocyte cell line, MO3.13
Results: Significant perturbations in metabolism were seen both *in vitro* and *in vivo* and included alterations in tryptophan metabolism and small molecule precursors involved in the synthesis of NAD+. This coenzyme is important for energy generation and as a substrate for enzymes involved in myelination. In order to test the importance of this pathway, we supplemented cuprizone-treated cells with nicotinamide and found that the addition of this compound could rescue cells from loss of viability.
Conclusions: Analysis of cuprizone-induced pathology in oligodendrocytes by metabolomics indicates that this compound perturbs multiple metabolic pathways and that its *in vivo* toxicity may involve more than its ability to chelate copper.

P595

Principal component analysis allows to cluster patients with multiple sclerosis on the basis of different subsets of CD8+ and iNKT-cells

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Background: Associations between Multiple Sclerosis (MS) and defects in different subsets of T cells, such as, invariant natural killer T cells (iNKT) or CD8+CD161^{++} T cells have been reported, but data is contrasting. A more detailed characterization of iNKT cell subsets and CD8+CD161+ T cells is needed to better clarify their role in MS.
Objectives: Aim of our study was to evaluate the amount of iNKT and CD8+CD161^{++} T cells in different forms of MS and following different treatments.
Methods: We studied 109 MS patients: 77 with a Relapsing Remitting (RR) form [16 treated with Interferon beta (IFN), 15 with Natalizumab (Nat), 14 with Glatiramer Acetate (Gla), 32 without treatment] and 32 with a Progressive (PR) MS: 17 Primary Progressive (PP) 15 Secondary Progressive (SP). Thirty-two age- and sex-matched subjects were used as healthy controls (CTR). S. Isolated PBMC were analyzed on a 6-color high speed acoustic focusing flow cytometer (Attune, Life Technologies, USA), using anti-Va24Ja18 TCR, -CD4, -CD8, -CD161, -CD3, -CD19 and -CD14 mAbs. MatLab was used for the Principal Component Analysis (PCA); statistical analyses were peformed by Stata 11.0 software.
Results: PCA revealed that some immunological parameters could be used to cluster patients, and identify their MS form. All MS patients displayed less CD8+ (p=0.003) and CD8+,CD161++ T

cells (p=0.023) compared to CTR. iNKT,CD8+,CD161+ (p=0.021) and iNKT,CD4+,CD161+ cells (p=0.005) were less represented in MS patients, who, however, showed a higher percentage of iNKT,CD4+ cells (p< 0.001) compared to CTR. In PRMS patients levels of iNKT CD4-,CD8- (p=0.002), iNKT CD8+,CD161++ (p=0.001) and CD4+,CD161^{++} (p=0.001) were lower in comparison to CTR. RR patients treated with IFN displayed lower percentages of CD8+ T cells compared to those treated with Nat (p< 0.001). Levels of CD3,CD8,CD161^{dim} cells were lower in IFN-treated patients (p< 0.001) and in patients treated with with Gla (p=0.001).
Conclusions: The use of PCA can allow the clusterization of MS patients based upon immunological parameters related to CD8+ and iNKT cell subsets. Though the role of CD4+ T cells in MS pathogenesis has been clearly established, much less is known about the role of CD8+ T cells or iNKT or about their polyfunctionality. The use of PCA strongly suggests that the role of these cells deserves further studies.

P596
Autoantibodies to IL-18 in multiple sclerosis: profit or damage?
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Background: The level of cytokines and anti-cytokine autoantibodies (auto-Abs) can considerably vary during autoimmune diseases. Increased serum concentration of IL-18 in multiple sclerosis (MS) was previously shown.
Objectives: to evaluate the level of anti-IL-18 auto-Abs in MS.
Methods: 51 patients with relapsing-remitting MS according to the McDonald criteria and 28 healthy donors (HD) were recruited to the study. 14 from MS patients received IFN-β1a therapy at least 1 year (MS/IFN-β group). Serum level of IL-18 was measured by ELISA using Interleukin-18-IFA-BEST kit (VectorBEST, Russia). Anti-IL-18 IgG auto-Abs were measured by ELISA using recombinant human IL-18.
Results: Serum concentration of IL-18 was significantly higher in MS patients (243.82 ± 29.32 pg/ml) than in HD (102.04 ± 12.53 pg/ml), $p< 0.05$. Serum level of anti-IL-18 auto-Abs in MS patients was lower (0.66 ± 0.04 o.u.) than in HD (1.01 ± 0.08 o.u.), $p< 0.05$. A strong positive correlation between serum concentration of IL-18 and age was found in HD (R=0.74; $p< 0.01$), but not in MS**.** We found a positive correlation between IL-18 concentration and level of anti-IL-18 auto-Abs (R=0.533, $p< 0.1$) in '>mean' HD group, which includes donors with IL-18 concentration lower than mean value. Inverse correlation between the same parameters was observed in '>mean' MS/IFN-β group (R= -0.995, $p< 0.1$).
Conclusions: Despite the increased IL-18 concentration, the level of anti-IL-18 auto-Abs was decreased in MS patients. Decreased level of anti-IL-18 auto-Abs may be due to their involvement in attenuation of inflammation in CNS or influence of IFN-β treatment.

P597
The role of endogenous interferon-beta in the pathogenesis of relapsing remitting multiple sclerosis
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Background: Our previous studies have demonstrated that interferon beta (IFNb) inhibits Th17-cell differentiation, as well as secretion of Th17 promoting cytokines (IL-1, IL-23) from B-cells and dendritic cells (DCs). Only a few studies have addressed the possible role of endogenous IFNb in the pathogenesis of relapsing remitting multiple sclerosis (RRMS).
Objectives: To investigate the role of endogenous IFNb in the regulation of Th17-mediated autoimmune response in RRMS.
Methods: Endogenous IFNb serum bioactivity was tested using IFN-inducable gene expression in human epithelial IFNAR^{+} WISH cell line in the serum from 20 RRMS patients and 20 healthy controls (HCs); flow cytometer studies have identified the percentage of pDCs in peripheral blood mononuclear cells (PBMCs) from 13 RRMS patients and 13 age-, sex- and race-matched HCs; Real time PCR (RT-PCR) was used to detect the expression of IFN-regulatory genes in separated pDCs (purity>98%) from 6 RRMS patients and 6 matched HCs.
Results: The expression of IFNb-induced genes *mixovirus resistance 1 (Mx1) and protein kinase RNA-regulated protein (PRKR)* was significantly decreased in the RRMS serum-treated WISH cells in comparison to HCs. In the context of a decreased percentage of plasmacytoid (p)DCs in PBMCs from RRMS patients, these results suggest a deficient endogenous IFNb secretion in the RRMS patients. *Ex-vivo* separated pDCs from RRMS patients showed significantly increased gene expression for IFNb secretion inhibitory molecule blood dendritic cell antigen (*BDCA)2* and decreased IFNβ-inducable *MX1* in comparison to HCs, reflecting a decreased endogenous IFNβ secretion and autocrine signaling in pDCs. Western blotting experiments on the CD4^{+} T-cells from RRMS patients revealed decreased baseline expression of pSTAT1, pSTAT3, IRF7 and MyD88 in comparison to the matched HCs, which may reflect decreased endogenous IFNb secretion. In addition, *in-vitro* treatment with exogenous IFNb-1a induced protein expression of pSTAT1, IRF7, and MyD88 that was less prominent in RRMS patients in comparison to the matched HCs, suggesting that IFNb signaling is decreased in CD4^{+} cells from RRMS patients in comparison to HCs.
Conclusions: A deficient IFNb expression in pDCs and deficient IFNb signaling in CD4^{+} T-cells from RRMS patients may contribute to the development of the Th17-mediated autoimmune response in RRMS.

P598
Sodium chloride-high diet promotes pro-inflammatory macrophage activation and aggravates central nervous system autoimmunity
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Background: It has long been debated whether environmental factors such as nutrition may have an influence on Multiple

Sclerosis (MS) incidence and severity. Recently it was shown that a diet rich in sodium chloride (NaCl) influences T cell responses during autoimmunity of the central nervous system (CNS) in the animal model of MS, i.e. experimental autoimmune encephalomyelitis (EAE).

Objectives: As the influence of NaCl on macrophages is largely unclear, we evaluated, whether NaCl could promote pro-inflammatory macrophage responses in the context of CNS autoimmunity.

Methods: Murine and human macrophage responses were investigated *in vitro* and *in vivo* under homeostatic conditions, upon stimulation as well as during MOG-induced EAE. Furthermore, the impact of transferred NaCl-treated macrophages on EAE disease severity was assessed.

Results: Murine NaCl-treated macrophages exhibited a strong pro-inflammatory phenotype upon stimulation characterized by significantly higher production of TNFa and IL-12 and increased expression of immune-stimulatory molecules. Mice receiving a NaCl-high diet showed significant aggravation in clinical EAE severity compared to mice receiving normal diet accompanied by a strongly activated phenotype of CNS macrophages at the peak of EAE. Transfer of NaCl-conditioned macrophages into EAE-diseased animals resulted in a significant aggravation of disease severity when compared to transfer of untreated macrophages, thus underlining the pathophysiological relevance of NaCl for macrophage activation in the context of EAE. Importantly, also in human monocytes, NaCl induced increased production of pro-inflammatory cytokines TNFa and IL-12 as well as enhaced expression of the immunmodulatory surface markers HLA-DR, CD80, CD86 and CD40.

Conclusions: NaCl-high diet promotes a pro-inflammatory phenotype in macrophages that aggravates CNS autoimmunity. Further studies are warranted to determine the relevance of increased dietary NaCl uptake in humans for MS disease incidence and disease severity.

P599

Suppression of IL-10 production by calcitriol in patients with multiple sclerosis

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Background: Risk for multiple sclerosis (MS) was found to decrease significantly with increasing serum 25-hydroxyvitamin D [25(OH)D] levels. On the other hand, it is still unclear whether immunoregulatory functions of vitamin D are altered in MS.

Objectives: The aim of this study was to investigate whether calcitriol (1,25-dihydroxyvitamin D) differentially modulates cytokine production by peripheral blood mononuclear cells (PBMCs) from MS patients compared with that from healthy controls. It was also examined the relationship between suppressive effects on cytokine production with vitamin D and MS severity.

Methods: Thirty-five patients with relapsing-remitting type MS (RRMS) or secondary-progressive type MS (SPMS) as diagnosed using the 2010 revised McDonald criteria, and age and sex-matched twenty-six healthy control (HC) subjects (female:male = 18:8, mean age at blood sampling = 42.2 ± 11.0 years) were recruited. In response to phytohemagglutinin (PHA) or lipopolysaccharide (LPS), cytokine level [IL-6, IL-10, IL-12/IL-23(p40), interferon (IFN)-gamma, tumor necrosis factor (TNF) and lymphotoxin (LT)] in a calcitriol-added sample was normalized to that in a calcitriol-absent sample, and this relative unit was compared.

Results: Calcitriol suppressed PHA- and LPS-evoked production of IL-6, IFNg, TNF, and LT in PBMCs from MS patients to the same extent as in healthy controls. However, PHA-stimulated IL-10 accumulation was significantly lower in calcitriol-treated PBMCs isolated from MS patients than in those isolated from healthy controls ($p = 0.0192$), suggesting a stronger suppressive effect of calcitriol on this anti-inflammatory cytokine in MS. Conversely, LPS-evoked IL-12/IL-23(p40) accumulation was significantly higher in calcitriol-treated PBMC cultures from MS patients ($p = 0.0088$), suggesting a reduced suppressive effect on production of this pro-inflammatory cytokine in MS. PHA-stimulated IL-10 production in the presence of calcitriol was negatively correlated with the Expanded Disability Status Scale (EDSS) scores, which means that stronger calcitriol-dependent suppression of IL-10 was associated with higher EDSS score.

Conclusions: In patients with MS, the immunoregulatory effects of vitamin D as well as serum levels of vitamin D may differ from healthy controls. Serum vitamin D levels and effects on immune cells also differ between ethnicities, providing a possible reason for the ethnic and geographic variability in MS incidence.

P600

Comparative efficacy between a generic (M356) and brand Copaxone® (glatiramer acetate injection) in an animal model of multiple sclerosis

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Background: M356 is being developed as a generic version of Copaxone® for the treatment of relapsing-remitting multiple sclerosis (RRMS) and is under FDA review. Equivalence between M356 and Copaxone® was evaluated using a comprehensive set of physicochemical (structural) and biological assays.

Objectives: To contribute to the evaluation of biologic equivalence of M356 and Copaxone® by using several variants of the experimental autoimmune encephalomyelitis (EAE) model, a longstanding and widely-used model of MS.

Methods: Three different mouse models were used: active immunization with proteolipid peptide (PLP; an RRMS model), with prophylactic dosing of study treatments; active immunization with myelin oligodendrocyte glycopeptide (MOG; a chronic MS model), with prophylactic dosing; and adoptive transfer of PLP specific T cells from immunized mice to naïve recipients, with therapeutic dosing. Symptoms of EAE progression were monitored daily. In the MOG model, histological analysis was also performed.

Results: Statistically significant ($p < 0.05$) differences between M356 and Copaxone® were not observed for symptom onset, disease intensity, and peak disease score. Histological analysis in the MOG model showed no statistically significant treatment differences in terms of inflammatory foci, apoptotic cells, and

demyelination. Both M356 and Copaxone® significantly delayed mean day of symptom onset relative to vehicle in all models: PLP/prophylactic: 13.0 to 15.3-15.4 days, $p < 0.001$; MOG/prophylactic: 12.0 to 28.5-29.0 days, $p < 0.001$; adoptive transfer/therapeutic, 8.3 to 12.8-16.4 days; $p \leq 0.01$.

Conclusions: The biologic equivalence of M356 and Copaxone® was demonstrated across several EAE models with different antigens and dosing regimens. These results were supportive of and consistent with results from a larger biocharacterization program, which consisted of multiple complementary molecular, cell-based, and in vivo assays across relevant biologic activities of glatiramer acetate.

P601

The ubiquitin-like modifier HLA-F adjacent transcript is upregulated in the central nervous system *in vitro* and in vivo by proinflammatory cytokines

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Background: FAT10, also called ubiquitin D or diubiquitin, is an ubiquitin-like modifier induced by pro-inflammatory Cyt. Like ubiquitin, FAT10 can signal protein degradation by the proteasome and perhaps through autophagy. Several targets involved in translation, protein folding, RNA processing, and macromolecular complex assembly have been identified in FAT10-transfected cells. No endogenous FAT10 conjugates are known and FAT10 functions are not clear. We reported gene expression for FAT10 is upregulated 400-fold in neurons following 6 hours of exposure to proinflammatory Th1 Cyt *in vitro,* confirmed upregulation by QRT-PCR, and protein expression by immunocytochemistry (IC).

Objectives: To examine regulation of HLA-F adjacent transcript 10 (FAT10) by cytokines (Cyt) in the central nervous system (CNS).

Methods: Mixed glial cultures, from neonatal rat brain, contained 40% differentiated oligodendroglia (OL), 40% astroglia (AS),10% microglia (MG), 10% OL progenitors and unidentified cells. Gene expression was measured with Affymetrix 230A 2.0 gene arrays after 6 hours of treatment with Cyt mixtures representative of Th1 cells, Th2 cells or macrophage/monocytes (M/M1). Experimental allergic encephalomyelitis (EAE) was induced in mice by immunization with myelin-oligodendrocyte glycoprotein.

Results: FAT10 message and protein are expressed in central nervous system (CNS) glia and in brains and spinal cords of mice with EAE, a model of multiple sclerosis (MS). In glial cultures, FAT10 message was upregulated 600-fold by Th1 Cyt, 8-fold by M/M1 Cyt and 13-fold by Th2 Cyt. By IC, untreated OL in culture expressed FAT 10 in cell bodies and myelin-like membrane sheets, and this expression increased after 24 hours of treatment with the Th1 Cyt. AS showed less immunostaining for FAT10; Th1, Th2 or M/M Cyt did not effect expression. MG in untreated cultures expressed high levels of FAT10, but this expression was not increased by Th1 Cyt. In vivo, FAT10 was expressed in meninges and submeningeal white matter of EAE mice.

Conclusions: FAT10 expression is rapidly and robustly upregulated in CNS glia in response to proinflammatory Cyt and is expressed in the CNS *in vivo*. FAT10 and its immediate targets are candidates for biomarkers of glial and neuronal responses to inflammation, and for therapeutic intervention in neuroinflammatory and neurodegenerative diseases.

P602

Effects of IFN-β-1b treatment on lymphocyte subpopulations and S1P-dependent migration in patients with multiple sclerosis

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Background: Migration of lymphocytes is important in all types of immune response including autoimmune diseases like multiple sclerosis (MS). Lymphocyte egress from lymphoid tissue into the blood is mediated by sphingosine-1-phosphate (S1P) and S1P-receptors (S1PR). Five types of S1PR (S1PR1-5) are known in humans. Only S1PR1 induces the emigration of B cells out of lymphoid organs.

Proinflammatory cytokines like interferons (IFN) can promote lymphocyte retention in lymphoid tissue by downmodulation of S1PR1. Although IFN-β has been used successfully in the treatment of MS patients for more than twenty years, the exact mode of action still remains unclear.

Objectives: Aim of this study was to assess the effect of IFN-β-1b treatment on the S1P-dependent lymphocyte circulation in MS patients as a possible mode of action.

Methods: Using flow cytometry, subpopulations of circulating B cells were analyzed in three groups: treatment naïve MS patients, MS patients under at least six months of IFN-β-1b treatment and healthy donors (HD). Additionally, S1P-dependent B cell migration in these groups was assessed with in-vitro transwell migration assays.

Results: 30 MS patients (15 treatment naïve, 15 under IFN) and 30 HD were analyzed. Lymphocyte subpopulations of treatment naïve patients resembled those of HD. IFN treatment led to significant changes in lymphocyte subpopulations: reduced memory B cells and marginal zone (MZ) B cells, but increased transitional and naïve B cells.

Treatment naïve MS patients revealed an impaired S1P-dependent migration of different B cell subtypes compared to HC, which normalized under IFN.

Conclusions: The reduction of MZ and potentially autoreactive memory B cells under IFN treatment could be explained through preferential differentiation into short-lived plasmablasts and consequent exhaustion. In compensation, more immature B cells are released from the bone marrow into circulation. This renewal of the B cell compartment could contribute to an alleviation of B cell-mediated autoreactive inflammation in MS.

The impaired S1P-dependent migration of B cells in treatment naïve MS patients should be confirmed in future studies with bigger sample sizes. That S1P-dependent migration improved under IFN treatment was surprising and should stimulate further research.

P603

Natalizumab and fingolimod differentially impact the alpha-4/beta-1 and alpha-L/beta-2 expression-related subset diversity of T-cells

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Background: The alpha-4/beta-1 (α4/ß1; VLA-4) and alpha-L/beta-2 (αL/ß2; LFA-1) integrins are crucially involved in the migration of immune cells into the central nervous system. Natalizumab (NZB) and fingolimod (FTY) are two efficacious treatment options in relapsing-remitting multiple sclerosis (MS). NZB blocks alpha-4-associated extravasation whereas the main effector mechanism of FTY is to block S1P-mediated recirculation of lymphocytes from lymph nodes.
Objectives: We report about α4/ß1 and αL/ß2 expression related subset diversities of T cells, which are differentially impacted by NZB and FTY.
Methods: Co-expression of α4 (anti-CD49d-PE, clone 9F10)/ß1 (anti-CD29-FITC) and αL (anti-CD11a-FITC)/ß2 (anti-CD18-PE) on naive ($CD45R0^{neg}$) and memory ($CD45R0^{pos}$) CD4+ and CD8+ T cells was investigated by flow cytometry. Peripheral blood mononuclear cells were analyzed prior to (T0) and after 3 months (T1) treatment with NZB (n=8) or FTY (n=11).
Results: Analyzing the co-expression of α4 and ß1 subunits of VLA-4 at T0 revealed two subsets ($\alpha4^+/ß1^{low}$, $\alpha4^+/ß1^+$) of naive CD4+, naive CD8+, and memory CD8+ T cells. A third subset ($\alpha4^-/ß1^+$) was observed in memory CD4+ T cells. NZB primarily effected a pronounced decrease in the frequency of $\alpha4^+/ß1^{low}$ subsets of naive CD4+ (T0:77%±10; T1:15%±9; $p< 0.001$) and CD8+ (T0:67%±21; T1:21%±6; $p< 0.01$) T cells. FTY primarily led to an increased frequency of $\alpha4^+/ß1^+$ subsets of naive CD4+ (T0:22%±%; T1:54%±13; $p< 0.01$) and CD8+ (T0:40%±16; T1:63%±1; $p< 0.05$) T cells. Analyzing the co-expression of αL and ß2 subunits of LFA-1 showed that αL expression largely corresponded with ß2 expression. Naive CD8+ T cells showed a clear differentiation into αL+/ß2+ and $\alpha L^{high}/ß2^{high}$ subsets, which served as cut-off. NZB decreased the proportion of $\alpha L^{high}/ß2^{high}$ memory CD4+ (T0:56%±13%; T1:26%±17; $p< 0.001$) and CD8+ (T0:90%±8%; T1:67%±16; $p< 0.05$) T cells. FTY increased the proportion of $\alpha L^{high}/ß2^{high}$ naive CD4+ (T0:7%±4%; T1:47±27%; $p< 0.05$) and CD8+ (T0:33%±18; T1:92%±3; $p< 0.001$) T cells.
Conclusions: NZB primarily acts as "brake" on the α4/ß1 expression of naive T cells and on the αL/ß2 expression of memory T cells. Contrary, the FTY-mediated redistribution of immune cells rather leads to a relative increase of naive T cells with high α4/ß1 and αL/ß2 expression levels. Further research about integrin-related T cell subsets between treatment groups might allow differentiating disease-promoting cells from those required for immune surveillance.

P604

Identification of novel protein candidates involved in immunomodulatory processes in therapy and pathomechanism of multiple sclerosis

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Background: One approved therapy of patients with relapsing-remitting MS is glatiramer acetate (GA). It is well established that GA among other effects induces a shift in the cytokine profile of T-cells, but the molecular mechanisms involved remain largely unknown.
Objectives: By investigating the protein profile of human GA and myelin basic protein (MBP) specific CD4+ T cells, we aimed to find single, Ag-specific expressed proteins that may be involved in regulatory processes of the immune system in MS.
Methods: We generated GA and MBP specific T cell lines (TCL) from healthy donors and from MS patients before and after 6 months of GA therapy. Proteomic analysis of activated and resting GA and MBP specific T cells was done by SDS-PAGE gel-electrophoresis and MALDI-TOF mass spectrometry. Additionally a high throughput human cytokine/chemokine magnetic bead assay was applied to supernatants of human PBMC cultured with protein candidates, to reveal potentially immunomodulatory effects on immune cells.
Results: 51 gel spots were identified as different proteins by MALDI-TOF mass spectrometry. Further analysis of these proteins by pathway software led us to prohibitin - a protein with anti-apoptotic, anti-proliferative and anti-inflammatory properties upregulated only during GA therapy. Other protein candidates (e. g. transgelin 2) were only found upregulated in GA specific T cells, but not in MBP specific TCL. One protein with prominent proinflammatory properties was detected with significant elevation only in MBP specific TCL compared to GA specific T cells. Further analysis via high throughput assays suggested a regulation of cytokines and chemokines by transgelin.
Conclusions: Our results indicate that proteomics are a valuable tool to detect differences in protein regulation among specific TCL in MS. Preliminary results with transgelin indicate so far unknown cytokine / chemokine interactions. Based on their expression and cytokine interaction profile, these proteins may be involved in immunological mechanisms operating in MS and/or during GA treatment.

P605

Short term effects of intravenous glucocorticoids on the expression of *Th17-related* genes on circulating CD4+ T-cells after multiple sclerosis relapse

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Background: CD4+Th17 cells may be the predominant effect of T cell population in the Central Nervous System (CNS) in multiple sclerosis (MS) lesions. However, no Th17 biomarkers have been consistently identified in relapsing-remitting (RRMS) patients or in response to intravenous methylprednisolone (IVMP), the standard treatment for MS relapses. The SMAD

family of proteins mediates signaling from the TGFßR to the nucleus, which is critically involved in TGF-ß pathway, a major regulator of T cell differentiation that governs the Th1/Th17 decision in autoimmune CNS inflammation.
Objectives: To identify Th17-related if there are deregulated genes in RRMS relapsing patients compared to healthy donors and to evaluate their potential as relapse and/or response biomarkers to IVMP treatment.
Methods: PBMCs were purified by gradient density from 20 ml of fresh blood. Then, CD4+ T lymphocytes were isolated by negative selection and magnetic separation. RNA was isolated from CD4+ T cells and cDNA transcribed. Th17 genes differentially transcribed in six RRMS patients during a relapse versus six healthy controls were identified using *Human T helper 17 (Th17) 96 StellARray qPCR Array*. Changes in selected genes were quantified by qRT-PCR in 18 RRMS patients in remission, 18 healthy donors (HD) and 38 RRMS patients during a relapse, 10 of them also after 3-5 days of IVMP 1gr/day. T-Test was calculated for comparisons between groups.
Results: Among more than 90 Th17 related genes we found that the expression of *SMAD7* was lower in RRMS patients during a relapse than in healthy donors. Other genes (*TNFA*, *CSF3*, *S1PR1*, *CEBPD*, *IL18R1*, *IL22* and *RORC*) were close to be statistically significant and were selected for further analysis. The mRNA expression of *SMAD7* (-2.5x-fold) and *S1PR1* (-1.4x-fold) was lower in RRMS patients when compared to healthy donors. *SMAD7* mRNA levels were 2-fold lower also after 3-5 days IVMP treatment.
Conclusions: Transcription of *SMAD7*, a repressor of TGF-ß response, and *S1PR1* is lower in RRMS patients during acute relapses and in remitting phases, suggesting a constitutive induction of Th17 immune response. *SMAD7* is the first response biomarker described in RRMS patients after treatment with IVMP. The additional down-regulation of *SMAD7* in RRMS patients after IVMP might have an important clinical implication.

Internet and social media

P606

Using social media for large-scale recruitment in a prospective multiple sclerosis (MS) inception cohort: the genes and environment in MS (GEMS) study

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Background: The Genes and Environment in Multiple Sclerosis (GEMS) study is a longitudinal, natural history study of an at-risk population, including subjects with at least one first-degree relative (FDR) with MS. We use an algorithm that incorporates validated genetic and environmental risk factors into a single estimate of a subject's risk of developing MS. We aim to recruit 5000 subjects and capture the transition from health to MS during the 20-year follow-up period. The traditional recruitment methods, relying on simple online advertising, clinic referrals, and mailings, were inefficient (100 participants enrolled in 12 months).
Objectives: To investigate the efficacy of social media as a recruitment tool.
Methods: We developed a facebook page and contacted organizations that targeted our subject population: having FDR with MS. We composed facebook and Twitter friendly posts that these organizations could share on their individual pages and websites to promote our study, particularly the National Multiple Sclerosis Society (NMSS). We also regularly posted engaging social media updates to keep enrolled subjects informed about study milestones and findings, MS-related news, and research by our group beyond the GEMS Study. The GEMS facebook page is monitored to eliminate subject self-disclosure and maintain confidentiality.
Results: The GEMS study has enrolled 2501 subjects (1898 females, 2269 Caucasians). 1678 subjects heard about our study through publicity provided by the NMSS and 594 subjects learned of our study via word of mouth and social media sharing. The GEMS facebook page has 1363 followers: 1223 live within the United States. The average viewership of each GEMS facebook posting by unique individuals was 182 during the first year after the launch of the site, 320 during the second year, and 497 in the current third year. 82% of GEMS facebook followers are female (compared to 46% of total facebook users who are female). Specifically, females between ages 18-54 make up 72% of the GEMS facebook followers.
Conclusions: The GEMS Study has been successful in leveraging social media for large-scale subject recruitment. By posting regular social media updates and engaging with other organizations in the MS community, we have created a tool to rapidly increase subject recruitment, maintain study visibility and engage enrolled subjects in this longitudinal study.

P607

A global analysis of the use of social media to discuss multiple sclerosis

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Background: Social media involves the rapid interaction and exchange of information within virtual communities. The number of social medial platforms and their use is increasing, with more time spent on social media sites than any other type of site. Individuals affected by multiple sclerosis (MS) and MS related organisations use social media to discuss the disease, but the extent and nature of the use of social media in MS is not known.
Objectives: To determine the global use of social media in relation to MS over time.
Methods: The social media web analytics tool TopsyPro was used to retrospectively analyse all "tweets" (messages of up to 140 characters) sent on the social networking site Twitter since its launch in March 2006. Search terms and hashtags related to MS generally, chronic cerebrospinal venous insufficiency (CCSVI), MS research, MS treatments and MS risk factors were identified using hashtagify.me, and analysed for the volume, frequency,

sentiment, influence and acceleration of tweets over time. Similarly, social media analytics tool DataSift was used to perform historical and real-time social data analysis across social media platforms including Tumblr, Blogger and public Facebook data. Where peaks of use of social media were identified, Google Trends was used to identify corresponding news stories and evaluate how these influence social media discussion of MS.

Results: Three search terms related to MS generally alone generated a total of 1,680,783 tweets from Mar 2006-Apr 2014. Tweets about MS are rapidly accelerating in frequency with 46% more tweets in Jan-Dec 2013 compared to Jan-Dec 2012. The sentiment of tweets is generally positive with 64% of tweets terms more positive than all other tweets sent in the same time frame. Five terms related to CCSVI were searched for tweets. A total of 61,808 tweets were identified which peaked April-May 2011 (3,314 tweets) following the announcement that $5 million would be spent in Manitoba on CCSVI trials. News stories, particularly when related to, or promoted by, celebrities were found to be particularly influential in social media use.

Conclusions: Social media platforms are widely, and increasingly, used to discuss MS and are an important source of information for individuals affected by MS. The use of social media is an invaluable and necessary opportunity for healthcare professionals to address misconceptions, sensationalist news stories and engage with the public. Further work should aim to advise professionals in MS on how this is effectively done.

P608

Social media and MS: a crowdsourcing-based approach

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Background: Internet is full of blogs and websites about MS where users can post any information that may acquire assertive and not debatable importance even without citing scientific or medical references. Patients with MS who use the web are well informed about the disease, but they are also vulnerable to the hope that can be fueled by false information, myths and therapies that have not been scientifically proven.

Objectives: Our aim is to create a web community where patients with Multiple Sclerosis (MS) can exchange information under the supervision of MS experts.

Methods: www.smsocialnetwork.com gives the opportunity to its users to publicly or privately interact using chat, messages and community wall. All the users can even contact doctors, psychologists and, through the streaming page, watch outpatient visits and medical conferences

Results: Started in March 2012, our web-community includes over 6.490 visitors and 894 active users. The total number of pages viewed is 149,963. Using a crowd-sourcing approach, a blind expert who was totally unaware of the sender, has examined 2762 posts of all public sections of our social network from March 2013 to March 2014. Two macrogroups of users have been clearly distinguished: A (leaders) and B (receivers). In group A there are users who propose innovations, suggestions and relevant medical information, and in group B there are users who share, partially approve or reject these standpoints. Moreover, between leaders, we have distinguished medical and social leaders: medical leaders were perceived "reliable" because of the useful information about treatment and drugs given to other users, while the leadership of "social expert" was due to the fact that they feed a great number of conversations (about movies, weather, music, walks, travels) not writing medical relevant posts but inducing a self-oriented approach to the disease.

Conclusions: The aim of the team of smsocialnetwork.com was to monitor and promote interactions between patients, controlling that information sharing was based on current medical science. We have created a virtual environment where users can talk about everything, under the supervision of MS experts. We believe that under these circumstances the exchange of medical information can be reliable and scientifically correct. It would be methodologically correct to compare our data with those of social networks dedicated to MS without any medical supervision.

P609

Consultation for difficult pediatric demyelinating cases via nationwide webinar

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Background: While pediatric demyelinating syndromes of the central nervous system (CNS) are often self-limited single events, some children experience recurrent demyelinating episodes or accumulate clinically silent MRI lesions, raising concern for multiple sclerosis (MS) or neuromyelitis optica (NMO). As treatments differ, it is critical to distinguish between MS and NMO as well as mimics of demyelination. To assist with these clinical conundrums, a monthly Difficult Case Webinar was established in 2012 with financial support from the National Multiple Sclerosis Society (NMSS) and logistical support from the United States Network of Pediatric MS Centers, currently comprised of 9 sites (Boston Children's, Loma Linda University, Mayo Clinic-Rochester, Massachusetts General, State University of New York (SUNY)-Buffalo, SUNY-Stony Brook, University of California-San Francisco, University of Alabama at Birmingham, Texas Children's) plus a data coordinating center at University of Utah. The webinar format allows the presenter to share their computer screen with participants to view clinical summaries and de-identified neuroimaging during the telephone presentation.

Objectives: Characterize webinar participants and cases presented to date.

Methods: Webinars recorded since December 2012 were reviewed and summarized with respect to cases and participants.

Results: From December 2012-April 2014, 44 patients were presented during 16 webinars which alternated between Mondays

12:00 EST and Fridays 4:00 pm EST. The one-hour webinars had a median of 9 participants (range 5-11) and 2-5 presentations per session. Most case presentations were diagnostic dilemmas with recurrent demyelinating events but MRIs atypical for MS or NMO. Of 90+ addresses on the email distribution list, 35 individuals from 23 institutions have logged onto the Difficult Case webinar at least once. Most were child neurologists (n=21) with 60% (13 of 21) from sites outside the US Pediatric MS Network. Other callers were adult neurologists (n=6, all from Pediatric MS Network sites), 1 pediatric rheumatologist, 3 staff members from NMSS and 4 unidentified participants. Of 28 physician participants, over 70% (n=20) presented at least one case (range 1-5 cases) and >50% participated in 2-14 webinars (median 3 sessions). Over half of participating physicians (n=16) completed their training < 10 years ago.

Conclusions: Webinar technology enables effective nationwide access to expert advice on challenging pediatric demyelinating disorders.

P610

Argentine´s experience in developing and implementing a blog, as a tool for better interaction between multiple sclerosis patients and their doctors

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Background: Globalization of information has helped to develop social media (SM) significantly. Health care practice is not excluded from this process. As the number of patients, health care professionals and students using social networks increases, the evidence to support the benefits of social media on health is beginning to grow In the area of patient care, SM has created the concept of e-patient (an **e-patient** is a health consumer who participates fully in his/her medical care an use electronic communication tools). In our case we focused on how SM can improve patient-doctor relations.

Objectives: The main purposes were to communicate accurate, independent and free information about MS, to report our experience in the development, implementation and follow up of a blog managed by a neurologist or a team specialized in MS. Secondary: feedback from patient communities locally and worldwide visits; to improve our knowledge of MS with the goal of improving the lives of patients in our country and better understanding family needs and their preferences related to MS. A major blog's benefit is that it encourages reflection and team work and provides us with an opportunity to assess its potential impact and plan for the future.

Methods: The starting point of the project was a MS patient workshop on communicating MS. Originally from creating a blog on June 2013 this came at a social network workshops organized for patients, families and other people with an interest in MS with more active participation in the different levels of health through questions, online surveys and reviews. Surveys: physical/work or therapy access, sleep diseases, nutrition/dietary, sexual dysfunction. The blog is fully in Spanish with Google translator.

Results: After 10 months from launch, the blog had 5500 visits till now among ARG: 1337: USA:1253; Spain:1251; Latam:372 and rest of the world:1287. Explorer %: Chrome 39; Firefox 18; Mobile Safari 16. Online Surveys: 213 responders; gender F/M (%): 62 /38; mean age: 43.6 ± 10.9.

Conclusions: SM is a facilitator for the exercise of the medical profession, increases interaction with our peers and access to better information for professionals and patients, both social and emotional support, although we must be careful in the quality of information and data confidentiality. Finally, we must recognize and analyse the changes that have been taking the patient-physician relationship, not to mention that they should remain the essence and purpose of our profession.

Microbiome

P611

Regulation of CNS demyelination by the gut microbiome

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Background: The host gut/symbiont microflora interaction or gut microbiome provide essential support by processing nutrients, controlling infections, and development of the immune system. Mounting evidence demonstrates the importance of the gut microbiome in a variety of experimental conditions including autoimmunity. Environmental factors such as antibiotic exposure, vaccination, diet and stress have been associated with a microfloral alteration or dysbiosis within the GI tract that lead to a disease state. We have assessed the capacity of current and novel therapies to influence immune regulation via the gut microbiome. Our lab first reported on the essential role of gut symbionts in the immune mediated regulation of EAE. We have identified a polysaccharide (PSA) produced by the human symbiont *B. fragilis* as a potent immunomodulator that protects in different models of EAE when administered orally, prophylactic and therapeutically. To date, this is the only purified symbiont product known to effectively protect against CNS inflammation.

Objectives: To determine the mechanisms by which current and novel gut microbiome-related therapies can mediate regulatory responses against EAE and human MS.

Methods: In vivo and *in vitro* culture systems were used to assay for murine gut associated lymphoid tissue (GALT) responses in EAE as well as human PBMC responses to commensal derived antigen and other IMD utilized in the treatment of human MS

Results: Our studies demonstrate the capacity of commensal agents and IMD to modulate the phenotype and functional regulation of specific immune cell compartments in both EAE and human PBMC. We have observed potent IL-10 responses elicited by regulatory CD4+ T cells that express enhanced levels of Foxp3 and CD39. The commensal microflora driven regulatory cells are essential to host protection against the experimental disease state. *In vitro* analysis of human PBMCs exposed to commensal antigen demonstrates a dose-dependent increase in IL-10-producing Tregs, and concomitant reduction of TNF-a. Antigen presentation by PSA was essential in the acquisition of this regulatory/IL-10 phenotype when compared with healthy donors.

Conclusions: These are the first reported findings that correlate the protection induced by a purified symbiont product in

EAE with the promotion of a regulatory T cell phenotype in humans. These findings provide *in vivo*/*vitro* data that support a novel mechanistic paradigm by which commensal products and IMD can establish GALT mediated protection in CNS demyelination.

P612

Intestinal microflora modified by Candida kefyr reduces the susceptibility to experimental autoimmune encephalomyelitis

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Background: Diet is recently drawn attention as a potential risk factor of developing autoimmune diseases. Especially, the rapid increase in MS incidence in Japan seems to be attributed to a change of dietary habit. Although several dietary risks and benefits of probiotics have been suggested in animal models, little is known about the effects of dietary yeast on health.
Objectives: The aim of our study is to examine the effects of dietary yeast on experimental autoimmune encephalomyelitis (EAE).
Methods: We chose 11 kinds of yeast which are contained in diet, then investigated the effects on EAE by oral administration. CD4-positive T cells in intestinal lamina propria and mesenteric lymph nodes (MLN) were analysed by FACS, and cytokine production from explants of intestine was assessed. The effects of Candida kefyr (C. kefyr) to intestinal microflora were analyzed by T-RFLP analysis of feces. Microflora transfer was performed by oral administration of diluted cecal contents after treatment with antibiotics cocktail.
Results: In yeast we examined, only C. kefyr exhibited significant clinical amelioration of EAE and the antigen-specific production of IFN-gamma and IL-17 by inguinal lymph nodes was decreased. Histological examination revealed the reduced inflammatory cellular infiltration into the central nerve system. The number of CD4-positive IL-17-producing cells was reduced in intestinal lamina propria. Intestinal tissue explant culture revealed that production of IL-6 was significantly suppressed in C. kefyr-treated group. In MLN, the number of Treg cells and CD103-posivtiv regulatory dendritic cells was significantly increased in C. kefyr-treated group. The analysis of 16s-rRNA revealed the increased *Lactobacillales* and decreased *Bacteroides*/*Prevotella* ratio compared to control flora. Transfer of intestinal microflora of C. kefyr-treated mice reproduced decreased *Bacteroides*/*Prevotella* ratio, and ameliorated EAE.
Conclusions: The effects of oral administration of yeast were varied depending on speices, and C. kefyr suppressed EAE by modifying microflora. Our findings suggest that dietary intake of C. kefyr may influence systemic immune state and has a beneficial effect on MS.

P613

Bacteria and their cell wall components uniformly co-activate IL-17-producing thymocytes

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Background: IL-17-producing T cells play a critical role in the immune response against microbial pathogens. Traditionally, experimental studies have focused on understanding the activity of IL-17-producing T cells which differentiate from naïve T cells in the peripheral immune system. However, we have previously demonstrated that IL-17-producing T cells are also present in the thymus of naïve wild-type mice and can be co-activated there by microbial stimuli. Other studies have supported the concept that IL-17-producing thymocytes have a specific role in the immediate defense against microbial pathogens, which is independent from the development of an adaptive immune response.
Objectives: Given a strong importance of the thymus in systemic bacterial infection and sepsis, we here investigate the effect of a broad spectrum of bacteria and cell wall components on thymocyte cytokine production.
Methods: Cytokine production of thymocytes was assessed with ELISPOT and with intracellular FACS analysis
Results: Surprisingly, we find that all types of bacteria investigated (including non-pathogenic species) uniformly activate IL-17-producing thymocytes upon α-CD3 stimulation. In contrast, there is a heterogeneous effect on IL-6 and IFN-gamma-production with Gram-negative bacteria inducing far higher frequencies of IL-6- and IFN-gamma-producing thymocytes than Gram-positive bacteria.
Conclusions: We conclude that IL-17-producing thymocytes constitute a "first line of recognition", but not a "first line of defense" against bacteria in general. Their activity might lead to immune activation, but not necessarily to a pathological inflammatory disease condition. The difference between these two states might be determined by other immunological effector molecules like IL-6 and IFN-gamma.

P614

A commensal symbiont product prevents murine CNS demyelination via TLR2-mediated expansion of migratory CD39+ T-cell subsets

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Background: Our recent research on the link between gut microbiome and immune- mediated CNS demyelination has shed light on human multiple sclerosis etiology and clinical utilization of microbiota products. We have reported that polysaccharide A (PSA), a product of the gut symbiont strain *Bacteroides fragilis*, protects against murine experimental autoimmune

encephalomyelitis (EAE) via TLR2 both prophylactically and therapeutically. TLR2 signaling is critical for PSA-mediated suppression of CNS inflammation and anatomically localized expansion of $CD39^+CD4$ T cells. $CD39^+CD4$ T cells express enhanced migratory markers and display increased migratory capacity both *in vitro* and *in vivo*. Deficiency of CD39 signaling compromises PSA protection of EAE, which is correlated by a reciprocal up- and down-regulation of CNS-infiltrating total leukocytes and Tregs.

Objectives: We investigate how *B. fragilis* PSA functions to poise immune balance during CNS autoimmunity.

Methods: We assessed the capacity of PSA to prevent EAE and modulate CD4 T cell phenotypes. EAE was induced in WT and $TLR2^{-/-}$ mice orally treated with purified PSA or PBS. Clinic scores were compared up to day 25. Histological assays were used to visualize CNS immune infiltrates and demyelination. Peak disease state CNS tissues were analyzed for the expression of inflammatory molecules by RT-PCR. FACS was used to measure CD39 level on CD4 T cells at various sites, and to profile migratory markers of $CD39^+$ versus $CD39^-$ T cell subsets. Immune infiltrates into the CNS were compared in PSA or PBS treated WT EAE mice. Finally, $CD39^{-/-}$ mice were compared with WT in PSA protection of EAE development.

Results: PSA significantly reduced murine EAE severity, restricted total leukocyte infiltration and demyelination in the CNS. PSA suppressed inflammatory cytokines and chemokines but reciprocally boosted CD39 signals in the disease-state CNS. Ablation of TLR2 negated the protective function of PSA. PSA preferentially expanded $CD39^+CD4$ T cells at CLN and MLN during EAE. $CD39^+$, as opposed to $CD39^-$ total CD4 T cells and Treg subsets, exhibited elevated level of CCR5, CCR6 and CXCR3. PSA protection of EAE and expansion of Tregs in the CNS were compromised in the absence of CD39.

Conclusions: Our results indicate that commensal products could limit CNS autoimmunity and enhance the tropism of regulatory $CD39^+$ T cell subsets. TLR2 recognition of microbiome could establish tolerogenic immune responses toward systemic inflammation.

P615

Gut microbiome in early pediatric multiple sclerosis: a case-control study

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Background: Alterations in the gut microbiome may be influential in neurological disease.

Objectives: We explored gut microbiome profiles in early pediatric MS and age and sex matched controls.

Methods: Children aged 18 years or under attending a University of California, San Francisco pediatric clinic were invited to participate in a genetic and environmental risk factors study (NS071463, PI Waubant). MS cases were within 2 years of symptom onset. Controls were free from autoimmune disorders (asthma and eczema allowed), with neither parent affected by MS or related disorders. The child's first stool of the day was shipped on ice to the laboratory and stored at -80C. The 16S rRNA gene was amplified from extracted DNA and bacterial profiles were generated using the PhyloChip G3 microarray (Second Genome, Inc., CA). Associations between the pediatric characteristics and variation in the bacterial community composition were assessed using nonmetric multidimensional scaling and permutational multivariate analysis of variance with distance matrices (Adonis function in the vegan R package).

Results: Between Nov/2011-Nov/2013, 20 MS (10 girls, 10 boys) and 16 controls (9 girls, 7 boys) provided stool samples for microbiome analysis. The mean age at collection was 13.2 years (SD=3.84; range 4-18) and 19/36 (14 cases, 5 controls) identified as non-white. Within two months pre-stool collection, 3 children (2 cases, 1 control) were exposed to an antibiotic, 10 (8 cases, 2 controls) to a corticosteroid and within three months, 12 to an immuno-modulatory or -suppressant drug (10 cases, 2 controls). For cases, the mean disease duration at stool collection was 11 months (range 2-24), the median EDSS at enrollment was 2.0 (range 0-4.0); all met McDonald criteria and had relapsing-remitting MS. Preliminary microbiome results indicated significant differences in community composition between cases and controls, with enrichment of Proteobacteria (*Shigella, Escherichia*) and Firmicutes (*Clostridium*) and depletion of Firmicutes (*Eubacterium rectale*) and Actinobacteria (*Corynebacterium*) observed in MS (all $p< 0.01$; false discovery rate, $q< 0.217$). Additional data analyses will be completed by Aug/2014.

Conclusions: Preliminary findings indicate that in very early pediatric MS, gut microbiome composition is significantly altered, with enrichment for microbiota known to be associated with gastrointestinal infectious processes.

P616

Gut microbiome is linked to immune cell phenotype in multiple sclerosis

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Background: The gut microbiome plays a key role in shaping the immune repertoire and plays an important role in disease susceptibility in the EAE model. The gut microbiome has been described in other diseases but not yet in MS.

Objectives: To determine if there are differences in the gut microbiome in MS and if gut microbiome could be linked to immune cell phenotype in MS.

Methods: MS patients from the Partners MS Center [untreated (n=22), glatiramer acetate treated (n=13), and IFN-β treated (n=18)] and healthy controls from the BWH PhenoGenetic project (n=44) were studied. Samples were profiled using two high throughput platforms (454 and Illumina 16s sequencing) to determine community structure and taxonomic composition of the gut microbome. The gut microbiome was also linked to immune cell profiling in peripheral blood using flow based and nanostring based assays.
Results: We found an increase in Archaea (Methanobrevibacteriaceae) in MS vs. controls ($p < 0.00001$ by 454 sequencing). Archaea are in a kingdom separate from bacteria and eukaryotes and in the human gut are dominated by Methanobrevibacter smithii, which make up 10% of colonic anaerobes in the gut. We also found two organisms with anti-inflammatory properties that were lower in MS vs. controls and which were increased with treatment. Specifically: 1) The Butyricimonas genus from Bacteroidetes phylum was lower in the untreated MS vs. controls. Butyricimonas are butyrate producers with anti-inflammatory effects; and 2) The Lachnospiraceae family from the Firmicutes phylum (which are also butyrate producers) was lower in untreated vs. treated MS irrespective of whether they were treated with IFN-β or glatiramer acetate. Immune cell analysis showed that the antigen presenting cells from MS patients have an activated phenotype that could be linked to presence or absence of Archaea. In addition, we found that the expression of T cell specific markers such as IFN-gamma, a proinflammatory cytokine associated with MS, is also linked to the presence or absence of Archaea.
Conclusions: Our results identify changes in both pro-and anti-inflammatory epigenetic factors in the gut microbiome of MS subjects that may contribute to disease pathogenesis and that could be linked to changes in immune cell phenotype.

P617

Commensal antigen induction of suppressive human Foxp3+ Tregs

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Background: Our GI tract harbors billions of microbial agents representing thousands of species. The interaction between the microflora and the host, or microbiome, boasts profound clinically relevant immunogenic potential. Our published studies in EAE demonstrate that the human commensal Bacteroides fragilis can generate IL-10 producing Foxp3+ regulatory T cells (Tregs) that are protective in both EAE and experimental IBD. Preliminary *in vitro* studies using human peripheral blood mononuclear cells (PBMCs) reveal the capacity of a single capsular polysaccharide (PSA) derived from B. fragilis to generate activated IL-10-secreting T cells of a regulatory phenotype that appears independent of Foxp3 co-expression. Our preliminary studies suggest a robust induction of IL-10 by PSA using PBMCs isolated from those with relapsing MS.
Objectives: To determine whether PSA enhances the frequency and/or function of human suppressive, IL-10-producing, Foxp3+ Tregs in those with relapsing MS when compared to normal age matched controls.
Methods: Whole blood was collected from those with MS either naïve or off current therapy (no oral or infused DMT) for at least 30 days or age matched control. Isolated APC and T cell subsets were cultured in the presence of PSA for up to 6 days. Detection and quantification of Treg subsets was achieved using flow cytometric analysis and supernatant collected for cytokine analysis.
Results: PSA induced CD39+HLA-DR+Foxp3+ Tregs, enhanced Treg suppressive potential and IL-10 production. PSA-generated Tregs were more capable of suppressing monocyte-produced TNF-alpha than controls. Studies currently underway are focused on PBMC derived from those with relapsing MS.
Conclusions: These preliminary studies support the capacity of a gut commensal antigen to induce both cytokine production and T cell conversion in healthy donors and those with relapsing MS. This is the first association of the gut microbiome and its capacity to induce an *in vitro* response consistent with immune regulation manifested phenotypically by an increase in suppressive CD39+HLA-DR+Foxp3+ Tregs. These findings suggest the feasibility of therapeutic application of products derived from the gut microbiome in the treatment of human multiple sclerosis (MS).

P618

The MS Microbiome Consortium (MSMC): an academic multi-disciplinary collaborative effort to elucidate the role of the gut microbiota in MS

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Background: The vast collection of microbial organisms that inhabit the human gut (collectively known as microbiota) can shape immune responses and modulate susceptibility to chronic diseases. When the balance that normally exists in the gut microbiota is altered (dysbiosis), a number of diseases may result. Recent studies have related gut dysbiosis with development or severity of Crohn's disease, type I diabetes, obesity and autism.
Objectives: To develop a multi-Center academic consortium that designs and implements a translational framework to evaluate the effect of gut dysbiosis in MS. Our goals include elucidating the role of gut microbiota in the different MS clinical phenotypes and response to disease modifying therapies (DMT) as well as to evaluate disease causality using animal models.
Methods: The MSMC is an IRB-sanctioned, multi-disciplinary collaboration composed of two translational and dedicated MS Centers (Mt Sinai and UCSF), and a microbiome-oriented basic/experimental program and sequencing/bioinformatics Core. The MSMC has collected hundreds of samples and is currently analyzing their gut microbiomes by 16S bacterial DNA by massively parallel sequencing. The analysis is primarily conducted using the QIIME pipeline and aims at identifying group differences at the genus-level. Variables being tracked include MS disease activity status, clinical phenotype, DMT use, gender, dietary habits, sample collection mode, and Center of origin.

Results: The MSMC has successfully implemented IRB-approved protocols to recruit MS patients and controls from both MS Centers and to process and analyze their blood and stool samples. Our initial results show significant genus-level differences in the microbiomes of patients treated with glatiramer acetate compared to untreated subjects. Significant enrichment of members of the Enterobacteriaceae family were identified when comparing female patients to gender-matched controls. Geographical differences were also identified when comparing samples collected in New York vs. the San Francisco Bay Area.
Conclusions: Founded by a group of leading MS and microbiome investigators, the MSMC is uniquely positioned to advance the study of the microbiome in MS. While still a modest-sized study, observed differences between cases and controls suggest a biological effect and warrant further investigation. The identification of regional differences in microbiota composition highlight the need to adequately control for geography, as well as dietary and socioeconomic factors.

Neurobiology

P619
Astroglial endocytosis of myelin causes microglia activation and dendritic loss of neurons

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Background: Reactive astrocytosis is a prominent feature in MS lesions. Hypertrophic astrocytes have been shown to contain myelin debris, suggesting that they participate in myelin removal and thereby may play a role in lesion development. Moreover, astroglial myelin uptake may compromise astroglial function and contribute to tissue damage in the lesion and adjacent white matter.
Objectives: To describe the molecular mechanism and regulation of astroglial myelin uptake and to examine the functional impact of myelin accumulation in astrocytes on microglia and neurons.
Methods: Rat cortical astrocytes were exposed to fluorescent-labeled myelin vesicles (MV), purified from rat spinal cord. Confocal immunofluorescent microscopy was used for qualitative analyses. Quantifications were performed using a fluorescent plate reader.
Results: Uptake of myelin debris by astrocytes was mediated by the cell surface scavenger receptor low density lipoprotein receptor-related protein 1 (*LRP1*) through endocytosis in a time- and concentration dependent manner. Activation of astrocytes with TNFα or IFNγ but not with LPS further increased myelin endocytosis. Myelin uptake was significantly reduced by a competitive LRP1-ligand, dynamin inhibition and by Fingolimod, a sphingosine 1-phosphate receptor modulator. Myelin vesicles co-localized with LRP1, early endosomes and lysosomes suggesting intracellular trafficking through the endosomal-lysosomal pathway. Astroglial myelin uptake induced elevated astroglial expression of GFAP and reactive oxygen species. Moreover, increased activation and migration of microglia cells was observed after incubation with supernatant of myelin-laden astrocytes. Finally, co-culture of myelin-laden astrocytes with hippocampal neurons caused significant loss of neuronal dendrites.
Conclusions: Our data indicate that astrocytes ingest myelin debris by LRP1-mediated endocytosis. Myelin uptake results in activation of astrocytes and ROS production, recruitment of microglia and loss of dendrites in neurons but not neuronal cell death.
These findings provide further evidence that astrocytes actively participate in lesion development and may therefore constitute a therapeutic target for MS treatment.

P620
Expression profiles of inflammation associated microRNAs in astrocytes from multiple sclerosis lesions

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Background: Astrocytes participate in both tissue injury and repair processes in the CNS. Astrocyte reactivity is a major feature of active MS lesions. MicroRNAs (miRNAs) are small ribose nucleic acid (RNA) molecules that function as post-transcriptional regulators of gene expression, including genes important in modulating inflammation. Studies of human fetal astrocytes *in vitro*, demonstrate that miR-146a is an inflammation-resolving miRNA, which targets interleukin-1 receptor-associated kinase-1 (IRAK-1) mRNA; miR-155 is a pro-inflammatory molecule that targets suppressor of cytokine signaling -1 (SOCS-1) mRNA. *In vitro*, IL-1β increases expression of both miR-146a and miR-155. *In situ*, these miRNAs are increased in active MS lesion samples.
Objectives: To assess expression of inflammation-related miRNAs in astrocytes derived from active MS lesions and compare levels of expression in cases of ischemic lesions and controls (cases without CNS disease).
Methods: Using laser capture microdissection (LCM), glial fibrillary acidic protein (GFAP) immunostained astrocytes were captured from formalin fixed paraffin embedded (FFPE) human brain tissue samples. Total RNA was extracted from captured cells and reverse transcribed (RT) in multiplexed reactions. Pre-amplification PCR (polymerase chain reaction) was performed on cDNAs before using them as templates in final quantitative PCR (qPCR).
Results: MiR-146a was down regulated overall in astrocytes derived from MS lesions as compared to control samples and was lower in astrocytes within MS lesions compared to those distant from the lesion site. MiR-146a was also down regulated in astrocytes captured from both grey and white matter regions of ischemic lesions compared to controls. In MS lesions, miR-155 expression levels in astrocytes were comparable to those from control tissues. Previously, we have observed that miR-155 expression was increased in myeloid cells in MS lesions compared to control tissues (Moore et al, 2013). MiR-155 expression levels did not differ between astrocytes from ischemia cases and normal controls, in either grey or white matter regions.
Conclusions: Defining distinct cell type specific expression profiles of miRNAs in human glial cells enhances our understanding of their contributions to mechanisms related to injury and repair in MS.

P621

Histamine H3 receptor negatively regulates oligodendrocyte differentiation and myelination

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Background: A huge unmet medical need exists in development of new treatment paradigms to repair myelin in patients with multiple sclerosis (MS). Agents which can promote oligodendrocyte precursor cell (OPC) differentiation are considered to possess the potential to accelerate/restore halted remyelination in MS patients, but so far very few therapeutic targets have been identified.
Objectives: To identify novel targets to induce remyelination, we established an *in vitro* differentiation assay with rat primary OPCs to screen GSK-proprietary annotated libraries.
Methods: The compounds and related targets were then validated through a series of *in vitro* and *in vivo* assays, i.e. cerebellar slice assay, gene-based manipulation, cuprizone model and pathology analysis with MS samples.
Results: Out of ~10,000 compounds screened, there were 21 hits that promoted OPC myelin basic protein (MBP) expression in a concentration-dependent manner, four of which were antagonists for the Gi/o-coupled receptor histamine H3 receptor (H3R). Further testing of additional structurally-diverse H3R modulators revealed that only inverse agonists but not neutral antagonists promoted oligodendrocyte differentiation and cerebellar slice myelination, which implied a key role of the constitutive activity of H3R. H3R was expressed in all stages of oligodendrocyte differentiation, and siRNA knock-down of H3R expression to ~40% of normal levels enhanced the expression of MBP and myelin associated glycoprotein (MAG), markers for mature oligodendrocytes. Furthermore, systemic administration of a brain-penetrable H3R inverse agonist, GSK247246 enhanced remyelination in the mouse cuprizone model. These data support that the constitutive activity of H3R negatively regulates oligodendrocyte differentiation. In humans, we detected oligodendroglial H3R expression in demyelinated MS lesions and observed genetic association between an exonic single nucleotide polymorphism in *HRH3* and susceptibility to MS (p=0.006, OR=1.22). GSK239512 is a highly potent, selective, orally bioavailable and brain penetrant H3 receptor inverse agonist with a good safety/tolerability profile; when tested in myelination-relevant assays, GSK239512 demonstrated *in vitro* and *in vivo* efficacy in promoting oligodendrocyte differentiation and myelination.
Conclusions: From phenotypic screen to human genetics, we provide evidence for H3R as a novel therapeutic target for myelination, paving the way for clinical testing of GSK239512 in MS and other demyelinating diseases.

P622

Proremyelinating properties of neurofilament peptide NFL-TBS.40-63 and axon cytoskeleton proteins *in vitro*

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Background: Axon neurofilaments (NF) are altered in demyelinated plaques in MS, and the concentration of NF subunits released in the CSF correlates with disease severity. Nevertheless the role of NF in the extraaxonal location is unknown. We have demonstrated that NF and tubulin rescue oligodendrocytes (OL) from toxic demyelination *in vitro* (Fressinaud and Eyer, Neurochem Int. 2013, 62:306-13).
Objectives: As NF are likely submitted to proteolysis, we have analyzed if synthetic peptides corresponding to the tubulin-binding site (TBS) sequence identified on light NF chain (NFL-TBS.40-63) could regulate OL fate *in vitro*.
Methods: Pure rat OL and astrocyte (AS) cultures, grown in chemically-defined medium were treated with biotinylated NFL-TBS.40-63 (YSSYSAPVSSSLSVRRSYSSSSGS), and NFL-scramble peptide (NFL-SCR2) with the same amino-acids that NFL-TBS.40-63, although in random sequence (10 μM, 24 h). Proliferation and differentiation were characterized using specific antibodies (to bromodeoxyuridine, A2B5, CNP, MBP, GFAP), and compared to untreated control cultures.
Results: NFL-TBS.40-63 significantly increased OL differentiation and maturation with more CNP+ and MBP+ cells characterized by numerous ramified processes, and putative myelin balls, whereas NFL-SCR2 was ineffective. The proliferation of OL progenitors was not affected, nor was AS proliferation and differentiation. Lysophosphatidyl choline (LPC, 2. 10^{-5} M, 24h) induced OL death (around 50%), whereas in cultures co-treated with LPC and NFL-TBS.40-63 OL survival, differentiation, and maturation were significantly rescued. The OL cytoskeleton network analyzed by immunocytochemistry with tubuline, tau and MAPs was not altered by the peptide treatment. The intracellular signalling pathway involved has not been identified yet, nevertheless biotinylated peptides were localized into the main processes and the perinuclear cytoplasm of CNP+ OL by confocal microscopy, suggesting endocytosis.
Conclusions: Thus if the release of peptides such as NFL-TBS.40-63 occurs in vivo following axonal damage, it could improve repair through increased OL differentiation and survival, which is a prerequisite for remyelination. The therapeutic potential of the peptide (which appears non toxic) will be determined in vivo by deep intrahemispheric microinjection.

P623

Differential glycosylation of KIR4.1 in glia cells affects binding of autoantibodies in multiple sclerosis

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Background: Inward rectifying potassium channel 4.1 (KIR4.1) specific serum antibodies were reported in a sub-population of multiple sclerosis (MS) patients. KIR4.1 is a glycosylated channel protein expressed in oligodendrocytes and a subset of astrocytes.
Objectives: To determine the posttranslational modifications of KIR4.1 occurring in human brain and their impact on autoantibody binding.
Methods: KIR4.1 protein was isolated from human subcortical white and cortical gray matter and transfected HEK293 cells. We

performed immunoblot, immunoprecipitation and N-deglycosylation assays to study its molecular heterogeneity and glycosylation. We analyzed the expression pattern of differently glycosylated KIR4.1 in human subcortical brain sections and transfected cells by immunochemistry using polyclonal and monoclonal antibodies (mAb) against the extracellular domain, the C-terminal domain, and KIR4.1-specific IgGs purified from MS sera (KIR4.1-IgG).

Results: KIR4.1 contains a functional N-glycosylation site in its first extracellular domain. In a cell-dependent fashion KIR4.1 undergoes simple or complex N-glycosylation. In astrocytes most of KIR4.1 is highly glycosylated (51-60 kD) while oligodendrocytes express predominantly lower glycosylated KIR4.1 (40-42kD). MAb generated against the extracellular domain and KIR4.1-IgG from MS sera specifically precipitate the lower glycosylated KIR4.1 but not higher glycosylated KIR4.1. This mAb and KIR4.1-IgG predominantly bind to oligodendrocytes and to a much lesser extent to astrocytes. In KIR4.1 transfected HEK293 cells most protein is highly glycosylated while only a minor fraction in the endoplasmatic reticulum is weakly glycosylated corresponding to the protein expressed in oligodendrocytes. Separation of different KIR4.1 glycosylation forms demonstrates that MS specific antibodies bind to weakly but not highly glycosylated KIR4.1.

Conclusions: Our findings demonstrate that KIR4.1 is differentially glycosylated in a cell specific manner. KIR4.1-IgG from MS sera bind weakly glycosylated KIR4.1 expressed predominantly in oligodendrocytes, while higher glycosylation of KIR4.1 interferes with binding. These findings demonstrate for the first time that posttranslational modification of an autoantigen can influence autoantibody binding and focus the antibody response to a particular subset of cells. These findings have important implications for the understanding of autoantibody responses and for the development of assays to detect autoantibodies in autoimmune diseases.

P624

Effect of inflammatory insults on cell viability and its regulation by epigenetic changes in oligodendrocytes

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Background: Oligodendrocyte injury and inflammatory demyelination are important pathological abnormalities of multiple sclerosis (MS) as well as in its animal model, the experimental autoimmune encephalomyelitis (EAE). Emerging evidence over the last decade suggests that oligodendrocytes are not merely immune targets but rather active participants in the neuroimmune network by regulating the events leading to inflammatory demyelination. Several studies have shown that the protection of oligodendrocytes against inflammatory injury results in protection against EAE as well.

Objectives: To study the effect of inflammatory insults on cell viability and its regulation by epigenetic changes in oligodendrocytes. We hypothesized that DNA methylating enzymes and acetylating enzymes may regulate cell viability as well as inflammatory responses.

Methods: We employed MTT and LDH assays to assess cell viability. Ectopic expression of DNMT and HDAC genes was achieved by stable transfection of oligodendrocytes. Epigenetic changes in oligodendrocytes were evaluated using Affematrix gene expression array and conformed by PCR.

Results: Since epigenetic modifications such as methylation and acetylation are involved in transcription, we explored and found changes taking place during the inflammatory stage of MS using methylation sensitive PCR. Further, in-vitro studies have shown that human oligodendroglioma (HOG) cells treated with cytokines caused cell death. We overexpressed DNMTs and HDACs in HOG cells. HOG cells overexpressing DNMT3B treated with cytokines resulted in increased cell viability. On the contrary, HOG cells overexpressing HDAC1 treated with cytokines resulted in decreased cell viability.

Conclusions: These results suggest that cytokines may inflict DNA epigenetic changes leading to inflammatory responses. We further postulate that understanding the significance of DNA methylation and acetylation in oligodendrocyte cell death may provide a basis for new intervention strategies for MS treatment.

P625

Azetidine-induced oligodendrogliopathy

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Background: The substitution (misincorporation) for proline of the non-protein imino acid Azetidine-2-carboxylic acid (Aze) can result in protein misfolding and destabilization. Aze induces increases of ubiquitinated and oxidized proteins and of heat shock protein levels in neural cells *in vitro*.Aze is abundant in sugar beets (SB). After sugar extraction, SB byproducts are fed to livestock, particularly dairy cows. The history and global spread of SB agriculture and the resulting entry of Aze into the human food chain closely parallel the history and global epidemiology of MS. We hypothesize that early life, (i.e. intrauterine, early childhood), exposure to Aze (e.g. in milk) results in misinicorporation in human oligodendrocytes (OGC) and myelin proteins, (which are normally highly stable thoughout life). Aze misincorporation might manifest later in life as enhanced vulnerability to cell stress leading to OGC degeneration that could be independent of or synergistic with cellular immunity in the CNS of MS patients (Rubenstein, 2008).

Objectives: To assess the effects of Aze exposure on the CNS of mice *in vivo.*

Methods: Pregnant females and newborn CD-1 mice pups were given doses of Aze or saline po by oral gavage. Juvenile mice were given Aze ip or po (gavage) qd or saline ip.

Clinical effects were monitored; pathologic analyses including immunohistochemistry (IHC) were performed.

Results: High dose Aze induced some systemic toxicity. High and moderate lower doses of Aze induced dose-dependent swelling of OGC nuclei throughout CNS white matter, OGC apoptosis (TUNEL and caspase-3 IHC), and MHC I induction. There was multifocal microglial activation in Aze-treated mice but there were no detectable effects on other CNS cells and no myelin injury by IHC.

Similar OGC nuclear swelling and apoptosis (Prineas, 2012), focal microglial activation and MHC I induction are found in MS normal-appearing white matter (NAWM) and adjacent to active lesions.

Conclusions:

1. Aze-induced oligodendrogliopathy uniquely models the metabolic OGC perturbations and tissue responses found in MS patient NAWM.
2. The induced alterations are consistent with Aze misincorporation leading to myelin protein instability *in vivo*.
3. Because historical and global epidemiology correlations suggest a relationship of SB agriculture to MS incidence, early life dietary exposure to Aze may be an environmental factor that contributes to MS pathogenesis, particularly lesion (and hence clinical), progression as a result of long term effects on OGC and CNS myelin.

P626

RGC-32 regulates TGF- β extracellular matrix expression in reactive astrocytes

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Background: Excessive extracellular matrix (ECM) deposition in active demyelinating multiple sclerosis (MS) lesions may impede axonal regeneration and can modify immune reactions. RGC-32 plays an important role in mediation of TGF-beta downstream effects but its role in gliosis has not been investigated.

Objectives: Our objective was to investigate the role of RGC-32 in mediation of TGF- β effects on astrocytes ECM production and expression of reactive markers.

Methods: We examined first the expression of collagen types I to V, decorin and RGC-32 in MS brains by immunohistochemistry and correlate its expression with that of astrocytes (glial fibrillary acidic protein positive cells). To gain more insight into the role played by RGC-32 in gliosis we then examined the role of RGC-32 in mediation of ECM and reactive astrocyte markers expression in cultured astrocytes. To silence RGC-32 expression, astrocytes were transfected with RGC-32 siRNA (Santa Cruz Biotech) and the results compared with that of control siRNA.

Results: Collagen I, III, IV, V and decorin expression was found in the perivascular space and in the parenchyma. Collagen I, IV and V are expressed by astrocytes which were also expressing RGC-32. Since RGC-32 was found to be involved in mediation of TGF- β effects, we investigated its role in TGF- β -induced ECM expression and reactive astrocyte marker α-smooth muscle actin (α-SMA). In cultured astrocytes, α-SMA, collagen I, IV and V as well as fibronectin were significantly induced at 18 h of stimulation with TGF-β. Next, we silenced RGC-32 expression by transfecting astrocytes with siRGC-32 and compared the effect of this treatment to that of control siRNA. We found that α-SMA expression was significantly reduced after RGC-32 silencing ($p< 0.05$). In addition we found that RGC-32 silencing resulted in a significant reduction in TGF-β induced collagen I ($p< 0.01$), collagen IV ($p< 0.02$), collagen V ($p< 0.05$) and fibronectin ($p< 0.05$) expression. Using astrocytes isolated from RGC-32 knockout (KO) mouse we found that TGF- β induced collagen IV and α-SMA expression was significantly reduced in RGC-32 KO when compared with wild type mouse.

Conclusions: The effect of RGC-32 silencing on α-SMA expression suggests that RGC-32 is required for the transition of astrocytes to a reactive state. Our data also indicate that RGC-32 plays an important role in the TGF-β-mediated induction of ECM expression in astrocytes. RGC-32 may therefore represent a useful new target for therapeutic intervention in MS.

P627

The relationship between neuronal S1P receptor modulation by Fingolimod and neuronal survival

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Background: Evidence from the experimental autoimmune encephalomyelitis (EAE) model reveals a nonimmunological effect of the sphingosine-1 phosphate (S1P) analog Fingolimod (FTY720) in the central nervous system (CNS), via astrocytes. Our observation of the in vivo modulation of neuronal S1P receptors (S1PR), in FTY720-treated EAE mice, prompted a study of the relationship between neuronal S1PR responses to FTY720 and neuronal survival.

Objectives: (a) To develop an experimental workflow for in vivo quantification of drug effects on neurons; (b) To evaluate the significance of neuronal S1PR modulation on stress and apoptosis pathways.

Methods: Myelin oligodendrocyte glycoprotein (MOG)-induced EAE was used, including untreated, vehicle-only placebo/FTY720 treated, MOG-injected placebo/FTY720 treated groups. Quantitative confocal microscopy was used for evaluation of markers of neuroinflammation, cellular stress and apoptosis. Laser capture microdissection (LCM) was used for isolation of defined CNS regions or neuronal populations, followed by cDNA generation and analysis on pathway-, or disease specific qPCR arrays. Intracellular cytokine staining (ICS) was used for profiling T cells.

Results: Quantitative confocal microscopy in white and grey matter separately showed generalized reduction in disease activity (over 75%), with highly significantly reduced T cell infiltration, microglial reactivity, axonal injury and demyelination in both compartments in MOG-injected/drug-treated, relative to placebo-treated counterparts. Residual T cell infiltration was associated with highly significantly reduced tissue levels of Th1 cytokines and chemokines, as shown on qPCR arrays. Interestingly ICS showed a reduced proportion of MOG-specific T cells in the brain and spinal cord (< 4%) relative to lymph nodes (25%). Quantification of neuronal S1PR by confocal microscopy, or qPCR from LCM-isolated neurons, showed highly significantly elevated S1P1, S1P3 and S1P5 in placebo-treated groups and differential down regulation (S1P1=S1P5=90%; S1P3=40%) in drug-treated counterparts. Profiling of neurons showed significantly elevated levels of stress and apoptosis markers in placebo-treated relative to control groups. Drug-treated counterparts showed down regulation of these markers, but significantly elevated levels of anti-apoptotic markers.

Conclusions: FTY720 treatment results in decreased neuronal S1PR expression, associated with anti-apoptotic marker upregulation. These complex responses may underlie an overall survival mechanism.

P628

Dose dependent protection of oligodendrocytes by IVIG

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Background: Intravenous immunoglobulin (IVIG) contains pooled, polyvalent IgG antibodies extracted from the plasma of thousands of blood donors. The modes of action of IVIG are still incompletely understood. While IVIG is an established and well tolerated treatment for various autoimmune conditions, treatment trials of IVIG for multiple sclerosis, using varying doses of IVIG, yielded controversial results.
Objectives: This study focusses on the effects of IVIG on demyelination in an in-vitro model of the CNS - immune interface.
Methods: Using organotypic slice cultures (OSC) from transgenic mice, which express green fluorescent protein (GFP) in oligodendrocytes and myelin, we induced extensive immune mediated demyelination and oligodendrocyte loss with an antibody specific for myelin oligodendrocyte glycoprotein (MOG) and complement. The effect of IVIG on demyelination was documented by live imaging of GFP expression, confocal microscopy and gene expression analysis.
Results: IVIG effectively preserved oligodendrocyte and myelin integrity from antibody induced damage. Staining of living OSC with propidium iodide (PI) confirmed that IVIG ameliorated antibody/complement-induced cell death. Immunohistochemistry with anti-CD68 antibodies indicated that IVIG treatment inhibited microglia activation. In contrast to whole IVIG, neither a monoclonal humanized IgG antibody nor equimolar IVIG-derived Fab-fragments exerted a protective effect, while Fc-fragments from a human polyclonal IgG preparation were as effective as whole IVIG. The protective effect of IVIG was dose dependent.
Conclusions: In this *in-vitro* model of the CNS - immune interface, whole IVIG, but not IVIG derived Fab fragments, protected from antibody-mediated demyelination, microglia activation and cell death. Our data show that IVIG mediated protection is dose dependent, indicating that the IVIG dose used in clinical trials may be critical for the results achieved. Future studies will further pinpoint the mechanisms of this protection and assess potential effects of IVIG on remyelination.

Neuromyelitis optica

P629

Neuromyelitis optica IgG in the cerebrospinal fluid induces blood brain barrier breakdown and NMO lesions in brain parenchymal white matter

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Background: Neuromyelitis optica (NMO) is associated with serum immunoglobulin G autoantibodies (NMO-IgG) directed towards the water channel aquaporin-4 (AQP4) [1]. AQP4 is densely localized in membranes of astrocytes and ependymal cells, which form the glia limitans of the blood brain barrier (BBB) and the cerebrospinal fluid (CSF)-parenchymal barrier [1] . We previously reported that intrathecal transfer of NMO-IgG together with human complement (C') into the CSF of mice was sufficient to induce NMO-like lesions at the pial surface and in periventricular areas, correlating with ependymal damage [2]. We also observed parenchymal lesions that did not always correlate to CSF-parenchymal barrier disruption.
Objectives: To examine whether NMO-IgG in CSF can access the brain parenchymal white matter and whether BBB breakdown and perivascular white matter lesions are induced by such NMO-IgG.
Methods: Purified NMO-IgG from an NMO patient was given to naïve mice as a single injection intrathecally into cisterna magna, with C'. A total of 14 mice received NMO-IgG+ C', 13 mice received normal-IgG + C' and 12 mice received NMO-IgG or normal-IgG (10) only. The mice were killed two days later. Tissue processing and immunostaining was performed as described previously [2]. BBB breakdown was assessed by iv horseradish peroxidase (HRP) injection [3]. Histological changes were scored semiquantitatively from 0-3 [2].
Results: Intraparenchymal perivascular deposition of human IgG was observed in 5/14 of the NMO-IgG+ C′ treated mice, two moderate (++) and three severe (+++), in deep white matter in cerebellum, cerebrum and brainstem. Low grade (+) deposition of IgG occurred focally in 2/12 of mice treated with NMO-IgG, 1/13 treated with normal-IgG + C′ and none in mice injected only with normal-IgG. Accumulation of IgG around parenchymal vessels coincided with HRP leakage into the parenchyma, which was extensive and widespread in NMO-IgG + C′ treated mice, but low grade and focal in NMO-IgG and normal-IgG + C′ treated mice. Astrocyte pathology with loss of AQP4 and glial fibrillary acidic protein were only identified in NMO-IgG + C′ treated mice.
Conclusions: NMO-IgG in CSF induced perivascular leakage of IgG in parenchymal vessels in deep white matter accompanied by HRP leakage in a considerable proportion of mice. The data are consistent with a paravascular route for access of CSF-derived NMO-IgG to exert pathologic effects in white matter that include BBB disruption.

P630

Neuromyelitis optica: Venezuelan multicentric epidemiologic study

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Background: Neuromyelitis Optica (NMO) is considered a rare disease in many countries.
Objectives: To estimates NMO relative frequency and epidemiological characteristic in the multi-ethnic Venezuelan population.
Methods: This is a retrospective and a multicentric study carried out in Venezuela, in five Multiple Sclerosis (MS) Centers reported patients with NMO and MS during 2011-2013.
Gender, age of the patient with the first outbreak, studies of Magnetic Resonance Image (MRI), Cerebral Spinal Fluid (CSF), evoked potentials, Ac. aquaporin 4 and treatment was analyzed.
Results: Was studied 750 patients of whom 122 were diagnose with NMO and 628 with MS. We report a relative frequency of 16%. NMO patients (108, female, 14 male) are evaluated. 92% mestizos, 5% white, 1% Afro-Americans, 2% natives. Age of the first outbreak first decade 1.6%, second decade 15.5%, third decade 27%, fourth decade 21%, fifth 20% and sixth decade 14%. Recurrent course 47%, monophasic 53%. Diagnostic tool: brain MRI 95%, spinal MRI 70%, CSF 87%, evoked potential 81%, anti-AQ4 75 samples (53% positive, 47% negative). Treatment with plasmapheresis and methylprednisolone in acute stage. Immunosuppressive therapy: 98% Azathioprine and 2% Rituximab.
Conclusions: In the multi-ethnic venezuelan NMO population is observed predominantly mestizos and females. Cases were observed in all age groups, predominantly the third and fourth decade of life. The 53% of anti AQ4 were positive. The relative frequency obtained is 16%.

P631

Neuromyelitis optica does not impact periventricular venous density - a 7 Tesla MRI study

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Background: Neuromyelitis optica (NMO) and Multiple sclerosis (MS) are autoimmune disorders of central nervous system. Previous studies have shown a reduced density of periventricular veins in MS on susceptibility-sensitive T2* imaging at ultra-high field MR correlating with disease severity and T2 lesion load; and such estimation is useful for evaluation of brain oxygen metabolic changes. Recently, there has been increasing recognition of brain involvement in NMO.
Objectives: In this multi-center study we aimed at characterizing whether there are venous density changes in NMO using 7 Tesla MRI with high-resolution and high-susceptibility contrast imaging.
Methods: Fifteen NMO patients (8 from Berlin and 7 from New York) underwent 7T MRI (Siemens, MAGNETOM) comprising high-resolution 2D susceptibility-sensitive gradient-echo T2*-weighted transversal imaging and fluid attenuated inversion recovery sequences (TIRM). For the quantification of small veins a region-of-interest based algorithm estimating periventricular venous area (PVA) was applied as previously described (Sinnecker et al., Mult Scler 2013).
Results: In total, we detected 138 lesions in 15 NMO patients (mean+/-SD 9.2+/-10.9, range 0-34). An initial visual analysis of T2*-weighted images did not reveal any abnormalities regarding periventricular venous density in patients with NMO. Hence, periventricular venous density as indicated by PVA was normal in NMO patients compared to significantly smaller measures of venous density in MS patients as reported previously (Berlin group: mean+/-SD PVA 2.1mm2 +/- 0.3mm2, range 1.8-2.7mm2; New York group: mean+/-SD PVA 1.5mm2 +/- 0.2mm2, range 1.3-2.0mm2; for MS data see Sinnecker et al., Mult Scler 2013).
Conclusions: Periventricular venous density alterations could not be detected in NMO despite the presence of brain parenchymal lesions mimicking MS, reflecting the different underlying pathophysiology between these two autoimmune CNS disorders. Since the visibility of venous density on T2* is associated with the oxygenation level in the venous blood, our finding may help to further elucidate the oxygen metabolic abnormalities and to differentiate between these disease entities.

P632

Plasmablasts as AQP4-Ab producers in the pathogenesis of neuromyelitis optica

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Background: Neuromyelitis optica (NMO) is an inflammatory disease of the central nervous system characterized by recurrent attacks of optic neuritis and myelitis. Although the probable autoantigen aquaporin-4 (AQP4) is intensively analyzed and clinical and pathological characterization of NMO has made the clinical entity of anti-AQP4 antibody (AQP4-Ab) spectrum disorders, the immune background remains unclear. We found autoantibody-producing plasmablasts (PBs) that are increased in the peripheral blood of patients.
Objectives: In this study, we tried to clarify the fate of circulating PBs in the peripheral blood of NMO.
Methods: A cohort of 20 AQP4-Ab-seropositive patients was recruited. Eight age-matched patients with MS and six healthy subjects were enrolled as controls. Peripheral blood mononuclear cells, cerebrospinal fluid (CSF) cells, and AQP4-Ab titers were analyzed using a flowcytometer. Single-cell sorted PBs were sequenced for the variable regions of IgG.
Results: We found that a subset of IgG-producing plasmablasts, which express CD138 and HLA-DR, was increased in the peripheral blood and enriched in the CSF during relapse in patients with NMO. These plasmablasts were found in the peripheral blood of healthy subjects or patients with NMO who received influenza vaccination.

The frequency of plasmablasts in a portion of patients with NMO who received H1N1 influenza vaccination were elevated from the baseline for more than 6 months after the vaccination, and they displayed frequent relapses compared with that before the vaccination. The AQP4-Ab titers derived from the serum of patients were comparable with the change in plasmablasts.

In a different analysis, we also found that peripheral plasmablasts during relapse of NMO upregulated CXCR3, directing cells to the inflammatory site, which suggests that PBs may traffic immune cells toward the central nervous system. We isolated plasmablasts for single-cell based sequencing of complementarity-determining regions of IgG from the peripheral blood and CSF of patients with NMO. We revealed that PBs clones in the peripheral blood and CSF from the same patients with NMO showed common complementarity-determining region rearrangements in the IgG heavy chains.

Conclusions: These results indicate that IgG-producing plasmablasts preferentially migrate to the CSF and could be involved in the following cascades of local inflammation during NMO relapse, which suggests a therapeutic strategy to target PBs and their migration.

P633

Neuropsychiatric features of neuromyelitis optica

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Background: Despite recent investigations of brain morphology in NMO there has yet to be an in-depth exploration of the prevalence and severity of psychopathology in NMO.

Objectives: The aims of this study were to investigate the prevalence and severity of both cognitive impairments and psychopathology in NMO.

Methods: Participants were administered a battery of neuropsychological tests and a structured psychiatric interview (MINI v6). Cognitive test performances were contrasted with age-adjusted published normative data.

Results: 32 individuals (25 female) meeting current NMO diagnostic criteria, with a mean age of 46.78 (SD 15.08, range 18-81), disease duration 9.21 years (SD 8.71, range .43-38.8) and EDSS 3.74 (SD 2.03) completed the assessment.

Some degree of cognitive impairment (at least 1 domain > 1.5 SD below normative populative mean) was observed in 75% of participants. 40.6% were classified with mild impairment (1 domain < -1.5 SD), 21.9% with moderate impairment (2 domains < -1.5 SD) and 12.5% with severe impairment (≥3 domains < -1.5SD).

31.3% of participants met diagnostic criteria for a current anxiety disorder and 16.7% for current depression. Lifetime prevalence of depression in the NMO sample was 50%.

No significant associations were found between presence or severity of cognitive impairment and clinical or demographic factors including age, disease duration, EDSS, or presence of depression or anxiety disorder.

Conclusions: Evidence of at least mild impairment in cognitive functioning appears common in NMO, with evidence of more global impairments across multiple cognitive domains observed in a substantial proportion of patients. In addition, substantial numbers of NMO patients met diagnostic criteria for depression and anxiety disorders. Rates of depression in NMO appear similar to those in MS but anxiety disorders may be more prevalent in NMO.

Greater understanding of the presentation and associated mechanisms of cognitive impairments in NMO are required. The high prevalence of psychiatric disorders in our population highlights the need for psychological screening and the development of targeted interventions for mood disorders in NMO.

P634

Comparison of peripheral and CNS B-cell pools in NMO, using next generation sequencing, and its implications for B-cell trafficking

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Background: Neuromyelitis optica (NMO) is a severe autoimmune disorder of the central nervous system (CNS) associated with an autoimmune antibody-response (AQP4-IgG) against the water channel aquaporin-4 (AQP4). To date, the origin, migration, and maturation of these CNS B cells, particularly AQP4-IgG producing B cells, is unknown.

Objectives: In order to establish B cell migration patterns between the periphery and CNS, we compared the heavy chain (VH) transcriptome from CSF plasmablasts with the VH transcriptome of peripheral B cell populations in 7 NMO patients.

Methods: CSF Ig transcriptome libraries were generated from isolated CSF plasmablasts by single-cell sorting, reverse-transcription polymerase chain reaction (RT-PCR), and DNA sequencing. Recombinant antibodies were generated from paired heavy- and light chain sequences and tested for AQP4-reactivity by a cell binding immunofluorescence assay. Peripheral B cells were FACS sorted into naïve (CD19+CD20+CD27-IgD+), memory (CD19+CD20+CD27+), plasmablast (CD19+CD20-CD27+IgD-CD38++), and triple-negative (CD19+CD20-CD27-IgD-) B cell populations. VH transcriptomes were subsequently generated by RT-PCR and next generation deep sequencing (Illumina MiSeq platform) using barcoded primers equipped with unique molecular identifiers (UMIs).

Results: In 5 out of 7 NMO patients, we found an overlap of clonally related B cells across the blood-brain-barrier. On average, 5% of the CSF Ig transcriptome sequences could be linked to blood Ig transcriptome sequences. The overlap between clonally related B cells could be established between CSF plasmablasts and peripheral blood memory B cells, triple-negative B cells, and plasmablasts. VH sequences associated with AQP4-specific CSF antibodies were observed in each of these B cell compartments; however, the pattern of somatic hypermutation in the peripheral blood memory and triple negative B cells were more closely matched to their CSF counterparts. In NMO patients, the proportion of peripheral triple-negative B cells was significantly elevated when compared to MS patients and healthy controls.

Conclusions: Our findings indicate that an exchange of B cells occurs between the peripheral and CNS compartment in NMO.

AQP4-specific memory and triple negative B cells may be a critical source of CNS AQP4-IgG producing plasmablasts and play a role in the initiation of NMO lesions. Monitoring these peripheral blood populations may prove useful for assessing disease activity and therapy.

P635

HLA class 11 alleles and environmental associations with neuromyelitis optica in Indian population

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Background: It is unclear whether there are genetic and environmental associations with neuromyelitis optica (NMO) particularly in Indian population.
Objectives: To determine HLA DRB1& DQB1 allele associations with NMO. Additionally the role of childhood exposure to smoking, infections, diet and sun exposure were evaluated.
Methods: 93 adult patients (including 24males) with relapsing NMO/ NMOS disorders were chosen. Among them 47.3% were seropositive for NMO-IgG by fixed cell based assay. They included NMO diagnosed by Wingerchuck 2006 criteria (61), recurrent myelitis with long cord lesions (11), recurrent optic neuritis (20) and sero positive recurrent tumefactive demyelination (1). DRB1& DQBI allele genotyping was performed in 93 patients and 200 healthy controls by PCR and analyzed by UNPHASED 3.0.8 software. Environmental exposure history was obtained in all patients and matched controls through a validated questionnaire by personal interviews and results analyzed by chi square test and multivariate analysis.
Results: NMO patients had significant allele frequency for DRB1*03 ($p= 4 \times 10^{-5}$, OR= 6.9, CI= 2.7-17.3) When NMO-IgG positive patients alone were analyzed this association persisted (p= 0.002, OR= 11.3, CI= 3.1- 40.6) . A similar association was seen for DRB1*10 allele (p= 0.0002, OR= 4.9, CI= 2.1- 12.2) which remained significant after adjusting for DRB1*03. The common haplotypes seen were DRB1*10- DQB1*0602 (p= 0.0003, OR= 5.2)and DRB1*03- DQB1*0201 (p= 0.03, OR= 1.74) . Vegetarian diet (p= 0.03), insufficient vitamin D rich food/ supplements in diet (p= 0.01) and poor sun exposure (p= 0.01) in childhood were significantly associated with NMO. History of childhood infections and smoking (active/ passive) showed no association.
Conclusions: Genetic and environmental association may be present for NMO in Indian population. Larger studies are necessary to replicate these preliminary findings.

P636

The increase of CD56 high NK cells and activated Treg-cells in patient with neuromyelitis optica after treatment with anti-IL-6R antibody tocilizumab

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Background: Neuromyelitis optica (NMO) is an autoimmune disease of the central nervous system, accompanying elevation of pathogenic autoantibodies against aquaporin 4 (AQP4). We previously showed that plasmablasts (PB), which are capable of producing anti-AQP4 antibodies, are increased in the peripheral blood of patients with NMO, and that the survival of PB is dependent on IL-6 receptor (IL-6R) signaling (Chihara et al. PNAS 2011). Therefore, we conducted an exploratory study to evaluate the safety and efficacy of tocilizumab (TCZ), a humanized anti-IL-6R monoclonal antibody, in NMO, and confirmed that TCZ treatment significantly reduced annualized relapse rate, as well as neurogenic pain and fatigue in seven patients with NMO (Araki et al. Neurology 2014). We also confirmed the reduction of PB numbers following injection of TCZ in two patients, suggesting that PB could be one of the targets of TCZ .
Objectives: However, endogenous IL-6 may influence functions of other immune cells, such as T cells and myeloid cells, directly or indirectly, which may lead to dysregulated immune balance in NMO.
Methods: To address this issue, we made monthly flow cytometer analysis of peripheral blood lymphocyte subsets derived from NMO patients who participated in the exploratory study.
Results: Here, we report that the TCZ treatment would induce a significant and continuous increase of CD56 high natural killer cells and of Foxp3 positive activated regulatory T cells during the first year of TCZ treatment.
Conclusions: Thus, correction of dysregulated immune network could be one of the mechanisms by which TCZ would regulate the disease activity of NMO.

P637

Evaluation of treatment response to plasmapheresis in acute exacerbations of neuromyelitis optica

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Background: Neuromyelitis optica is an autoimmune disease of the central nervous system that presents predominantly with optic neuritis (ON) and myelitis. The association of the IgG class antibodies directed against the aquaporin-4 channel (AQP4-IgG) allows early diagnosis of limited forms, such as ON or myelitis, considered part of the spectrum of NMO disorders (NMOSD). Acute exacerbations are treated with methylprednisolone, followed by plasmapheresis in refractory patients. AQP4-IgG seropositivity supports the use of therapy based on humoral immunity, such as plasmapheresis, but differences in the response of seronegative patients are poorly described.
Objectives: To evaluate the efficacy and safety of plasma exchange in NMO patients resistant to treatment by methylprednisolone.
Methods: A prospective analysis of 16 patients with NMO and NMOSD (2006 diagnostic criteria) refractory to methylprednisolone, treated between May 2013 and January 2014. The Expanded Disability Status Scale (EDSS) and the Visual Outcome Scale (VOS) were used to compare results both pre- and post-treatment. Keegan´s criteria were associated to assess improvement in functionality. Patients were evaluated at intervals fixed pre, 1, 15, 30, 60 and 90 days after plasmapheresis.

Results: Of the 16 patients, 3 were male, and the mean age was 36,9 years. Patients with a diagnosis of NMO numbered 6 and 10 had NMOSD. 17 relapses were treated: 7 ON, 7 longitudinally extensive transverse myelitis, 2 with both clinical symptoms and 1 with brainstem symptoms (nausea and vomiting). The mean interval between the onset of relapse and the beginning of plasmapheresis was 27,8 days and the average number of sessions was 6 (range 2-7) in a period of 2 weeks. The mean EDSS score pretreatment was 6,3 (range3,5 to 8.5) and post-treatment was 5.9 (range 3 to 8). It was observed subsequent improvement of neurological function and functional capacity, and 1 patient presented additional improvement is the sixth month evaluation. Complications related to the procedure were encountered by 4 patients. AQP4-IgG status was positive for 8 patients and 3 were waiting for the result.
Conclusions: It is concluded that plasmapheresis is effective and well tolerated by most patients with NMO and NMOSD, independent of their AQP4-IgG serum status.

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Relevance of cervical cord atrophy and 3rd. ventricle widening to clinical disability in neuromyelitis optica

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Background: Neuromyelitis optica (NMO) can present similarly to multiple sclerosis (MS) and involvement of the cervical cord is common in both diseases. Its impact on clinical disability has been studied in MS but only rarely in NMO. Furthermore the involvement of the periventricular system in NMO has been a focus of interest in recent publications, however the relevance of ventricular widening as an indirect measure for central atrophy to clinical disability remains unknown.
Objectives: To identify different spinal cord and brain atrophy patterns in NMO and MS patients and assess their impact on clinical disability.
Methods: 18 NMO patients, 20 MS patients and 26 healthy controls, matched for age and disease duration (patients) were included retrospectively. Patients' disability status was scored according to the expanded disability status scale (EDSS). Quantitative MRI included semi-automated volumetry of the upper cervical cord (UCCA), brain grey (GM) and white matter (WM), lateral, 3rd. (3VV) and 4rth. ventricle (4VV), cerebellum and brainstem. Differences between the groups and associations between volumetry and clinical status were investigated by t-tests, Spearman rho correlation analyses and univariate respectively multivariate regression analyses.
Results: NMO patients and MS patients had similar levels of UCCA atrophy (75.2 mm^2 respectively 76.5 mm^2 versus 84.1 mm^2 in controls) although the MS patients were only moderately clinically affected (EDSS: 3.3 +-1.1 versus 4.9 +-2.2 in NMO). 3VV was increased in both diseases, while 4VV widening was specific for MS. Correlations between UCCA and EDSS were significant in NMO and in MS. Multivariate regression analyses including all MRI parameters which were significantly associated with EDSS in the univariate regression and age and disease duration resulted in UCCA and 3VV (R^2=0.461) as most significant to determine EDSS in NMO, while in the MS group GM and 4VV (R^2=0.483) were the most explanatory MRI parameters for the disability score.
Conclusions: Both upper cervical cord atrophy and 3VV enlargement have been found to be relevant for clinical disability in NMO. Periependymal dysfunctions may cause widening of the 3VV in NMO. Longitudinal studies are required to confirm 3VV as well as cervical cord atrophy as prognostic relevant MRI markers in NMO and to gain a better understanding of the pathologic processes in NMO patients.

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Neuromyelitis optica and neuromyelitis optica spectrum disorder patients in Turkish cohort: demographic, clinical, laboratory and radiological features

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Background: Neuromyelitis Optica (NMO) and Neuromyelitis Optica Spectrum Disorders (NMOSD) are both considered to be disabling diseases of the central nervous system.
Objectives: In this study we aimed to determine demographic, clinical, laboratory and radiological features and treatment strategies of NMO and NMOSD in Turkish cohort to better understanding the disease and coping with disability in NMO and NMOSD.
Methods: In this study a total of 182 patients who were followed by different medical schools and educational hospitals in Turkey were included. For each patient; the age at onset, gender, onset symptoms, nervous systems involvement in each episode, number of attacks, intervals between each episodes, brain and spinal cord MRIs, analysis of the cerebrospinal fluid (CSF), serum anti-AQP4 antibodies, evoked potentials, collagen vascular disease screenings, treatment, past medical and family histories were collected from the patients' medical records. The patients were divided into six subgroups as follows: Classical NMO, Single or recurrent attacks of transverse myelitis with longitudinally extending spinal cord lesions (LETM), Recurrent optic neuritis (ON) with normal brain MRI or cranial MRI lesions not compatible with multiple

sclerosis (MS), Optico-spinal MS (OSMS), ON or TM accompanying systemic autoimmune diseases, ON or TM with brain lesions suggesting NMO.
Results: One hundred and forty nine (81.9%) female and thirty three (18.1%) male patients were included. The mean age was 38.43±12.40 (between 13-75 years), the mean age at disease onset was 31.29±12.40 (between 10-74). The age at disease onset was < 50 years in 166 patients (91.2%) while it was ≥ 50 years (Late Onset NMO/NMOSD) in 16 (8.8%) patients. The mean disease duration was 64.65±69.17 months. Twenty four (14%) patients had other autoimmune diseases. In classical NMO group, NMO - IgG and oligoclonal band positivity was 62.5% and 20.4% respectively. Annual progression index was significantly higher in the LETM group with respect to classical NMO and recurrent optic neuritis (0.70±1.73 vs 0.17±0.34 and 0.08±0.12). The mean EDSS and progression index values were higher in the LONMO group. Seventy four patients (41.34%) of whole group were under long term treatment.
Conclusions: Our cohort points out the more severe disability in NMO/NMOSD patients presenting with transverse myelitis and late onset subgroups.

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Therapy of neuromyelitis optica exacerbations: a retrospective evaluation of 840 episodes with 1168 treatment cycles
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Background: Neuromyelitis optica (NMO) and its spectrum disorders (NMOSD) typically take a relapsing disease course. In comparison to multiple sclerosis, exacerbations are often more severe, respond less to therapy and leave a residual deficit. High-dose IV steroid therapy (HD-S) and plasma exchange (PLEX) are recommended treatments of NMO relapses.
Objectives: To describe frequency and sequence of therapies used for NMO relapses and to evaluate the remission status upon these therapies.
Methods: Using a nation-wide registry (www.nemos-net.de), we retrospectively reviewed records of NMO patients with a standardized evaluation form to assess demographic and diagnostic data, relapses (clinical appearance, severity, therapies), and the short-term remission status rated as either complete, incomplete, none, or missing data (MD). We included all patients with Wingerchuk NMO or aquaporin-4-positive NMOSD who had ≥1 relapse with sufficient documentation. Patients or relapses with insufficient data were excluded.
Results: We identified 186 patients (143 NMO, 43 NMOSD, 152 female) with a total of 1124 relapses. Mean age at disease onset was 40 years, mean duration of follow-up 7 years. The annualized relapse rate was 1.06 (95% CI 0.96-1.12). 150 patients had ≥1 optic neuritis (ON) and 179 patients had ≥1 myelitis (MY). Episodes of isolated MY (59%) were more frequent than isolated ON (28%), MY+ON (10%), and other presentations (1%). ON was unilateral in 82% and bilateral in 18%. After exclusion of 284 relapses due to insufficient data, non-standard or no therapy, 840 relapses with a total of 1168 treatment cycles were assessed. These comprised HD-S (n=856), PLEX (n=192), immunoadsorption (n=38), intrathecal steroids (n=23), intravenous immunoglobulins (n=21), and others (n=51). First-line treatment induced complete remission in 17%, incomplete in 60%, and no remission in 16% (7% MD). Frequency of relapses requiring a second, third, forth, and fifth line therapy were 30%, 8%, 1%, 1%, respectively. After the last treatment cycle, 19% of relapses showed complete, 67% partial, and 7% no remission (7% MD). Remission rates were higher for PLEX and immunoadsorption compared to HD-S. ON responded significantly better than MY.
Conclusions: NMO and NMOSD exacerbations are frequent and have poor remission rates, in particular MY. Treatment strategies are highly heterogeneous and HD-S and PLEX most commonly used. Escalation of relapse therapy improves remission in some patients.

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Clinical and radiological profiles of anterior visual pathway involvement in neuromyelitis optica
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Background: The anterior visual pathway is a frequent site of injury, as shown by optic neuritis (ON) during the course of neuromyelitis optica (NMO); however, the details of pathomechanism in ON of NMO remain unclear.
Objectives: To elucidate the clinical and radiological characteristics of anterior visual pathway involvement in NMO.
Methods: We performed a retrospective study of 17 NMO spectrum disorder (NMOsd) patients (28 attacks) and 16 MS patients (25 attacks) with history of visual impairments, and investigated visual acuity (VA), visual field (VF), optical coherence tomography (OCT), and MRI findings. In this study, serum testing yielded positive results for aquaporin-4 (AQP4) antibodies in all patients with NMOsd, and negative results in all patients with MS.
Results: The mean VA and mean deviation (MD) of VF at ON attacks were significantly worse in eyes of NMOsd patients than those of MS patients ($P < 0.001$). According to the VF classification used in the Optic Neuritis Treatment Trial, the frequency of NMOsd patients with total loss of vision was significantly higher than that of MS patients at ON attacks ($P < 0.01$), but the frequency of NMOsd patients with central abnormalities was significantly lower than that of MS at ON attacks ($P < 0.05$). Contrast-enhanced MRI assessments at ON attacks indicated that the length of abnormally enhanced lesions in patients with NMOsd was significantly longer than that in patients with MS ($P < 0.05$). Moreover, optic perineuritis, referred to as 'tramtrack sign' on axial views of MRI and 'doughnut sign' on coronal views, was present in not only MS patients (29%) but also NMOsd patients (46%), suggesting evidence of optic nerve sheath inflammation. OCT assessment in NMOsd demonstrated a thinner retinal nerve fiber layer than in MS ($P < 0.05$). Importantly, ON eyes with NMOsd patients had more prolonged time from ON onset to

achieving VA to 1.0 logMAR than those with MS patients ($P < 0.001$), suggesting poorer visual outcome in NMOsd patients.
Conclusions: These data suggest that anterior visual pathway involvement in NMOsd was characterized by AQP4-mediated astrocytopathy with more widespread axonal injury and optic nerve sheath inflammation. This concept in NMOsd eyes might be identical to that in NMOsd spinal cords.

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Factor H autoantibodies in neuromyelitis optica

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Background: Neuromyelitis optica (NMO) is an inflammatory disease characterized by pathogenic complement-activating autoantibodies against aquaporin 4 (AQP4), the main water channel of the central nervous system.
Objectives: NMO is frequently associated with other autoantibodies and antibody-mediated diseases. Since autoantibodies against complement regulating factors may contribute to the activation of the complement system, here we examined the presence of antibodies against the complement regulator protein factor H in the serum of patients with NMO.
Methods: The study cohort included 45 patients with NMO, who were all seropositive for AQP4 autoantibodies, 25 healthy controls and control sera from patients with hemolytic uremic syndrome (HUS) seropositive for factor H antibodies. Serum samples were screened by ELISA for autoantibodies using purified factor H as target antigen and human serum albumin as control antigen.
Results: Specific IgG binding to factor H was detected in 4 samples (~9%), while no IgG binding to factor H was detected from 25 control sera. We could also detect factor H-IgG complexes using a monoclonal antibody to factor H as a catch antibody in ELISA. The binding sites of the factor H autoantibodies were mapped using recombinant constructs representing various factor H domains. All autoantibodies bound to factor H domains 19-20, and also recognized the homologous protein CFHR1. This binding pattern was similar to factor H autoantibodies associated with atypical HUS, used as positive controls in the assays.
Conclusions: Our results demonstrate that factor H autoantibodies are not uncommon in NMO. Our data also suggest that generation of antibodies against complement regulating factors among other autoantibodies may contribute to the complement-mediated damage.

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Retrospective review of optimal treatment for acute relapses in neuromyelitis optica

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Background: Patients with neuromyelitis optica (NMO) are known to suffer from severe relapses that are only partially responsive to steroids. Although the use of plasma exchange (PLEX) is a common practice in many centers, little is known about the additional benefit obtainable by adding PLEX to steroid therapy.
Objectives: To compare the efficacy of high dose intravenous methylprednisolone (IVMP) versus high dose IVMP + PLEX in the treatment of acute relapses in neuromyelitis optica (NMO).
Methods: We conducted a retrospective review of 85 admissions of NMO patients to the Johns Hopkins Hospital with confirmed acute relapses of transverse myelitis, brainstem lesions and/or optic neuritis treated with either high dose IVMP alone versus high dose IVMP + PLEX. The clinical decision to escalate treatment to PLEX was based on poor response to IVMP. Extended disability status score (EDSS) was calculated for each patient prior to admission, at presentation, at discharge from the hospital and on follow-up in clinic (if available).
Results: Nineteen NMO relapses were treated with a course of high dose IVMP while 65 relapses were treated with high dose IVMP + PLEX. In the IVMP-alone group, the median baseline EDSS was 2.5, which increased to 6.0 on presentation. The degree of improvement on discharge reached a median of 5.0 but this improvement was not maintained on follow up in clinic. In the IVMP + PLEX group, the median baseline EDSS was 5.5, the median EDSS on presentation was 7.5 which decreased to a median of 6.5 on discharge and maintained at a median of 6.5 on follow up visit within 1 year of discharge. Sixty five percent of IVMP + PLEX patients achieved an EDSS that is equal or below baseline at follow up while only 35% of the IVMP-only patients achieved their baseline EDSS (odds ratio=0.2961, 95% CI 0.0946 to 0.9266, P= 0.0365). A delay of more than 3 weeks from onset of relapse to initiation of plasma exchange was associated with a lower likelihood of success.
Conclusions: High dose IVMP provides only a moderate degree of neurologic recovery from acute NMO relapses. Plasma exchange implemented in a timely manner improves the outcome on follow-up in cases where IVMP alone is insufficient.

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Pathological study of tumefactive brain lesions in neuromyelitis optica

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Background: Neuromyelitis optica(NMO) with tumefactive brain lesions is a relatively uncommon condition in NMO patients. Diagnosis is different for its clinical and imageological features resemble that of other inflammatory-demyelinating diseases and brain tumors. Pathological study of tumefactive brain lesions is rare.
Objectives: To investigate the pathological characteristics of tumefactive brain lesions in neuromyelitis optica(NMO) and provide clues to differential diagnosis of tumefactive brain lesions.
Methods: 2 NMO patients,1 recurrent optic neuritis(RON) patient and 1 multiple sclerosis(MS) patient with tumefactive brain lesions underwent biopsy operations for being clinically suspected brain tumors. The tissue slices were haematoxylin and eosin(HE) stained and immunohistochemical stained.

Results: 1) Lymphocytes and foam-like macrophages infiltrated in brain lesions in NMO, especially in perivascular region, with vascular walls thickening and hyaline changing; 2) Necrosis was more significant in NMO lesions than in non-NMO lesions; 3) Aquaprin4(AQP4), glial fibrillary acidic protein(GFAP) immunoreactivity were lost in acute NMO lesions, expecially in perivascular region, indicated humoral immunity associated astrocytes impairment, however, myelin basic protein(MBP) immunoreactivity was relatively preserved, indicated that demyelination might occur secondary to astrocytes impairment. Astrocytes impairment is not obvious in non-NMO patients; 4) Axon damage in NMO lesions is more severe than non-NMO lesions.

Conclusions: Pathological characteristics of brain lesions in NMO are different from other inflammatory-demyelinating diseases, indicates that they have different pathogenesis. NMO-IgG and humoral immunity associated astrocytes impairment is important in the pathogenesis of NMO. Considering the distinct treatment strategies of NMO, other inflammatory-demyelinating diseases and brain tumors, early performing serum NMO-IgG test, an effective method to diagnosis NMO, may benefit the tumefactive brain lesion patients.

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Is late-onset neuromyelitis optica spectrum disorder associated with a worse outcome?

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Background: Neuromyelitis optica (NMO) is a rare inflammatory disease affecting predominantly the optic nerve and the spinal cord and the recently discovered disease-specific autoantibody, aquaporin-4 (AQP4-ab) has been able to broaden this disease category; neuromyelitis optica spectrum disorders (NMOSD). Recent studies suggested that the age of onset appeared to be an important predictor of disability type and late-onset (LO) NMO/NMOSD is particularly severe, with a high rate of motor impairment and death.

Objectives: We tried to elucidate the characteristics of LONMOSD (50≥ years) vs adult onset (AO) NMOSD (16 ~ 49 years).

Methods: We included 46 AQP4-ab positive LONMOSD patients (mean age, 57.70±5.97 years) and 51 AQP4-ab positive AONMOSD (mean age, 31.49±9.70) in 10 tertiary Hospitals, South Korea, from March 2005 to March 2014. Clinical and demographic characteristics were compared between LONMOSD and AONMOSD. We analyzed Expanded Disability Status Scale (EDSS) score permanent bilateral visual loss, and wheelchair dependence at the last visit, as well as annual relapse rate (ARR) and progression index (PI) (i.e. the EDSS/disease duration in years).

Results: The interval from onset to relapse was significantly shorter in the LONMOSD group than in the AONMOSD group (13.03±17.30 vs 28.67±39.62 months) ($p < 0.05$), although ARR was not significantly different between the two groups. The Final EDSS score was significantly lower in LONMOSD group, than in the AONMOSD group (3.27±1.95 vs 4.26±2.60) ($p < 0.05$), particularly in the bowel/bladder (0.58±0.95 vs 1.21±1.48) ($p < 0.05$), and visual functions (1.13±1.65 vs 2.29±2.37) ($p < 0.01$). However, the PI was much higher in the LONMOSD, particularly in the sensory (1.64±3.16 vs 0.39±0.53) ($p < 0.01$), and pyramidal functions (1.09±1.72 vs 0.49±0.94) ($p < 0.05$). The frequencies of wheelchair dependency and permanent bilateral visual loss at the last visit were not significantly different between the two groups. In addition, other concomitant autoimmune diseases, such as Sjögren's syndrome were significantly less involved in the LONMOSD group, than in the AONMOSD group (OR=0.148, p=0.001).

Conclusions: We found that patients with LONMOSD have a more severe disease than patients with AONMOSD, particularly in the pyramidal and sensory functions and with shorter interval from onset to the relapse. Furthermore, a large-cohort and prospective study is needed.

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Presence of HLA DR10 in Mexican patients with neuromyelitis optica (Devic's disease)

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Background: Neuromyelitis optica (NMO) is an autoimmune channelopathy (AQP-4) that affects young adults (35-47 years), infrequent in Caucasians and affecting mainly the optic nerve and spinal cord with monophasic or relapsing course.

There are reports of some association with some HLA genes, mainly DPB1*0501 but this can be different across populations.

Different frequencies of Devic`s disease could be explained (at least in part) by some genetic background susceptibility.

Here, we report the association of HLADR10 with Devic`s disease in Mexican mestizo patients.

Objectives: To describe frequency of HLA DR10 in patients with Neuromyelitis optica (Devic ´s Disease) in Mexico.

Methods: Descriptive transversal study.

Population and sample: Patients from out patient clinic of demyelinating diseases with NMO diagnosis in agreement with Mayo clinic proposed criteria and NMO-IgG status (Devic's disease diagnosis). We obtained demographic and clinical data from medical records. After signing the informed consent, we take 10 mL of blood sample to attain DNA aliquots for amplification, we also took a sample for controls, which were patients who are attended for

other diagnoses no including MS or another autoimmune disease. The samples in the laboratory following standardized procedures. The blood is separated and stored at -70 ° C. The DNA was extracted from the pellet of leucocytes extracted with ACD as anticoagulant. The DNA extracted by the technique of modified Covarrubias Cuevas Buffone and Darlington; Millar and Plesky; Maniatis and Fritsch. Afterwards hybridization was performed by adding the specific PCR product for each locus to investigate. Later the samples acquired with Fluoroanalyzer Luminex ®.

Results: 63 Neuromyelitis Optica patients and 198 controls were ampled. The average age was 43.3 years (20-67) and 82% were women.

HLA DR10 was present in 5 cases (0.08%) and only in 1 control (0.005%), with a p:0.001, OR 19.3.

Conclusions: In our study HLADR10 is more frequent in patients with Devic's disease than in controls with statistical significance, which is relevant since the haplotype DRB1 gene has been described as non-Caucasian so may confer a risk factor for developing optic neuromyelitis in our population. Even more, this allele has been described in other autoimmune diseases such as rheumatoid arthritis and it seems to be protective for MS in an spanish study when its combined with HLA DRB1*15.

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The plasma antiaquaporin antibodies and the outcome of myelitis in neuromyelitis optica and neuromyelitis optica spectrum disorders: any relationship?

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Background: Some patients with neuromyelitis optica (NMO) and NMO spectrum disorders (NMOSD) have similar clinical features do not have Anti-Aquaporin-4 (AQP4) antibody and may have a different condition with different outcomes with regard to motor disability.

Objectives: To determine whether the AQP4 antibody has a relationship with the prognosis of transverse myelitis in terms of motor disability.

Methods: Sera of 34 patients with NMO (n=27) and NMOSD with isolated or recurrent myelitis (n=7) were all investigated for the presence of AQP4 antibody by a cell-based indirect immunofluorescence assay (IIFA). The prognostic values of anti-AQP4 antibody were evaluated in terms of a good motor outcome (able to walk unaided for at least 100 metres) and a poor motor outcome (with aid to walk at least 100 metres or a worse condition).

Results: In our study, the anti-AQP4 antibody´s seropositivity in all cases was 61.8% (n=21), was 59.3% in NMO and 71.4% in NMOSD cases. Anti-AQP4 antibody seropositivites had older disease onset (39±13.5 vs 27.9±8.7, p=0.009). And 33.3% of the seropositive patients and 38.5% of the seronegative patients had a poor motor disability, during a follow-up period of 102±79.1 months. There was no significant difference that existed between anti-AQP4 antibody seropositivity and seronegative in terms of motor disability (p=0.770).

Conclusions: As compared with anti-AQP4 antibody-negative ones, anti-AQP4 antibody-positive patients show significantly older disease onset. The outcome of myelitis in terms of motor disability was similar in both anti-AQP4 antibody-positive and negative patients.

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Autoantibodies in patients with neuromyelitis optica

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Background: Neuromyelitis optica (NMO) is an inflammatory disease of the central nervous system that predominantly affects the optic nerves and the spinal cord. NMO is associated with antibodies that target aquaporin-4, other autoimmune disorders and multiple other autoantibodies. A studied identified 22 autoimmune conditions associated with NMO.

Objectives: The aim of this study was to investigate autoantibodies in patients with NMO from Southern Brazil.

Methods: Patients with NMO were diagnosed according to the 2006 revised diagnostic criteria. Demographic and clinical data were obtained from 22 patients with NMO using a standard questionnaire and from medical records. All patients were under use of prednisone in combination with other immunosuppressive drug (azathioprine or mycophenolato mofetil). Patients were divided in group 1, consisted of 13 patients under use of 10 mg/day of prednisone; and Group 2, consisted of 9 patients under use of >10mg/day of prednisone. Peripheral blood samples were collected to test antibodies to aquaporin-4 (anti-AQP4), thyroid-stimulating hormone (TSH) receptor antibodies (TRAb), antinuclear antibodies (ANA), antithyroid peroxidase antibodies (antiTPO), antitireoglobulin antibodies and antibodies to double stranded DNA (anti-dsDNA). The disability was evaluated using the Expanded Disability Status Scale (EDSS).

Results: No difference was found in age, gender, ethnicity, body mass index, and corticosteroids therapy between the groups (p>0.05). The median age of disease onset and median disease duration was higher on group 1 than group 2 (48.5 vs 37.0 years; p=0.0482; 7.0 vs 2 years, p=0.0240). Although patients presented others autoimmune diseases (systemic lupus erythematosus, systemic sclerosis, juvenile rheumatoid arthritis, hypothyroidism, hyperthyroidism and Raynaud's phenomenon), no statistically significant differences were found in anti-AQP4, TRAb, ANA, antiTPO and antitireoglobulin antibodies (p>0.05). All patients were negative for anti-dsDNA titers. Patients from this cohort presented only 50% of anti-AQP4 positivity. No differences in EDSS were found (p>0.05).

Conclusions: Patients with NMO presented a controversial clinical and laboratorial course. More studies should be performed to clarify the clinical and laboratorial course of the disease.

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Neuromyelitis optica: annual relapse rates off and on immunosuppression and the relationship to attack type and ethnicity

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Background: Due to the rarity of neuromyelitis optica (NMO) the prognosis on and off treatment is unclear. This data is important in making individual treatment decisions and in designing clinical trials.
Objectives: Analyse relapse-rates (RR) in a well-defined NMO cohort.
Methods: Data from 82 aquaporin-4 antibody positive patients clinically assessed within the nationally commissioned NMO service in Oxford were analysed.
Results: Median onset age was 39 years, median follow up was 6 years and 85% were female. The cohort included 45 Caucasians, 15 Afro-Caribbean's, 9 Asians and 13 mixed race /other. Only 3.6% had both transverse myelitis (TM) and optic neuritis at onset. The median time from onset to diagnosis had reduced from 12.4 years pre 2004 to 0.1 years post 2009 (when awareness of NMO was higher and the Oxford National clinical and assay service were established).

The lowest RRs were seen in Asian patients (44% with the lowest RR quartile) and the highest in the Afro-Caribbean patients (67% had the highest RR quartile). The latter may be related to a higher rate of relapses in brain/brainstem attacks. Younger patients (< 18yrs) had higher RR than those over 18 years: 1.53 versus 0.82.

The 'pre-all-treatment' RR was 0.87 versus an on-immunosuppressive treatment RR of 0.42. However delaying treatment did not appear to affect the on treatment RR. Annual RRs for 13 patients untreated for ≥ 4 years were: yr 1: 1.46 (including onset attack), yr 2: 0.23, yr 3: 0.15 and yr 4: 0.15 (probably biased by milder patients being more likely to stay off treatment). The pre-all-treatment RR compared to the 'on' individual treatment rates (± prednisolone) were: azathioprine (n=66, 63 1st line) 0.93:0.21, methotrexate (n=16, 6 1st line) 1.9:0.44, mycophenolate mofetil (n=17, 4 1st line) 0.76:0.50, rituximab (n=10, 1 1st line) 0.76:0.46.

Immunosuppressive treatment appeared to reduce the residual disability caused by attacks of TM: Mean downstream minus pre relapse EDMUS scores and % with no residual change in EDMUS were pre-treatment + 0.8, 54% vs - 0.05, 88%.
Conclusions: Immunosuppressive therapy is the 'current standard of care' to prevent relapses. Observational studies like ours support their use but will not exclude other factors. Immunosuppressive therapy may also reduce the residual damage from relapses. Our data suggests that relapse frequency is influenced by ethnicity, onset age and attack type.

P650

Magnetic resonance imaging features of optic neuritis distinguishing neuromyelitis optica from multiple sclerosis

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Background: Optic neuritis (ON) may be the initial manifestations of multiple sclerosis (MS) and neuromyelitis optic (NMO). Differential diagnosis between these two disorders is important because of difference in prognosis and treatment. Although several clinical characteristics of ON may help differentiate NMO from MS, an episode of NMO-related ON is often indistinguishable from MS-related ON.
Objectives: The purpose of this pilot study was to evaluate the magnetic resonance imaging (MRI) features of optic nerve during acute ON in NMO and MS patients.
Methods: We identified brain and orbit MRI of 11 MS (3 males and 8 females; mean age at ON 31.8 ± 12.0) and 17 NMO (2 males and 15 females; mean age at ON = 34.0 ± 10.6) patients who presented with ON. All MRI obtained within 6 weeks of ON and were reviewed by two neuroradiologists masked to the diagnosis. The parameters for MRI analysis included the presence and degree optic nerve enhancement, optic nerve thickening, and the extent and location of optic nerve involvement. The length, cross section area (CSA) and T2 signal intensity of the affected part of optic nerve were measured. The length was measured using curved multiplanar reconstruction from 3D FLAIR data. CSA were compared with unaffected part of optic nerve (contralateral unaffected nerve or inpsilateral unaffected part in bilateral ON cases). The T2 signal intensity ratio between affected optic nerve segment and unaffected cerebral cortex was calculated.
Results: The length of affected segment was significantly longer in NMO-related ON than MS-related ON (33.6 mm ± 15.0 vs. 20.8 mm ± 11.6, $p = 0.024$). T2 signal intensity ratio between affected optic nerve segment and unaffected cerebral cortex was significantly higher in NMO-related ON than MS-related ON (1.3 ± 0.2 vs. 0.9 ± 0.1, $p = < 0.001$). No significant differences were demonstrated in the CSA of affected segment, degree of optic nerve enhancement, and location of optic nerve involvement between MS- and NMO-related ON.
Conclusions: These findings suggests that the length of affected segment and T2 signal intensity ratio between affected optic nerve and unaffected cerebral cortex may help distinguish between MS-related ON and NMO-related ON. NMO-related ON appears to have more extensive and more inflammatory lesion than MS-related ON.

P651

Involvement of cerebral cortex in anti-aquaporin-4 antibody seropositive neuromyelitis optica spectrum disorder patients

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Background: Brain lesions with characteristic locations and configurations are helpful in the diagnosis of neuromyelitis optica spectrum disorder (NMOSD). It was previously reported that cortical lesions were not found in patients with NMO, which was contrast to multiple sclerosis (MS).
Objectives: We aimed to determine whether involvement of cerebral cortex is present on conventional brain MRI in

anti-aquaporin-4 antibody positive NMOSD patients and to describe their imaging and clinical characteristics.

Methods: In this study, 215 anti-aquaporin-4 antibody positive NMOSD patients from six referral hospitals were involved. We retrospectively analyzed demographic, clinical, and MRI findings of the enrolled patients. All MRI scans were performed on either a 1.5- or a 3.0-T machine. Abnormal lesions involving cerebral cortex on brain MRIs were identified, by consensus of three experienced observers, a neuroradiologist and two neurologists.

Results: Among 215 enrolled patients, 87% were female. Median age at onset was 33.3 (5-60) years and mean follow-up duration was 123 months. During follow-up, brain MRI was performed in 194 patients, and brain lesions were found in 143 patients (74%). Brain lesions involving cerebral cortex were identified in six patients (3.1%). Five of them (83.3%) were female, and their mean age was 29.5 (16-46) years when the cortical lesions were presented. Interestingly, four of them showed leptomeningeal enhancement in the lesions. The lesions showed heterogeneous signal intensity and blurred margins. The cortical lesions were located most commonly in frontal and occipital lobe, and presented with other previously described brain lesions characteristic of NMOSD. At the presentation of the cortical lesions, three of them were not treated, two were treated with beta-interferon, and one with oral prednisolone. Two patients were presented with encephalopathy, one showed myoclonus of the contralateral arm, and three did not show obvious symptoms correlated with the cortical lesions.

Conclusions: This study shows that although very rare, cortical involvement occurs in NMOSD patients and is commonly combined with leptomeningeal enhancement. It is speculated that this rare findings may occur only in patients who are not properly treated.

P652

NMO and NMOSD: clinical, imaging. Laboratory and CSF characteristics: a cohort from Iran

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Background: A little information has been published about the clinical,imaging and laboratory characteristics of NMO in the middle east region. We reviewed on the characteristics of a cohort of 50 Neuromyelitis optica (NMO) patients in our center In Tehran, Iran.

Objectives: NMO is relatively rare disease in Iran.Our estimation is 1 to 400-700 of MS cases.So it might not be more than 800 all over the country.

Methods: 50 fulfiled the 2006 criteria, analyzed for the presenting symptoms, number of recurrences, associated disorders, CSF abnormalities, anti NMO and anti MOG antibody, imaging and outcome.

Results: 13% were male, 87% female. Mean age was 36.76 years. Mean disease duration was 71.08 months and the mean follow up time 27.60. 34.8% had Optic Neuritis as the presenting symptom. 43.5% were not affected by Myelitis. 50% had Cervical myelitis and 6.5% both cervical and thoracic myelitis. 23.9% had atypical brain symptoms .80.4% had experienced recurrence from which 62.16% had one time.24.32% 2 or 3 and 4.3% more than 3.

50.1% had EDSS between 0-2 at presentation. 26.1% 2 to 4. 23.9, 4 to 7 at presentation.

17.4% indicated positive NMO Abs. 30 patients did Anti MOG antibody, positive results appeared in 86%.

24.5%had CSF samples, from which 23.9% were OCB positive and 23.9% had elevated IgG index.

54.3%had normal brain in imaging. 37% atypical brain abnormalities in MRI.37.9% LEMS.

We found pain in 69.6%, .28.3% were misdiagnosed as MS.

Conclusions: Despite the relatively high recurrence rate Outcome revealed no significant disability.Inspite of low positivity of NMO antibody, mantiMOG antibody was remarkably positive in our patients, however, Further studies seem essential to prove the exact sensitivity of these laboratory tests.

P653

An unusual case of neurofibromatosis type 1, high titer antinuclear autoantibodies and neuromyelitis optica

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Background: Neurofibromatosis type 1 (NF1) is an autosomal dominant disease with a wide range of neurological manifestations. Associations of autoimmune diseases with NF1 have rarely been described. There are 5 case reports of NF1 with systemic lupus erythematosus (SLE) and 6 case reports of NF1 with multiple sclerosis in the medical literature up-to-date. Neuromyelitis optica (NMO) spectrum disorders include a series of syndromes associated with aquaporin-4 seropositivity. NMO with concomitant SLE or high titer antinuclear autoantibodies (ANA) has been described, but association of NMO with NF1 has never been reported.

Objectives: To report a case of a patient with NF1 and high titer ANA, who developed NMO.

Methods: Longitudinal case report.

Results: We report a case of a 65-year-old African-American female with NF1, who presented with 8 weeks of bilateral leg stiffness, paresthesia up to breast line, urinary retention and bowel incontinence in June 2006. Cerebrospinal fluid (CSF) was negative for oligoclonal bands or malignancy. She had positive ANA, but did not fulfill criteria for SLE. Imaging studies showed normal brain, abnormal T2 hyperintensity from T2-T6, as seen in myelitis and L3-L5 root lesions which were proven to be neurofibromas. She regained urinary control and her gait improved after intravenous corticosteroid treatment. A diagnosis of autoimmune myelitis was made.

Patient was treated with oral steroids and mycophenolate mofetil (MM) for 4 years and continued to be stable on MM alone for 2 years. In December 2012, after reducing the MM, she had a relapse of her symproms with an incomplete response to intravenous corticosteroid treatment. Her previous dose of MM was restarted. In April 2013 she developed right-sided hemiparesis. Neuroimaging showed worsening of her thoracic spine lesion and a new cervical spine lesion from C3-C6. Patient had seropositive

aquaporin-4 antibody and was diagnosed with NMO spectrum disorder.
Conclusions: This is the first case of an NF1 patient with NMO spectrum disorder described in the literature. Whether these associations reflect a causal relationship or are coincidental needs further study.

P654
Neuromyelitis optica and neuromyelitis optica spectrum disorders: the evaluation of 86 patients followed by Istanbul Bilim University
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Background: Neuromyelitis optica (NMO) and its spectrum disorders (NMOSD) are relatively rare disorders when compared to multiple sclerosis (MS).
Objectives: We aimed to evaluate clinical characteristics and disease course of the NMOSD patients followed at our department.
Methods: All the patients with the diagnosis of NMO/NMOSD followed since the establishment of our MS clinic in April 2011, were evaluated.
Results: There were 86 patients (66 female, 20 male) with NMO/NMOSD followed at our MS unit; 24 had NMO, 42 had recurrent optic neuritis (RON); and 20 had longitudinally extensive transverse myelitis (LETM). The mean age of the patients was 40.1±14.1 (12-77) years. The disease duration was 4.5±4.5 years. The disease course was relapsing in 70 patients (81%). The first attack was bilateral ON (BON) and TM in 3 patients, ON and TM in 1 patient, ON in 50 patients (bilateral in 6) and TM in 26 patients. The mean of the duration between the first two relapses was 14.02±31.1 months. The mean EDSS score was 2.75±1.7 at the last visit. NMO IgG was positive in 12 patients with NMO (55%), 4 patients with LETM (25%), and 8 patients with RON (22%). Oligoclonal band was positive in 15 out of 44 patients (4 each with NMO and ON, two with ON and TM and three with LETM; 34%). In NMO/NMOSD patients, cranial magnetic resonance imaging (MRI) showed no abnormality in 48; nonspecific lesions in 37; and 1 patient had hypothalamic lesion. In spinal MRIs, 36 patients had LETM; six had suspected hyperintense T2 lesion in C5.
Conclusions: This is one of the largest single center series collected over 3 years. NMO/NMOSD seems to be over-represented in our center since it is one of the few where NMO IgG testing is available. In NMO/NMOSD, early diagnosis and treatment, as well as differentiation from MS, is important to prevent the patient from the permanent disability.

Neuro-ophthalmology and OCT

P655
Optic neuritis related to tumour necrosis factor-a antagonists: description of 30 cases in a nationwide pharmacovigilance database
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Background: Tumour necrosis factor-a antagonists (anti-TNFa) indicated in different inflammatory diseases including rheumatic and bowel diseases may have neurological side-effects. Among them, few cases of optic neuritis (ON) have been reported whatever anti-TNFa category.
Objectives: The objective was to study the french reported cases of anti-TNFa-related ON.
Methods: We included all spontaneous reports of ON related to anti-TNFa recorded in the french pharmacovigilance database until may 2013 or included in databases of the french Multiple Sclerosis centers network (Club Francophone de la Sclérose en Plaques).
Results: 30 cases of ON related to anti-TNFa (in 28 patients [25F/3M] with mean age at 43 years [+/- 14.8 years] were identified. Among these cases, 12 (42.85%) occured during exposure to infliximab, 10 (35.7%) during adalimumab and 8 (28.57%) during etanercept, mainly required because of inflammatory bowel and rheumatologic diseases. Mean duration of treatment was respectively 8.5, 13.5 and 9 months for infliximab, adalimumab and etanercept. Clinical characteristics of ON were not specific, pain was rarely found and ON was unilateral in 18 patients (64,28%) and bilateral in others. Data concerning visual field, cerebral MRI, visual evoked potential tests and CSF will be reported in the poster. In most cases, anti-TNFa was discontinuated, and less than half of the patients received corticosteroids. Improvement was observed in 21/28 (75%) patients and none of them developed Multiple Sclerosis.
Conclusions: ON is probably the most frequent but rare demyelinating event related to TNF-a antagonists. No specific clinical, biological or radiological sign was identified. Prognosis seems favorable after anti-TNFa discontinuation. It is important to systematically report adverse drug reactions to pharmacovigilance databases to obtain robust data for future guidelines.

P656
Multicolor retinal imaging in acute optic neuritis: a new potential biomarker for multiple sclerosis
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Background: MultiColor laser imaging [MCLI, Heidelberg Engineering] of the retina is a new imaging modality that passes blue, green, and red LED light from 450 to 900 nm across the retina en face anteriorly to posteriorly resulting in topographic maps that exceed the visible spectrum of the human eye. By precisely focusing upon these three retinal zones, MCLI imaging provides increased resolution and detail compared to fundus photography with monochromatic light.

Objectives: To investigate the potential role for MLCI in patients with acute optic neuritis.
Methods: We investigated 15 patients with acute optic neuritis at the time of their presentation and 3 months after their initial visual symptoms. The event of optic neuritis was the first clinical demyelinating event in 8 patients and in the other 7 the event occurred in patients already diagnosed with RRMS.
Results: In 10 of 15 patients with acute optic neuritis, MCLI demonstrated hyper-reflective zones encircling the fovea and macular area in the blue and green laser zones. Comparison of the MLCI changes to the histology of the foveal and macular areas indicated that these zones represented probable inflammatory and/or edematous changes within the retinal nerve fiber, ganglion cell, and inner nuclear layers as well as the inner plexiform regions.

In addition, 10 patients also demonstrated hyper-reflectivity following the anatomic course of the retinal veins, suggesting a possible perivenular site of neuro-inflammation as has been observed in histopathologically with RRMS as well as in in vivo models of antibody mediated optic nerve demyelination. Primary photoreceptor, outer retinal pathology was observed in both the macular and extra-macular areas in PPMS patients and in some patients with RRMS.
Conclusions: MLCI is a new high resolution, non-contact, non-invasive retinal imaging technique for RRMS and PPMS that should serve as a correlative biomarker to SD-OCT and magnetic resonance imaging for both clinical practice and clinical trials. In addition, this technology demonstrates retinal and retinal vascular changes in MS not seen with SD-OCT and monochromatic fundus photography.

P657

White matter damage is associated with optic neuritis related retinal nerve fiber and ganglion cell loss in neuromyelitis optica spectrum disorders

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Background: Neuromyelitis optica (NMO) is an autoimmune CNS disease characterized by optic neuritis (ON) and myelitis. Attacks can be devastating, especially damage by ON is more severe than in multiple sclerosis. Previous studies reported retinal nerve fiber layer thinning in ON eyes of NMO patients but data on cerebral grey and white matter damage are limited.
Objectives: To investigate whether RNFL (retinal nerve fiber layer) and GCL (ganglion cell layer) thinning in eyes after ON is associated with white or grey matter brain atrophy.
Methods: 24 patients with NMO and NMO spectrum disorders with ON and 21 sex and age matched healthy control subjects were enrolled. All subjects underwent spectral domain OCT examinations with RNFL and GCL analysis and 3T MRI. T1w scans were lesion-corrected before estimating normalized grey and white brain volumes (NGMV, NWMV) with FSL SIENAX and Voxel Based Morphometry (VBM). DTI scans were analyzed by Tract Based Spatial Statistics (TBSS).
Results: Normalized white matter volume was significantly reduced in the NMOSD group compared to healthy subjects (725 cm^3 vs. 753 cm^3, ANOVA p=0.022), while no significant difference in grey matter was found. VBM did not identify any grey matter regions with significantly reduced concentration compared to controls. RNFL thickness (58 ± 17 µm, GEE: B=0.153; SE=0.05; p=0.002) and GCL thickness (26 ± 6 µm, GEE: B=0.071; SE=0.02; p=0.001) in eyes with a previous ON correlated with NWMV but not with NGMV. We found no correlation between RNFL or GCL and brain volume in healthy controls. TBSS data will be included upon presentation.
Conclusions: Our results support a role for ON -associated white matter damage and is in line with a lack of overt grey matter damage in NMO. Potential explanations include degeneration of white matter tracts of the visual pathway subsequent to an ON event.

P658

Optical coherence tomography (OCT) as a predictive and longitudinal *in vivo* biomarker of disease and repair in a mouse model of multiple sclerosis

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Background: Identifying predictive and longitudinal *in vivo* biomarkers to assay therapeutic efficacy is integral to developing treatments for neurodegenerative diseases such as multiple sclerosis (MS). Optic neuritis (ON) is a common acute manifestation of MS onset; similarly, experimental autoimmune encephalomyelitis (EAE) mice, a rodent model of MS, exhibit ON. Optical coherence tomography (OCT) allows for direct visualization and measurement of optic nerve head topography and measurement of layer thickness between retinal nerve fiber layer (RNFL) and retinal pigment epithelium (RPE). Quantification of retinal layers by OCT provides an indirect measure of axonal and neuronal loss in anterior visual pathways. Optic neuropathies and numerous neurologic disorders, including MS, show abnormal retinal layer thickness.
Objectives: We investigated the effects of late myelin oligodendrocyte glycoprotein (MOG_{35-55})-induced chronic EAE and therapeutic treatment of chronic EAE mice with the highly selective estrogen receptor β ligand Indazole-Cl (Ind-Cl) on retina and optic nerve health.
Methods: Fundus imaging and serial high-resolution spectral domain optical coherence tomography (sdOCT), followed by immunohistochemistry and electron microscopy analysis, were performed on normal animals and chronic EAE mice therapeutically treated with Ind-Cl or vehicle (i.e., after peak clinical disease). Image analysis using template-based marking of retinal layers (RNFL, GCL, nuclear layers, and retinal pigment epithelium-RPE) was performed using Bioptigen software (Durham, NC).

Results: Significant changes in RNFL, GCL and RPE retinal layers between normal, vehicle-, and Ind-Cl-treated EAE groups were observed. Preliminary immunohistochemical and electron microscopy data indicate increased inflammation in the retina and optic nerve and decreased axon myelination in the optic nerves of EAE mice. As compared to vehicle treatment, therapeutic treatment with Ind-Cl decreased retinal and optic nerve inflammation and improved optic nerve axon myelination.
Conclusions: These data support OCT and optic nerve analysis as strongly translational in vivo biomarkers with which to assess the efficacies of potential neuroprotective agents for the treatment of inflammatory neurodegenerative diseases. Further, these data support therapeutic Ind-Cl as a treatment capable of improving widespread, quality-of-life-diminishing MS-related retinal and optic nerve pathology.

P659

Retrograde axonal and neuronal degeneration of the retina in acute optic neuritis

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Background: A detailed description of the diffusion of retinal damage in acute optic neuritis (ON) and its correlation with visual disability is relevant for the understanding and clinical management of axonal and neuronal degeneration in ON.
Objectives: To assess the dynamics of retinal damage after ON by Optical Coherence Tomography (OCT) and to identify early OCT predictors of visual disability.
Methods: We analyzed 31 consecutive patients with incident acute ON (idiopathic or associated to MS) from clinical onset to month 6. Patients were recruited by their physicians after signing informed consent. We performed retinal OCT (Spectralis) at baseline and every month (7 visits) evaluating thicknesses of peripapilar retinal nerve fiber layer (pRNFLTH) and macular layers with device´s software and best-corrected high contrast (HCVA), low contrast (LCVA) and color visual acuity (CVA), and visual field testing (VF) every 2 months from baseline (4 visits). The predictive value of early OCT measures as biomarkers of visual disability was tested by logistic regression.
Results: After 6 months, ON-eyes decreased 39.6 μm in pRNFLTH and 14.8 μm in macular thickness. Macular atrophy was due to decrease of macular RNFLTH (- 7.4 μm) and ganglion cell layer + inner plexiform layer complex thickness (GCIPTH) (-10.2 μm), while outer layers thickness increased slightly. Most pRNFLTH reduction occurred in first 2 months while macular thickness decreased progressively along 3-6 months. We observed different responses for inner and outer macular layers: while RNFLTH and GCIPTH decreased every month (specially in first 2 months), thickness of outer layers increased in first 2 months (partially compensating RNFL and GCIP thinning), and decreased thereafter by month 3 to 6. Macular atrophy was more severe and homogeneous in internal sectors (parafovea) and dependent on GCIP thinning, and atrophy of external sectors was more related to RNFL thinning in papillomacular bundle. Change in GCIPTH from baseline to month 1 predicted visual impairment at month 6: a decrease ≥4.5 μm (6.5%) predicted poor 2.5%-LCVA (sensitivity 91% and specificity 88%), and ≥ 7 μm (10%) predicted poor VF and CVA (sens. 78% and 100% respectively and specif. 71%).
Conclusions: After acute optic nerve damage in ON, retrograde axonal degeneration and atrophy of ganglion cells develop within 3 months, while outer layers suffer a transient compensatory thickening. The atrophy of GCIP after 1 month of ON clinical onset is predictive of short-term visual disability.

P660

Mechanisms of fatigue in multiple sclerosis: the role of neuronal loss in the visual system

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Background: Fatigue is frequently acknowledged to be one of the most common and disabling symptoms in Multiple Sclerosis (MS). Injury to the anterior visual pathway is also extremely common in MS. The retino-hypothalamic pathway is important biologically in that it entrains the circadian rhythm. Injury to the anterior visual pathway has not been explored as a contributor to fatigue in patients with MS.
Objectives: We examine visual pathway neuronal and axonal loss as predictors of fatigue in two cohorts of MS patients.
Methods: Retinal nerve fiber layer (RNFL), total macular volume (TMV) and Ganglion cell Layer (GCL) were measured using Optical coherence tomography (OCT) in two independent cohorts. In the first cohort, fatigue was captured by the modified fatigue impact scale (MFIS). Participants scoring ≥ 38 were categorized as fatigued. In the second cohort, fatigue was captured with a yes/no question.

Logistic regression, accounting for important disease covariates such as age, race, duration and subtype, was used to calculate an odds ratio (OR) for fatigue.
Results: Among 138 participants in the first cohort, 44% were categorized as fatigued. Forty eight percent of fatigued participants and 45% of non-fatigued participants had a history of optic neuritis (ON). Ninety five percent and 5% of participants in both groups had relapsing remitting MS (RRMS) and secondary progressive MS (SPMS), respectively. GCL but not RNFL thinning predicted the presence of fatigue (OR 1.4, 95% CI 1.04 - 1.87, p=0.028 and OR 1.15, 95% CI 0.98 - 1.35, p=0.079, respectively.

Among 331 participants in the second cohort, 64% reported fatigue. Forty percent of fatigued participant and 42% of non-fatigued participants had a history of ON. Ninety one percent and 9% in the fatigue group had RRMS and SPMS, respectively. Ninety three percent and 9% in the non-fatigued group had RRMS and SPMS, respectively. TMV loss, but not RNFL thinning, predicted the presence of fatigue (OR 1.78, 95% CI 1.06 - 3, p=0.031 and OR 1.01, 95% CI 0.92 - 1.12, p=0.784, respectively).

Conclusions: Loss of retinal ganglion cell thickness in the retina demonstrates stronger associations with fatigue than ganglion cell axonal injury in two independent cohorts of patients with MS. It remains to be elucidated if this reflects the greater importance of gray matter injury in mediating fatigue in MS or if instead this reflects differences in how well macular vs. RNFL measures on OCT capture injury to the retino-hypothalamic system.

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Retinal structural injury is worse in African-Americans than Caucasians with multiple sclerosis

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Background: Nearly 200 OCT studies in MS have been published since 1999. Retinal structure injury studies have evolved as investigational outcomes to study disease pathology in MS. Several clinical and MRI studies have shown that African-Americans with MS (AA-MS) experience more aggressive disease course and greater tissue injury in the CNS than Caucasians with MS (CAU-MS). However, no study has been conducted to examine retinal structure injury in AA-MS and compared to CAU-MS.

Objectives: To examine retinal structure including RNFL and macular volume in AAMS. To compare retinal structure injury in CAU-MS.

Methods: Records of all relapsing MS patients who underwent OCT between January 2013 and March 2014 at our MS Center, were examined, after obtaining local IRB approval. Clinical history including EDSS scores were obtained from patient records. Brain MRI and CSF data were also reviewed when available. All patients and healthy controls (HC) underwent Spectral Domain OCT (SD-OCT) with the Heidelberg Spectralis (HRT+OCT). Images were processed by Heidelberg software version 5.4. Segmentation of macular scan was performed by Heidelberg, blinded to study participant clinical status or race.

Results: 150 MS patients and 55 HC were enrolled. Of the 150 MS patients, 61 were AA-MS (mean age 41.8 years, EDSS 3.8, disease duration 8.1years, DMT exposure 5.2 years) and 89 were CAU-MS (mean age 43.5 years, EDSS 2.3, disease duration 10 years, DMT exposure 5.5 years). Mean RNFL global thickness was 85.2 mm^3 in AA-MS and 89.5 mm^3 in CAU-MS (p=0.06). Mean macular volume was 8.16 mm^3 in AA-MS vs 8.38 mm^3 in CAU-MS (p=0.0004). Mean RNFL volume was 0.77 mm^3 in AA-MS and 0.84 mm^3 in CAU-MS (p=.002). Sub-analysis performed in patients with no history of ON, showed AA-MS had macular volume of 8.19 mm^3 and CAU-MS 8.44 mm^3 ($p< 0.0001$). Compared to HC, MS patients demonstrated significantly reduced RNFL thickness and macular volume. Preliminary analysis also indicate significant correlation between RNFL and CSF IgG Index. Further analysis is ongoing.

Conclusions: This is the first study to examine retinal structure injury in AA-MS. Our study demonstrates that AA-MS experience greater RNFL and macular atrophy than CAU-MS. In patients without history of optic neuritis, our results extend previous observations of greater irreversible tissue loss in AA-MS compared to CAU-MS. Collectively, these findings confirm previous clinical and MRI observations suggesting greater tissue injury in the CNS in AA-MS compared to CAU-MS.

P662

Impaired color vision as determined by Farnsworth Munsell 100 Hue testing is tightly associated with retinal thinning in multiple sclerosis

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Background: Color vision abnormalities are common among patients with multiple sclerosis. This has been established using several screening tests including Hardy Rand Ritler (HRR) plates, Lanthony D-15 desaturated tests (LD15), and the Farnsworth Munsell 100 Hue Test (FM-100). The FM-100 is probably the definitive test for impaired color vision, but it is time-consuming to administer and has not been formally assessed in conjunction with retinal nerve fiber layer (RNFL) thickness as measured by optical coherence tomography (OCT) and other tests of visual function.

Objectives: We sought to determine the relationship between the FM-100 and other visual outcome measures in multiple sclerosis (MS) patients who had previously suffered optic neuritis (ON).

Methods: Forty six MS patients with stable ON for at least 3 months were enrolled as part of a clinical trial. They underwent comprehensive visual analysis, including high- and low-contrast visual acuity (HCVA and LCVA) testing, visual evoked potentials (VEPs), OCT, and FM-100 testing during two separate visits over 8 weeks.

Results: The majority of MS eyes (86% of ON eyes and 64% of non-ON eyes) had abnormal color vision as determined by FM-100. FM-100 scores correlated strongly with other measures of visual function, including HCVA (ρ=-0.68, $p< 0.001$), LCVA (ρ=-0.671, $p< 0.001$), RNFL thickness (ρ=-0.574, $p< 0.001$), and VEP 60-minute amplitudes (ρ=-0.353, p=0.001) but did not correlate with P-100 latencies on VEP testing. FM-100 scores also correlated with visual function quality of life testing (ρ=-0.219, p=0.039). FM-100 scores were reproducible over multiple visits. The magnitude of the correlation between color vision disturbance and average RNFL thickness as determined by the current study was ρ=-0.574,$p< 0.001$, similar to that previously reported using HRR plates (ρ=0.594, $p< 0.0001$) and stronger than that established using LD15 scores (ρ=-0.424, p=0.035).

Conclusions: FM-100 scores correlate strongly with other quantitative measures of visual function and less strongly with visual quality of life testing. HRR plates may be a simpler and less time-consuming method of screening color vision when compared to the FM-100.

P663

Retinal ganglion cell layer thinning and vision outcome in optic neuritis over six months

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Background: We showed the retina ganglion cell layer (GCL) thickness is normal (if calculated by 3D but not by commercially available 2D segmentation) at presentation and thinning occurs within one month of acute optic neuritis (ON), long before retinal nerve fiber layer (RNFL) thinning or complete loss of acute swelling are seen (ARVO 2013). The utility of GCL measurement as a structural biomarker for ON is unknown.
Objectives: We investigated the trajectory of GCL thinning over 6 months in order to determine when most of the loss occurs and how the amount of GCL loss relates to the vision outcome.
Methods: Using spectral domain optical coherence tomography (SD-OCT) of the optic nerve head and macula areas we prospectively evaluated 33 patients (age 36±10) with ON within 2 weeks of vision loss, at one month and at 6 months. We used 3D-segmentation to calculate the average GCL plus interplexiform layer thickness (IPL) for each macula image.
Results: The maximum amount of GCL thinning occurred at 1 month with mean loss of 9.33 μm± 5.27. A modest, further thinning of the GCL occurred between 3 and 6 months (2.68 μm and 0.58 μm, respectively). The amount of GCL thinning at 1 month strongly correlated with the amount of GCL loss at 6 months (r=0.837). There was no correlation between the GCL thickness at one month and the mean deviation of the visual field (r=0.033). The RNFL was thickened at presentation and 1 month and did not correlate with mean deviation of the visual field at that time point. The RNFL did not show thinning until the 3 and 6-month time points. GCL thickness at 1 or 6 months did not correlate with the 6 month visual acuity or mean deviation, which had markedly improved in all eyes. Thinning of the RNFL and GCL at 6 months were moderately correlated (r=0.48, p =0.02).
Conclusions: For acute optic neuritis, the largest proportion of GCL loss has already occurred by one month and suggests that neural preservation or protection therapy must be delivered earlier to significantly prevent loss of retinal ganglion cells. GCL measurement appears to be a reliable biomarker to demonstrate early loss; and as an outcome measure of optic neuritis, it shows residual injury better than the RNFL or perimetry or high contrast visual acuity assessment.

P664

Ocular motility: a potential method of quantifying progressive cerebral dysfunction in multiple sclerosis and clinically isolated syndrome

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Background: Multiple Sclerosis (MS) is an inflammatory disease of the central nervous system. However, emerging evidence suggests that neurodegenerative changes are also an important disease process, occurring at the earliest stages of MS, including clinically isolated syndrome (CIS). These changes often predate explicit lesion formation as evident on standard clinical scans, and affect the connectivity of distributed cerebral networks. With neurodegenerative pathology known to increase with disease duration, often in the absence of increasing lesion pathology, sensitively monitoring these changes from the earliest stages is imperative for the analysis of progression. The ocular motor (OM) network provides a unique opportunity to sensitively quantify both disseminated (neurodegenerative) and discrete (lesions) changes providing a comprehensive measure of cerebral functioning.
Objectives: We aimed to determine whether OM assessment sensitively dissociates deficit as a function of disease duration in MS including from initial presentation of the disease (CIS).
Methods: Our study used 5 discrete OM tasks, each implicating cognitive processes commonly affected in MS; visually guided saccades, baseline task; antisaccades, inhibition of an exogenously elicited response; endogenously cued saccades, inhibition of an endogenously elicited response; memory guided saccades, working memory (WM) and inhibition; spatial *n*-back task, WM deficit as a function of increasing WM load. 25 patients with a CIS suggestive of MS, 25 MS patients within 7 years of diagnosis (CDMS early), 25 MS patients greater than 7 years from diagnosis (CDMS late), and 25 healthy controls were compared on all tasks, including neurpsychological tasks.
Results: All patient groups made a significantly higher proportion of errors across all OM tasks compared with controls. Proportion of WM errors on the *n*-back task and latencies of visually guided, memory guided and endogenously cued saccades increased linearly across patient groups as a function of disease duration. In contrast, performance on neuropsychological tasks did not increase proportional to disease duration.
Conclusions: This methodology provides a highly sensitive means of measuring progressive changes in MS and CIS, with the potential for development as either or both clinical or research tools for measuring disease progression or treatment efficacy.

P665

Retrospective comparison of mfERG and SD-OCT between RRMS and PPMS patients with and without optic neuritis

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Background: Relapsing remitting multiple sclerosis (RRMS) and primary progressive multiple sclerosis (PPMS) are characterized by distinct clinical presentations, genetic profiles, histopathology, and neuroimaging findings. RRMS patients frequently experience visual loss caused by optic neuritis (ON). In contrast, PPMS patients develop ON much less frequently but often complain of photosensitivity. Despite these clinical differences, very little data compare the anatomic and electrophysiological findings of the afferent visual system for PPMS and RRMS. This comparison could provide useful biomarkers for future clinical trials.
Objectives: To compare retinal electrophysiologic and anatomic metrics between RRMS and PPMS patients in eyes with and without a history of ON.
Methods: Charts of Neuro-Ophthalmology Service patients were retrospectively reviewed. Only PPMS and RRMS patients who underwent 103-hexagon multifocal electroretinogram (mfERG) and spectral domain optical coherence tomography (SD-OCT)

within a three month span were included. All eyes were divided into four groups: [1] RRMS and [2] PPMS eyes with no history of ON and [3] RRMS and [4] PPMS eyes with a history of ON. Response densities of mfERG amplitudes and latencies were averaged into six concentric rings [R1-R6]. Retinal nerve fiber layer (RNFL) and macular thicknesses were obtained via SD-OCT. Statistical comparisons were made between groups [1] versus [2] and groups [3] versus [4] with Welch's t-test.

Results: Fifteen RRMS patients (30 eyes, of which 7 had ON) and 8 PPMS patients (16 eyes, of which 4 had ON) were included. In eyes without a history of ON, inner ring amplitudes (N1 [R2-R4], P1 [R1-R4], N2 [R1-R5]) were decreased in PPMS compared to RRMS ($p < 0.02$). N1, P1 and N2 latencies for outer rings [R4-R6] were increased in PPMS patients ($p < 0.04$). Additionally, eyes of PPMS patients disclosed decreased RNFL and macular thicknesses compared to RRMS patients ($p < 0.04$). In PPMS and RRMS patient eyes with a history of ON, no significant differences were found with mfERG ($p > 0.10$) or SD-OCT ($p > 0.34$) metrics.

Conclusions: Decreased mfERG amplitudes, increased latencies, and decreased RNFL and macular thicknesses in eyes of PPMS patients without a history of ON suggest primary macular dysfunction in this subtype of MS. Lack of significant retinal electrophysiologic or anatomic differences between PPMS and RRMS patients with a history of ON suggests that both groups develop secondary macular pathology in association with a primary optic nerve lesion.

P666

Reliability of different point estimates for intra-retinal layer thickness determination

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Background: Intra-retinal layer segmentation of optical coherence tomography (OCT) measurements has recently become increasingly prominent in MS research. In the published literature, different approaches for intra-retinal layer thicknes estimation have been used. Previously it was shown that different point estimates can provide comparable data. However, no data exist on the reliability or reproducibility of different point estimates, which is crucial for their future application in clinical studies.

Objectives: To evaluate the reliability of different point estimates for intra-retinal thickness determination.

Methods: We searched pubmed for articles that have used intra-retinal layer segmentation of OCT data in patients with MS. To analyze the reproducibility of the methods published with the Spectralis OCT (Heidelberg Engineering), we generated a simulation dataset consisting of 15 healthy subjects, each measured 3 times with all scans previously used in published studies. Segmentation was performed automatically with Heidelberg Eye Explorer (version 6.0.0.7) and manually corrected in cases of errors. We simulated each point estimate on the repeated sessions and analyzed reproducibility with intraclass correlation coefficients (ICC).

Results: We identified 27 research articles, which presented results for intra-retinal layer analysis of MS patients. 11 of them used Spectralis OCT, 10 Cirrus OCT (Carl Zeiss Meditec), 2 studies used both devices, 2 studies RTVue-100 (Optovue) and one study used 2 research devices. For the Spectralis OCT, 9 different point estimates were used. In the simulation dataset, approaches using the mean thickness of the area around the fovea (6 mm and 3 mm diameter ETDRS area) showed excellent reproducibility (ICC > 0.91) for all retinal layers except for the outer plexiform layer (OPL), which had a different appearance in each repeated session and was not segmented consistently. Point estimates using only single values or limited number of values at pre-defined locations performed generally weaker in the reproducibility analysis.

Conclusions: Point estimates based on ETDRS areas showed good reliability and should be favored when OCT is used in clinical research. On the contrary, point estimates with single layer analysis should be avoided based on their weak reliability. Assessment of the OPL was weak in all investigated point estimates and needs further investigation before OPL thickness can be used as a reliable parameter.

P667

Longitudinal correlation of retinal nerve fiber layer and timed 25 foot walk in a large MS cohort

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Background: There is a need to identify anatomical biomarkers of disease progression in Multiple Sclerosis (MS) that are practical, inexpensive, and easy to perform. Measurement of the retinal nerve fiber layer (RNFL) by Optical Coherence Tomography (OCT) correlates with brain volume loss as measured by MRI. In addition, correlation between these types of anatomical parameters with measurements of clinical function is needed in order to establish practical implications for patient management. The timed 25 foot walk (T25FW) is a simple bedside test that has been identified as having a relationship with functional level, gait, and disease progression.

Objectives: Identify if RNFL measurement by OCT correlates with T25FW and serves as a surrogate anatomical marker of functional decline in individuals with MS.

Methods: This was a longitudinal study of 299 MS patients with mean age of 48.7 (± .73). The sample consisted of 238 females and 61 males. Each subject had OCT and T25FW measured at two time-points, separated by 1 to 3 years.

Results: A small but statistically significant negative relationship was found for RNFL and T25FW at first year (Spearman rho = -0.13, p = .049). The results were similar at the second time period (Spearman rho = -0.16, p = .009). Similar results were found after categorizing T25FW scores using benchmarks reported in the literature as being clinically meaningful, and comparing RNFL scores using ANOVA ($p \sim 0.02$, omega squared = .02 at both time periods). The mean T25FW delta score was not significantly lower from walk one to walk two (mean delta = 0.20 seconds, 95% CI =

-0.10 to 0.51), but RNFL delta score was significantly lower on the second test (mean delta = -1.7, 95% CI= -2.29 to -1.10).
Conclusions: This study examined the relationship between RNFL and T25FW over a follow of up to three years. Although a significant relationship was found between RNFL and T25FW, the effect was very small making it difficult to infer any clinical relevance from it. Larger cohorts and longer time frames are warranted to be able to establish significant relevance for clinical purpose.

P668
Relapsing inflammatory optic neuropathy: clinical description in a serie of 16 patients
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Background: Chronic relapsing inflammatory optic neuropathy (CRION), recently described, is characterized by subacute inflammatory optic neuropathy, bilateral often with prominent pain, and a clinical course with recurrences and remissions. The main feature is early response to systemic corticosteroids and recurrence on steroid withdrawal. The diagnosis requires that there not be a evidence of additional neurological deficit, sarcoidosis or systemic autoimmune disease.
Objectives: To perform a clinical description of CRION by analyzing a serie of patients with recurrent optic neuritis (ON).
Methods: A transversal study was carried out on patients who were selected for having recurring ON, at least two, after exclusion of demyelination and other autoimmune disorders. Clinical and demographic characteristics of the patients were analysed and described (age, sex, evolution period, number of recurrences, magnetic resonance imaging (MRI), oligoclonal bands, AQP4 antibodies, MOG antibodies, antinuclear antibodies (ANA), angiotensin converting enzyme (ACE) and treatment).
Results: Out of the 18 patients initially tested, 16 fulfilled the selection criteria. Median age: 32 (13-56) years old, 62.5% were women, the average evolution period was 7.7 years. Median of total number of ON in all patients was 3.5 (2-7). 68.6% had a recurrence on steroid withdrawal. IgG oligoclonal bands were detected in two patients. MRI was normal in 62.5%, MRI that were not normal not meet criteria for multiple sclerosis. ANA positivity was found only in one patient, ACE was normal in all of them. AQP4 antibodies were negative in all patients, and MOG antibodies were positive in 4 patients. At the present time, seven patients of this serie are being treated with immunosuppressive treatment (IS) or Intravenous immunoglobulin (IVIg), four of them had recurrences with another IS therapies before. Most patients received long-term and low dose corticosteroids.
Conclusions: No specific laboratory or clinical criteria have been described for the diagnosis of CRION. Clinical indicators and relapse on steroid withdrawal is helpful. Anti-MOG antibodies have not been investigated in CRION patients to date, to our knowledge. We assessed the presence of MOG-IgG antibodies in the serum of 16 patients with recurrent ON, and these are detected in 4 of them, suggesting that MOG antibodies test may help identify these patients.

P669
Multimodal clinical trial paradigm to assess neuroprotection in optic neuritis: baseline data
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Background: Recent basic science experiments suggest that acid sensing ion channel blockade by amiloride is neuro- and myeloprotective in models of MS. The Amiloride Clinical Trial in Optic Neuritis (ACTION) has been recruiting since April 2013. The aim is to assess the neuroprotective efficacy of amiloride in acute optic neuritis.
Objectives: To evaluate structural and electrophysiological correlates of the anterior visual system with clinical visual outcomes in the setting of acute optic neuritis, within a phase II neuroprotection trial.
Methods: Patients were recruited with a first episode of optic neuritis, and baseline assessments of vision, retinal nerve fibre layer thickness (RNFL), MRI brain and electrophysioslogy were performed.
Results: To date, 25 (76% female) participants have been recruited within a mean of 14.6 days from onset of visual symptoms. The presenting letter score visual acuity of the affected eye (mean ± SD) was 52.5 ± 30.4 letters, snellen equivalent of 20/100. RNFL as measured by scanning laser polarimetry (SLP) and optical coherence tomography (OCT), was significantly increased in the affected eyes (123 ± 46.8μ OCT, 57.4 ± 7.14μ SLP) compared to fellow eyes (99 ± 10.6μ OCT, 55 ± 6.3μ SLP) in both modalities (p0.01 OCT, p0.02 SLP). In our cohort, SLP thickness appears to have a significant positive correlation with letter score in affected eyes (r=0.537, p< 0.01). No comparable correlation was found in OCT measures.
All 25 patients had identifiable waveforms in pattern-electroretinogram (PERG). The N95 component was significantly lower (p< 0.01) in the affected eyes (4.1 ± 1.5μV) compared to unaffected eyes (5.0 ± 1.65μV). Pattern visual evoked potential (PVEP) waveforms were identified in 17/25 affected eyes. PVEP P100 time to peak was significantly (p< 0.01) delayed in the affected eyes (126 ± 19ms) compared to the unaffected eyes (103 ± 6.4ms). P100 amplitude was significantly lower (p< 0.01) in the affected eyes (8.3 ± 5.14μV) compared to unaffected eyes (14.0 ± 5.9 μV).
Conclusions: Initial analysis shows that PERG is more consistently recorded than the PVEP in acute optic neuritis. Inclusion of PERG increases electrophysiological data capture at baseline assessment in clinical trials of optic neuritis, and may provide useful structure-function information. SLP derived RNFL thickness, which is less confounded by swelling than OCT in the acute phase of optic neuritis, appears to have a significant correlation with vision in this preliminary analysis of our baseline data.

P670

Sector-specific macular volume compromise in relapsing & remitting multiple sclerosis as measured by optical coherence tomography

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Background: Optical coherence tomography (OCT) is a non-invasive imaging technique that can quantify the layers of the retina and subsequent the total macular volume (TMV), which contains all neuronal retinal tissue. OCT has become increasingly popular in ophthalmology and in neurology examining diseases such as Multiple Sclerosis (MS). In addition, OCT is recognized as a potential marker for axonal loss and neurodegeneration.
Objectives: To examine mean and sector macular volume in Relapsing-Remitting MS (RR-MS) patients with or without prior optic neuritis (ON), and compare the results to healthy controls.
Methods: OCT measures of a total of 622 eyes from 311 subjects were evaluated and partitioned into the following groups; MS ON: 218 eyes from 109 patients with MS and previous ON (46%) (MS affected), 256 eyes from 128 patients with MS and with no previous ON (54%) (MS unaffected), and healthy controls: 148 eyes from 74 healthy controls. MS patients were relapse free for at least 6 months prior to the studies. Using OCT spectral domain and specialized software, TMV as well as inner, outer and center sector volumes can be measured around the macula.
Results: TMV was significantly higher in control (8.6 ± 0.4 mm^3) eyes relative to MS unaffected (8.4 ± 0.4 mm^3) and MS affected eyes (8.1 ± 0.5 mm^3) ($p < 0.0001$). Inner macular volume was significantly higher in the control group (2.2 ± 0.1 mm^3) compared to MS unaffected (2.1 ± 0.1 mm^3) and MS affected groups (2.0 ± 0.1 mm^3) ($p < 0.0001$). Similarly, the outer macular volume was significantly higher in the control eyes (6.3 ± 0.3 mm^3) relative to MS unaffected (6.1 ± 0.3 mm^3) and MS affected eyes (5.9 ± 0.3 mm^3) ($p < 0.0001$). Controls were compared to MS unaffected and MS affected eyes, all eight sector volumes were significantly lower ($p \leq 0.013$ and $p \leq 0.0001$ respectively) in the MS groups. In addition, all individual sector volumes in the MS affected eyes versus the MS unaffected eyes ($p \leq 0.003$) reached significance.
Conclusions: TMV and all the sector volumes are reduced in RR-MS eyes compared to controls, and more so in the MS patients with prior history of ON. In addition, ON findings are consistent with a uniform neurodegeneration in the perimacular retina. Future OCT studies may focus on the TMV as well as the individual sector volumes as sensitive measures of neurodegeneration in MS and ON.

P671

The utility of optical coherence tomography in acute monocular visual loss: is it optic nerve or retina?

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Background: Acute monocular visual loss is common in multiple sclerosis (MS). Differential diagnoses include optic neuritis (ON), arteritic or non-arteritic ischemic optic neuropathy (AION/NAION), and central or branch retinal artery occlusion (CRAO/BRAO). Distinguishing ON from BRAO may be challenging because acute retinal whitening can be subtle and transient and optic atrophy may develop chronically from retrograde axonal degeneration.
Objectives: We present two patients referred to our center for optic neuropathy where spectral-domain optical coherence tomography (SD-OCT) provided vital clues to help make the diagnosis of BRAO.
Methods: Exam, fundoscopy and vision testing were performed by a neuro-ophthalmologist. Visual fields (VF) and SD-OCT were performed by a trained technician. Careful evaluation of RNFL thickness, macular volume and retinal layer thickness using manually corrected automated segmentation algorithms was performed.
Results:
Case 1: A 52 year old man with type I Gaucher's disease and acute monocular visual loss in his left eye was referred with a diagnosis of optic neuritis. Examination revealed reduced visual acuity, color impairment, a paracentral VF defect and a normal appearing optic nerve. Static perimetry showed superior paracentral scotoma. SD-OCT imaging (Cirrus; Zeiss) showed temporal inferior macular elevation consistent with acute BRAO. MRI of the brain and orbits revealed a left internal carotid dissection.
Case 2: A 55-year old man with a diagnosis of NAION and history of a thoracic dissecting aortic aneurysm 2 years prior was referred for evaluation of optic disc atrophy and a superior nasal defect in the left eye. Exam revealed normal acuity with a left afferent pupillary defect and segmental atrophy of the lower part of the left optic nerve. SD-OCT(Spectralis; Heidelberg) showed marked thinning of the inferior temporal macula and inner layer architecture disruption. Using proprietary segmentation software, severe loss of ganglion cell, inner plexiform, and bipolar cell layers in the inferior temporal macula was observed. Strikingly, the photoreceptor layer thickness was intact. These findings on OCT were most consistent with left BRAO, not NAION.
Conclusions: OCT imaging of macular layers can help distinguish optic neuropathy, a common finding in MS, from a primary retinal process for which the treatment and patient management are drastically different. It is important to obtain images of both the RNFL of MS patients and the macula to rule out retinal causes of acute vision loss.

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Vogt Koyanagi Harada disease, our experience

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Background: Vogt Koyanagi Harada disease (VKHD) is a rare autoinmune disease in which the main target are melanocytes, mainly ones in the uvea and the central nervous system (CNS). The clinical presentation is characterized by uveitis, aseptic meningitis, hearing loss and sometimes vitiligo and poliosis. It has no specific markers and white matter lesions are found in neuroimaging. The treatment of choice is immunosupresive therapy.

Objectives: We present three clinical cases, two men aged 53 and 55 and a woman aged 52 with visual loss that were diagnosed of uveitis.
Methods: The studies performed were not suggestive of an autoinmune disease nor of sarcoidosis. The woman had had a previous event of severe headache and the eldest of both men had been suffering from a vertiginous syndrome for the previous months. Two of them had pleocytosis in cerebrospinal fluid and all three showed white matter lesions in magnetic resonance imaging (MRI).
Results: Once VKHD was suspected corticosteroids were initiated with clinical improvement. The woman and the eldest man developed relapsing forms, whilst the latter required initiating azathioprine. Only the eldest man has developed a severe visual loss and has an ongoing vertiginous syndrome.
Conclusions: The importance of an early diagnosis of VKHD is underlined by the fact that uveitis can exert a catastrophic effect on the visual function, yet the lack of markers make it a difficult task. Other diseases that affect the visual pathway and the central nervous system like multiple sclerosis (MS) or Cogan disease should be ruled out. In any patient with aseptic meningitis, white matter lesions and hearing loss or vertigo, VKHD should be suspected.

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Comparing two and three way receiver operating characterization (ROC) analyses for optic neuritis transfer function characterization

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Background: Optic neuritis (ON) represents a single-lesion model to explore multiple sclerosis (MS). Functional magnetic resonance imaging transfer function (fMRI-TF) measures the resting state (no-activity) gain between different brain regions that are correlated in visual activity. fMRI-TF offers a route to generate useful markers for identifying the cortical contribution to recovery from ON.
Objectives: To evaluate the predictive capability of fMRI-TF markers applied in (1) a two analysis to distinguish between healthy persons and ON patients; and (2) a three way analysis to distinguish between healthy persons, ON patients with relapsing remitting MS (RRMS) and ON patients with a clinically isolated syndrome (CIS).
Methods: In this prospective cohort study, resting state fMRI data was collected from 21 patients (11 CIS; 10 RRMS) and 12 age-matched healthy volunteers using T2*-weighted echo planar fMRI images. A feed-forward propagation of visual information was assumed, between the lateral geniculate nuclei (LGN) and the primary visual cortex (V1) so that fMRI-TF = DFT(V1) / DFT(LGN) where DFT is the discrete Fourier transform. Power spectral ratios were calculated for several frequency bands. Preliminary identifiers for the best transfer function metrics were A_z and *VUS;* respectively, the area and volumes under the two- and three-way receiver operating characteristics (ROC) curve and surface.
Results: The ROC analysis showed significant differences between ON patients and healthy persons.The results for LGN to V1 signal propagation in resting state eyes closed condition for 0-0.1Hz power transfer are reported here. Two way ROC analysis showed power transfer greater in controls than patients ($A_z = 0.19$, negative predictor less than 0.5). Power transfer was highest in healthy subjects, followed by patients with MS and then patients with CIS in the three way ROC analysis for LGN to V1 propagation in the 0-0.1Hz region. However the three way ROC differentiates patient groups a predictive level of $VUS = 0.46$, less than the two-way.
Conclusions: Both two way and three way ROC results suggest that ON TF analysis has potential in identifying ON presence. Methods, such as artificial neural networks, are needed to combine fMRI-TF results from multiple visual pathways, to boost transfer function metric accuracy. Bootstrap analysis approaches are being used to provide reliability estimates. The current baseline results are to be compared to 6 month results to determine if fMRI-TF measures can track ON recovery.

P674

25-hydroxyvitamin D levels in acute optic neuritis. Relation to paraclinical findings, demographic characteristics and risk of MS

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Background: The importance of vitamin D in development of multiple sclerosis (MS) is becoming increasingly accepted. Optic neuritis is a common first symptom of MS and only few studies have so far investigated vitamin D at this early stage of the disease.
Objectives: To examine 25-hydroxyvitamin D (25HVITD) levels in patients in the acute phase of optic neuritis (ON) with comparisons to a general MS population and to determine whether 25HVITD levels in acute ON are associated with paraclinical findings (MRI and CSF findings) suggestive of MS and with risk of RRMS.
Methods: A cross-sectional study of 25HVITD levels in ON (n=165) and MS (n=948) with retrospective follow-up of the ON patients. Mean 25HVITD differences and differences in prevalence of 25HVITD deficiency (< 50nmol/L) between ON and MS (two-sample t-test, chi-square test) were examined. Associations between 25HVITD levels, paraclinical findings and demographic characteristics in ON patients (logistic regression) and hazards of MS development (cox regression analysis) were assessed. In all analysis deseasonalized 25HVITD levels were employed and adjustment was made for possible confounders.
Results: Mean levels were 47.7 (ON) and 63.9 (MS) nmol/L ($p< 0.0001$) and a significantly higher prevalence of 25HVITD deficiency in ON (56 % vs. 35%)($p< 0.0001$), most pronounced in females, was shown. In the acute ON 25HVITD levels were associated with increased odds of paraclinical findings suggestive of MS i.e. MS-type lesions on MRI (OR: 0.984 [95 % CI:

0.970-0.998], p:0.037) and presence of elevated igG index in CSF (OR: 0.979 [0.961-0.997], p:0.018) although not significant with regards to presence of oligoclonal bands (OR:0.990 [0.976-1.005], p:0.208). 23 ON patients developed MS during the study. Univariate and multivariate survival analysis showed a trend towards decreased hazard of MS with increasing 25HVITD levels albeit insignificant (HR: 0.987 [0.968;1.006], p:0.202 and HR: 0.988 [0.969;1.007], p:0.217 respectively).
Conclusions: The present study shows for the first time in patients in the acute phase of ON an association between vitamin D levels and paraclinical findings suggestive of MS. Also shown is a high prevalence of 25HVITD deficiency indicating low 25HVITD levels in ON although the latter could not confirmed due to lack of a healthy control group. No significant association between 25HVITD levels and risk of MS was shown in acute ON. The study indicates a possible role of vitamin D in the early stages of MS.

P675
Optic nerve head volume as a marker for neuronal damage after optic neuritis in multiple sclerosis and neuromyelitis optica
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Background: Optic neuritis in multiple sclerosis (MS) and neuromyelitis optica (NMO) leads to thinning of the retinal nerve fiber layer (RNFL). Whereas MS is thought to be a T cell mediated disease against myelin, NMO's pathophysiologic hallmark are antibodies against aquaporin-4, an astrocytic water channel. Within the retinal nerve fiber layer astrocytes are mainly concentrated in the optic nerve head (ONH), which lead us to hypothesize that the optic nerve head volume and the peripheral retinal nerve fiber layer might be differentially affected in both diseases.
Objectives: To compare ONH volume and RNFL thickness as markers for optic neurodegeneration in eyes with a history of optic neuritis in NMO and relapsing-remitting MS (RRMS).
Methods: Seventy-one patients with a history of optic neuritis (49 RRMS and 22 NMO) and 39 healthy controls underwent retinal optical coherence tomography. RNFL was measured with a standard peripapillary ring scan. ONH volume (ONHV) and ONH excavation volume (ECV) were determined from 3D ONH volume scans with a custom protocol (145 B-scans, scanning angle of 15°x15°, 384 A-scans per B-scan) and a custom built algorithm. Statistical analysis was performed with Generalized Estimating Equation models (GEE) and linear regression models (LR).
Results: ONHV was significantly reduced in patients' eyes with a history of optic neuritis in comparison to patients' eyes without in MS (B=0.295, p< 0.001, GEE) and NMO (B=0.700, p< 0.001, GEE). Similarly, ECV was significantly increased. ONHV was significantly stronger affected in ON eyes from NMO patients than in MS (B=0.278, p=0.030, GEE). ONHV correlated well with RNFL in MS (r=0.601, p< 0.001, LR) and NMO (r=0.623, p< 0.001, LR). Neither association differed significantly in offset or slope. However, in NMO three eyes showed a higher ONHV in comparison to RNFL than would have been expected from the models. Two of these eyes were suspect for papilledema, one eye had a history of ON three months prior to measurement.
Conclusions: The relation between ONHV and peripheral RNFL affection did not differ between NMO and MS patients' eyes. However, ONHV might be more or temporally differently affected from swelling during episode of ON than RNFL. This suggests ONHV in conjunction with RNFL as a potentially sensitive marker to detect subclinical or subacute ON in both diseases.

P676
Optic neuritis associated with multiple sclerosis: VEPs sensitive in acute phase, OCT useful in chronic phase
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Background: Visual involvement is a frequent feature of Multiple Sclerosis.
Objectives: To evaluate the relative value of optical coherence tomography (OCT) and visual evoked potentials (VEPs) in assessing visual involvement in patients with multiple sclerosis (MS).
Methods: Cross-sectional study of 121 consecutive subjects with MS. Of 242 eyes, 166 had no previous history of optic neuritis (ON), 22 had a single recent ON episode (< 3 months); 54 had chronic ON (at least 1 episode >3 months before). All patients underwent assessment of EDSS, visual acuity (VA), OCT retinal nerve fiber layer (RNFL) thickness and VEP.
Results: In eyes with recent ON, the sensitivity of OCT was 5.6% considering only RNFL thickness increase, 38.9% considering also RNFL reduction, with a higher sensitivity of VEP (77.3%; McNemar p< 0.0001 and 0.02). In eyes with chronic ON, no significant difference was found between OCT (68.5%) and VEP (81.5%) sensitivity (VEP/OCT 88.9%). In asymptomatic eyes, VEPs had a higher sensitivity (31.7%) vs OCT (19.9%; p=0.005); VEP/OCT combined detected abnormalities in 39.2%. In this subgroup, VEP score and global RNFL thickness were significantly correlated with EDSS, disease duration, not with VA.
Conclusions: The present findings confirm a higher sensitivity of VEPs in the subacute phases of optic neuritis and in asymptomatic eyes. This discrepancy fades off after more than 3 months from the ON episode. Finally, the correlation with disability and DD favours the usefulness of both techniques in monitoring MS patients, to be verified through longitudinal studies.

P677
Optical coherence tomography after first optic neuritis for the differentiation between neuromyelitis optica and multiple sclerosis
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Background: Retinal nerve fiber layer thickness (RNFLT) measured by optical coherence tomography (OCT) has been suggested to be useful in discrimination between neuromyelitis optica (NMO) and multiple sclerosis (MS). However, multiple episodes of optic neuritis (ON) result in cumulative severe reduction of RNFLT, making differentiation of these two diseases difficult.
Objectives: In this study, we compared the visual functions and retinal thickness measure by OCT in the eyes with single episode of ON in NMO or MS to discriminate the diseases in early phase.
Methods: Total 111 subjects (73 patients with NMO and 38 patients with MS) without recent ON events within 3 months underwent neuro-ophthalmic evaluation, including best corrective high contrast visual acuity (HCVA), low contrast visual acuity (LCVA), RNFLT, and total macular volume (MV) by optical coherence tomography (OCT).
Results: There were 60 eyes of NMO and 33 eyes of MS with single episode of ON among 101 affected eyes of NMO and 38 affected eyes of MS. With single episode of ON, HCVA and RNFLT were decreased significantly more in NMO than MS ($p < 0.001$). However, when NMO eyes with single episode of ON and MS eyes with multiple episodes of ON were compared, HCVA and RNFLT were not significantly different. In a patient after first episode of ON, RNFLT of less than 78.9 μm suggests the possibility of NMO with 93.9% specificity, and RNFLT of less than 78.9 μm with HCVA of less than 0.35 decimal were 100% specific for NMO.
Conclusions: Evaluation of eyes with single episode of ON has an advantage on detecting the differences of insult on optic nerves by NMO or MS because multiple episodes of ON obscure the disease specific changes. There was significantly more severe axonal injury in optic nerves caused by NMO than MS after the first episode of ON, which could establish cutoff value. This study suggests that peripapillary RNFLT value and HCVA after first episode of ON can help us to differentiate ON of NMO from MS in early phase.

P678

Longitudinal time-domain optic coherence study of retinal nerve fiber layer of IFNβ-treated and untreated MS patients

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Background: Quantification of the retinal nerve fiber layer by optical coherence tomography (OCT) is proposed to provide an indirect appraisal for retinal axonal loss.
Objectives: This prospective longitudinal OCT study was aimed to find evidence whether interferon beta (IFNβ) treatment impedes retinal axonal loss.
Methods: We enrolled a total of 96 eyes from 48 patients with multiple sclerosis (48 IFNβ-1b-treated and 48 untreated eyes) and 24 eyes from 12 healthy controls. OCT measurements were performed at baseline, and at 3-, 6-, and 12-month follow-up. At all visits, we additionally performed full-field visual evoked potential (VEP), the Paced Auditory Serial Addition Test (PASAT), and Expanded Disability Status Scale (EDSS). We used a cubic polynomial regression model to adjust for disease duration and total number of relapses. By means of treatment duration an ideal treatment group was defined.
Results: Over a period of one year, we observed a decrease by 2.47 μm in untreated and 1.66 μm in ideal IFNβ-treated MS eyes. Clinical parameters showed only inconsistent (EDSS, PASAT 3", VEP amplitudes) or no correlations (total number of relapses, PASAT 2", cup to disc ratio). Reliable negative correlations were found between RNFL thickness and VEP latencies.
Conclusions: Our data support a protective role for IFNβ to impede thinning of the RNFL, probably by preventing MS relapses. However, since the pathophysiological dynamics in RNFL decrease are still largely unknown, longitudinal studies that comprise a larger time frame are warranted to assign differences in RNFL loss to the effect of a drug.

Neuropathology

P679

Macrophage/microglia differentiation in slowly expanding lesions of progressive MS

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Background: Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system. The exact pathological basis of disease progression has not yet been defined. Slowly expanding white matter lesions were previously described as one characteristic pathological feature of progressive MS. These lesions reveal a distinct accumulation of macrophages/microglia cells at the lesion border with slowly ongoing myelin degradation and axonal degeneration.
Objectives: The present study aimed at characterizing the immunological and molecular profile of macrophages/microglia at the lesion border of slowly expanding lesions with respect to their M1/M2 differentiation.
Methods: Immunohistochemical analysis of a range of slowly expanding white matter lesions from MS autopsy cases with progressive MS was performed. Slowly expanding lesions were identified by accumulation of macrophages/microglia using the pan macrophage/microglia marker Ki-M1P. Ongoing myelin breakdown was demonstrated by the detection of myelin degradation in macrophages/microglia using conventional LFB-PAS staining or immunohistochemistry for myelin proteins (MBP). Macrophages/microglia were characterized with markers indicating either M1 (iNOS, CD40) or M2 (CD163, CD206) differentiation.
Results: Quantitative evaluation revealed a preferential M1 differentiation of macrophages/microglia in slowly expanding lesions.
Conclusions: These data suggest that more damage-associated phenotypes of macrophages/microglia may play a crucial role in the expansion of white matter lesions during the progressive disease stage. Further experiments will aim at defining the molecular expression profile in slowly expanding lesions.

P680

Meningeal inflammation affects the balance of TNF signalling in cortical grey matter in progressive multiple sclerosis

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Background: Neuroimaging and neuropathology studies indicate that accumulating grey matter (GM) pathology is the best correlate of clinical progression in multiple sclerosis (MS). Recent studies of cortical pathology in secondary progressive MS (SPMS) have shown that a more severe clinical course and the presence of extensive subpial GM lesions (GMLs) with significant neuronal/glia loss and microglial activation are associated with diffuse inflammation and lymphoid-like structures in the subarachnoid space.
Objectives: We investigated the hypothesis that inflammatory/cytotoxic molecules diffusing from the meninges lead to pathological changes in signalling pathways within the underlying GM that could explain the extensive pathology.
Methods: By using Illumina HumanRef8 Beadchip arrays, we defined differentially expressed genes/pathways in subpial GMLs and normal appearing GM (NAGM) of the motor cortex from 20 post-mortem MS brains with and without meningeal inflammation and 10 non-neurological controls, previously characterised for cellular pathology (Magliozzi et al, Ann. Neurol. 2010). Real time RT-PCR was used to verify changes in gene expression, while Western-blot analysis and immunohistochemical techniques (IHC/IFC) were performed to validate the expression and cell source of molecules of interest.
Results: Gene expression profiling of GMLs and NAGM not only confirmed the substantial GM pathological cell changes, but also demonstrated the upregulation of multiple genes/pathways associated with the inflammatory response. In particular, genes involved in TNF mediated apoptosis/survival signalling were found to be significantly deregulated in MS cases compared to controls. In particular, increased meningeal inflammation was associated with a shift in the balance of TNF signalling away from the TNFR2 and NFkB anti-apoptotic pathway towards TNFR1 mediated RIP1/RIP3 dependent pro-apoptotic and necroptotic signalling. Western blot analysis confirmed the differential protein expression of TNFR1/TNFR2 in the GM of SPMS linked to meningeal inflammation. Furthermore, IHC/IFC showed the expression of TNFR1 predominantly by oligodendrocytes and neurons and of TNFR2 mainly by astrocytes in the same GM areas and MS cases.
Conclusions: These results support the hypothesis that the inflammatory milieu, generated in the subarachnoid space by leptomeningeal immune cell infiltration, has a fundamental role in the genesis of subpial GM pathology in progressive MS and that TNF may be a key mediator of cortical tissue damage.

P681

The relationship between axonal loss and demyelination in the MS spinal cord

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Background: Multiple sclerosis (MS) is a chronic inflammatory and neurodegenerative disease affecting the whole central nervous system (CNS). Limb dysfunction due to damage and loss of cortico-spinal tracts (CSTs) axons is common among people with MS (pwMS). The relationship between demyelination and CST axon remains unclear, possibly due to variation in sampling techniques.
Objectives: To accurately quantify the loss of CST axons in the MS spinal cord and assess its relationship with demyelination.
Methods: Formalin fixed spinal cords of nine people with secondary progressive MS (5 women and 4 men, age = 62 ± 3 years, disease duration= 24 ± 3 years) and three reference cases (2 women and 1 man, age= 84 ± 8 years) with no known neurological disease were studied. Spinal cords were dissected into ≈0.5 cm thick axial tissue blocks across the entire length (total no. of blocks = 294). Sequential 10μm-thick sections were stained for myelin basic protein (MBP) to identify demyelinated areas and SMI-31 to identify axons using established protocols. Images of axons in four microscopic fields (40x), randomly cast on each lateral CST area, were acquired and then quantified using ImageJ software. The density of axons in each CST was estimated bilaterally as the density of axons inside the counting fields multiplied by the corresponding cross-sectional CST area in mm^2.
Results: Reduction in CST axonal density in pwMS was 62% ($p < 0.0001$), 49% ($p < 0.0001$), and 50% ($p = 0.0018$) at cervical, thoracic and lumbar level respectively. The percentage of demyelinated grey matter (GM) was significantly higher than in white matter (WM): 26% and 11% ($p = 0.012$), 47% and 12% ($p < 0.0001$), 13% and 3% ($p = 0.032$) at cervical, thoracic and lumbar level, respectively. A moderate negative correlation was detected between the density of axons and the extent of demyelination at cervical level only ($r= -0.2456$, $p= 0.05$).
Conclusions: Comprehensive sampling is required to draw more definitive conclusions about the relationship between major components of pathology in the MS spinal cord. We observed axonal loss of at least 49% throughout the spinal cord, however only at the cervical level was with loss - moderately - associated with demyelination. Wallerian or tract specific degeneration may explain lack of association at lower cord levels. Further work is underway to explore these mechanisms and to correlate these findings in the spinal cord with cortical demyelination and neuronal loss in the brain.

P682

Astrocytes upregulate interleukin-17 receptor expression in white matter lesions in multiple sclerosis

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Background: Interleukin-17 (IL17) is a pro-inflammatory cytokine, produced by T helper 17 cells, that has recently attracted attention for its putative role in disease pathogenesis in multiple sclerosis (MS). In humans, post-mortem tissue studies in MS have shown expression of IL17 in areas of active demyelination. However, the target cells of IL17 in the brain in MS have not yet been identified.
Objectives: To identify the cells that express IL17 receptor A (IL17RA) and IL17 receptor B (IL17RB), and to study their distribution, in post-mortem brain tissue in MS and non-MS controls.
Methods: 52 post-mortem brain tissue blocks from 21 patients with MS and 5 non-MS controls were studied using immunohistochemistry. The phenotype of IL17R+ cells was determined using double immunofluorescence; using primary antibodies against IL17RA, IL17RB, MOG, Iba-1, GFAP, S100B, Olig-2, CD8, CD20, CD3, NeuN, and NF-L. The density of IL17-R+ cells was then compared between active demyelinating lesions, chronic-active demyelinating lesions, inactive demyelinating lesions, normal appearing white matter, grey matter lesions, and normal appearing grey matter.
Results: Abundant IL17R+ cells were found in MS white matter, but not in non-MS controls ($p< 0.01$). The vast majority of these cells were S100B/GFAP+ astrocytes. The distribution of IL17R+ astrocytes matched exactly the distribution of white matter lesions. The number of IL17R+ astrocytes was greatest in active demyelinating lesions, followed by chronic-active lesions, followed by inactive lesions, with very few found in normal appearing white matter ($p< 0.01$). IL17R+ astrocytes were not present in grey matter lesions, or in normal appearing grey matter. Surprisingly, IL17R+ staining was also observed in occasional axons in the most actively demyelinating white matter lesions. IL17R+ neuronal cell bodies were present in both grey matter lesions and normal appearing grey matter, in both MS cases, and non-MS controls.
Conclusions: This study provides evidence that astrocytes are the main target cells for IL17 in the MS white matter lesion. Moreover, the distribution of IL17R+ astrocytes appears closely related to the demyelinating and inflammatory activity, suggesting a role in the pathophysiology. In addition, this study provides evidence of IL17R staining on neurons. The precise function of IL17 signalling in the MS white matter lesion requires further study in order to determine the potential effects of anti-IL17 therapeutics.

P683
Induction of ion channel and transporter transcripts in normal appearing grey matter of chronic multiple sclerosis patients
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Background: Although grey matter (GM) pathology correlates with the progression of multiple sclerosis (MS), the question remains how does GM pathology arise? Several pathological mechanisms have been proposed, like meningeal inflammation and glutamate excitotoxicity. In addition many genes involved in synaptic plasticity and glutamate neurotransmission are dysregulated in MS. However the precise signaling pathways involved in these pathological processes are still relatively unclear.
Objectives: We investigated underlying signaling pathways of neurodegeneration, including abnormalities in ion channels and transporters, neurotrophins and receptors, and synaptic plasticity, in normal appearing grey matter (NAGM).
Methods: Tissue blocks from MS and control cases were obtained after rapid autopsy. Based on anti-proteolipid protein (PLP) immunohistochemistry, frozen normally myelinated NAGM cingulate cortex from 5 chronic MS cases and 5 controls cases were selected for real time quantitative PCR arrays. Three RT^2 profiler PCR arrays (Qiagen) were used to examine mRNA levels of genes involved in ion channels & transporters, neurotrophins & receptors and synaptic plasticity. Data was normalized with housekeepings genes with the most consistent expression in all samples and significant differences in gene expression were investigated using Student's t-test.
Results: Expression of genes coding for ion channels and transporters were markedly increased. They include glycine transporter type 1 (Fold Regulation=2.1, $p=0.03$); chloride channel 7 (FR=2.9, $p=0.04$); potassium channel 4 (FR=6.9, $p=0.002$), and potassium inwardly rectifying channel 11 (FR=4.4, $p< 0.001$). In contrast, expression of several genes involved in synaptic plasticity was consistently downregulated, including JUNB (FR= -2.2, $p=0.2$), FOS (FR=-3.0, $p=0.06$) and PRKG1 (FR=-1.4, $p=0.01$). PRKG1, also known as cGMP dependent protein kinase type 1 has been shown to promote long-term potentiation (LTP) via CREB phosphorylation, probably by increasing intracellular Ca2+. Interestingly, neurotrophins and neurotrophin receptors were not differently regulated in MS cortex compared to controls.
Conclusions: So far, the qPCR array results suggest a downregulation of genes involved in synaptic plasticity and an upregulation of genes encoding ion channels and transporters in NAGM. The expression of neurotrophins and receptors remain unchanged. Currently, experiments are ongoing to validate the data on protein level.

P684
Podoplanin is expressed in multiple sclerosis meninges and perivascular infiltrates and regulates T-cell proliferation and Th17 differentiation
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Background: The transmembrane glycoprotein podoplanin (PDPN) plays a critical role in the development of multiple organs

including the lymphatic system and in the biology of immune cells. In multiple sclerosis (MS), formation of ectopic meningeal lymphoid follicles is associated with accelerated neurodegeneration and disability progression. Recent work in EAE mice suggests that PDPN is necessary for ectopic lymphoid follicle formation and is specifically expressed on Th17 cells. These findings suggest a critical but unknown role for PDPN in multiple sclerosis.

Objectives: To characterize

(1) expression of PDPN and its ligands in MS autopsy tissue and
(2) function of PDPN in human lymphocytes.

Methods: Paraffin-embedded sections of MS and other neurological disease brain were immunohistochemically labeled with antibodies against PDPN and its ligands CLEC-2 and galectin-8, and with markers for immune cells and myelin. For *in vitro* experiments, peripheral blood mononuclear cells from healthy controls were isolated by Ficoll density centrifugation, and naïve CD4+ T cells were sorted by FACSAria. Cells were cultured with αCD3, αCD28, IL-1β, TGF-β, IL-6, IL-23 for 1 week with or without CLEC-2 or sodium chloride. Flow cytometry, qRT-PCR, and ELISA were performed.

Results: PDPN was present in MS in meningeal and perivascular infiltrating lymphocytes and meningeal CD34+ stromal cells. The PDPN ligands CLEC-2 and galectin-8 were expressed by a subset of T and B cells. *In vitro*, PDPN was expressed on a sub-population of CD4+ IL-17A- IFN-g- T cells under Th17 polarizing conditions. Stimulation of these cells *in vitro* with CLEC-2 promoted proliferation and a shift away from a Th17 phenotype. We have previously shown that salt promotes Th17 induction and may represent a risk factor for MS. Addition of salt to Th17 cultures decreased PDPN expression, further suggesting a reciprocal relationship between IL-17 and PDPN.

Conclusions: Our results suggest that PDPN and its ligands are expressed in MS brain, where they may contribute to ectopic follicle formation. Moreover, CLEC-2 signaling through PDPN enhances T cell proliferation and decreases Th17 differentiation. Thus, in inflamed CNS, CLEC-2 and galectin-8 may signal through PDPN to regulate Th17-associated inflammation. Our work suggests a dual mechanism for PDPN that could be exploited therapeutically for the treatment of MS patients.

P685

Epigenetic changes control memory function following demyelination in multiple sclerosis

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Background: Multiple Sclerosis (MS) is an immune-mediated demyelinating disease of the human central nervous system. Among a wide array of symptoms, 30-40% of MS patients demonstrate memory impairment.

Objectives: To investigate possible mechanisms of memory impairment in MS patients, we compared morphological and molecular changes in MS hippocampus. We previously reported that myelin status altered expression of mRNAs associated with axonal transport, neurotransmission and memory function as well as microRNAs which target these genes in MS and rodent model of demyelination.

Methods: Epigenetics defines modulation of gene expression in a manner that is not dependent on changes in DNA sequence, and is a term widely used to describe mechanisms of transcriptional and translational regulation within the cell. DNA methylation is a well-studied mechanism of epigenetic gene regulation, and has been associated with several neurological diseases. We compared global methylation and mRNA profiles to determine if epigenetic modifications alter gene expression in MS hippocampus.

Results: Genes encoding the DNA methyl transferases (DNMTs) were decreased significantly following demyelination in MS hippocampus. Global profiling and analysis of DNA methylation sites has revealed several targets including key genes involved in neuronal survival, synaptic plasticity and remyelination. mRNA targets of altered methylation were validated in both human tissue and rodent model of demyelination.

Conclusions: Taken together, our results provide evidence of complex epigenetic interactions in MS hippocampus following demyelination.

P686

***Clostridium perfringens* epsilon toxin: a model for the newly forming MS lesion**

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Background: The newly forming Multiple Sclerosis lesion is remarkably specific and characterized by blood-brain barrier breakdown, oligodendrocyte death, relative preservation of myelin and an absence of adaptive immune infiltrates. Since the majority of MS lesions surround a central venule, the concept that MS lesions begin from a blood born toxin has existed since the time of early histologists.

Objectives: We propose *Clostridium perfringen*s epsilon toxin as a candidate toxin responsible for new lesion formation in MS and in this study sought to provide mechanistic evidence in support of this hypothesis.

Methods: *C. perfringens* epsilon toxin has previously been shown to cause disruption of the blood brain barrier and in other work from our lab we provide evidence showing the specificity of epsilon toxin for CNS endothelial cells. In this study we examined epsilon toxin binding and toxicity in primary CNS cultures and in organotypic cerebellar slice explants.

Results: In primary cultures of enriched mouse oligodendrocytes, astrocytes or microglia, specific binding of epsilon toxin only occurs with oligodendrocytes. Similarly, epsilon toxin is a potent inducer of oligodendrocyte cell death in a dose dependent fashion but has no effect on astrocytes or microglia. The effects of epsilon toxin on the oligodendrocyte lineage are dependent on maturation as epsilon toxin neither binds nor is toxic to oligodendrocyte progenitor cells. Cerebellar slice explants maintain the normal cytoarchitecture of the CNS including spatial and temporal patterns of myelination. Fully myelinated cerebellar explants treated with epsilon toxin show dose and time dependent specific death of

oligodendrocytes and demyelination with preservation of neurons, astrocytes and microglia. Neutralizing antibodies to epsilon toxin protect against toxin mediated oligodendrocyte death and demyelination.

Conclusions: In the CNS *Clostridium perfringens* epsilon toxin specifically targets oligodendrocytes and myelin leading to demyelination with preservation of other neural elements. In conjunction with its specificity for CNS endothelial cells, the actions of epsilon toxin are fully compatible with the know targets identified in newly forming lesions: blood-brain barrier and oligodendrocytes. Since epsilon toxin enters the CNS from the blood stream this pathophysiologic step is consistent with the vasocentric concept of new lesion formation.

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Postmortem MRI to guide pathological localization: individualized, 3D-printed cutting boxes for fixed brains

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Background: Interfacing MRI and pathology is critically important to understand the pathological basis of MRI signal changes in vivo and for clinicopathological correlation. Postmortem MRI is an intermediate step in this process, but unfortunately relating the data to standard hand-cut pathological sections, which are relatively thick and often non-parallel, is both time-consuming and insufficiently accurate. This represents a major limitation in performing radiology-pathology correlational studies.

Objectives: To develop technology to integrate postmortem, high-resolution, whole-brain MRI into the planning and execution of the pathological analysis through precise localization of the target and coordinates of cut.

Methods: We built customized, 3D-printed cutting boxes for formalin-fixed whole-brains. According to specific findings of interest, the position, orientation, and thickness of each slab were determined a priori and designed using 7tesla postmortem MRI images. Three brains were donated for research purposes (two patients affected by multiple sclerosis and one by anti-NMDA-r encephalitis): two were sectioned using customized cutting boxes (Pat1 and 3) and, for comparison, one brain underwent the standard pathological sectioning (Pat2).

Results: There was a marked improvement in uniformity of thickness (6mm vs.~1cm) and skewness of the slabs obtained with the cutting box compared to the traditional sectioning method: the match with the MRI was judged to be not accurate, respectively, in ~25% (Pat1 and 3) vs. 86% slab surfaces (Pat2). Outstanding results were achieved with a cutting box customized for the brainstem-cerebellum (Pat1). MRI targets of interest were correctly localized after the cutting box-guided sectioning and characterized histologically for further research purposes (demyelinated lesions in Pat1 and hippocampi in Pat3).

Conclusions: Compared to standard pathological sectioning, the use of individualized, 3D-printed cutting boxes, designed based on postmortem MRI of fixed whole-brains, improves the speed, quality, and accuracy of radio-pathological correlation, and specifically the histopathological localization of imaging findings. This technology is easily implemented and applicable to any brain disorder. From the point of view of the pathologist, this technique can improve localization of small abnormalities, whereas from the point of view of the radiologist, it has the potential to improve understanding of MRI signal changes observed in disease.

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S100B levels are increased in multiple sclerosis and modulates demyelination, glial reactivity and inflammation

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Background: S100B was identified in cerebrospinal fluid (CSF) and post-mortem brain tissue of multiple sclerosis (MS) patients and correlated with glia reactivity upon demyelination. Although S100B is seen as an astrogliosis marker it is also expressed by maturing oligodendrocytes and is necessary for their differentiation into myelinating cells. Interestingly, S100B has neurotrophic properties at low levels, but its increase upon injury may induce glial reactivity and tissue damage.

Objectives: We aimed to unravel the role of S100B as a biomarker and therapeutic target in MS.

Methods: S100B levels were detected in cerebrospinal fluid (CSF) of MS patients by ELISA and S100B and its receptor RAGE expression were analyzed in post-mortem samples of MS patients by immunohistochemistry. We induced demyelination in cerebellar organotypic slice cultures (COSC) using lysophosphatidylcholine (LPC) and evaluated the release of S100B by ELISA. S100B action was inhibited by anti-S100B, the extent of demyelination and glial reactivity were assayed by immunohistochemistry and expression of inflammatory mediators HMGB1 and IL-18 by qRT-PCR.

Results: S100B was significantly elevated in CSF samples from MS patients at diagnosis (>1.6-fold, $p < 0.05$, Mann-Whitney test). Active MS lesions showed increased S100B and RAGE expressions, while chronic lesions displayed S100B expression in demyelinated areas with a much lower expression of RAGE in the rim. Interestingly, reactive astrocytes were identified as the predominant cellular source of S100B, whereas RAGE was mainly expressed by activated microglia/macrophages in active lesions. Treatment of COSC with LPC induced a marked elevation of S100B (~5-fold, $p < 0.01$), which co-localized mostly with astrocytes. Inhibition of S100B action reduced LPC-induced demyelination by more than 20% ($p < 0.01$), prevented astrocyte reactivity

and promoted microglia migration to demyelination areas. In parallel, anti-S100B abrogated the expression of HMGB1 and IL-18 ($p< 0.01$) to control levels.
Conclusions: Our data suggest that S100B may be a potential new biomarker for MS diagnosis given its high production during disease episodes and its presence in active MS lesions. Moreover, S100B may also be a potential therapeutic target to reduce damage during the course of MS, based on the beneficial outcome of its inhibition in COSC studies.

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Enrichment of retroviral sequences in brain tissue from patients with progressive multiple sclerosis

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Background: Our group has used deep sequencing to identify viral RNA signatures in frozen human brain specimens. We have previously used this method to detect RNA and DNA viruses in brain tissue from deceased donors. Deep sequencing was performed on brain specimens from a cohort of patients who died with progressive forms of MS, revealing evidence of increased human endogenous retrovirus (HERV) expression.
Objectives: identify RNA sequences and new antigens involved in the pathogenesis of MS
Methods: Viral and HERV sequence databases were prepared from publicly available resources. Deep sequencing of total RNA extracted from 12 primary progressive MS, 2 neuromyelitis optica, and 14 normal control frozen brain specimens was performed using the Illumina HiSeq 2000 instrument. The resulting single ended 50 bp sequence reads were compared to the viral and retroviral databases. Normalized hit rates (HR) of the MS samples to HERV domains and exogenous viruses were compared to those of the control specimens.
Results: Two-20 million high quality reads per specimen were obtained. Sequence comparisons revealed numerous significant HR differences for several HERV domains and retroelements. Clustered average HRs of retroviral domains (i.e. GAG, ENV, RT, etc.) discriminated MS from control specimens (P=0.009). Based on overexpression in the MS samples, 16 different MS candidate GAG and ENV domains have been selected for follow up investigation. Quantitative PCR comparisons of HERV expression in brain samples between the MS and control groups are presented.
Conclusions: These data demonstrate that HERV and retroelement sequences are significantly overrepresented in these MS brain tissue specimens. This supports the hypothesis that expression of endogenous retroviral sequences is contributing to MS pathogenesis, potentially reconciling the autoimmune and infectious nature of this challenging disease.

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Differential oxidative stress and cytokine profile between progressive and relapsing-remitting multiple sclerosis patients

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Background: Although inflammation is the driving force for brain injury in multiple sclerosis (MS), studies suggest that oxidative stress may also modulate the disease.
Objectives: The aim of the present study was to evaluate the role of redox and cytokine profile in progressive forms of MS patients.
Methods: 126 patients with RRMS and 34 with progressive MS were recruited. The disability was evaluated using the Expanded Disability Status Scale (EDSS) and the disease activity was evaluated using the resonance magnetic imaging (RMI). Cytokines were measured by a sandwich enzyme-linked immunosorbent assay; hydroperoxide (CL-LOOH) was evaluated by chemiluminescence; Advanced Oxidation Protein Products (AOPP) was determined using the semi-automated method; carbonyl protein was measured as an estimate of protein oxidative injury; sulfhydryl groups of proteins were evaluated by a spectrophotometric assay; nitric oxide metabolites (NOx) levels were assessed by nitrite and nitrate concentration, and total radical-trapping antioxidant parameter (TRAP) was determined by chemiluminescence.
Results: Progressive MS patients differed from RRMS patients in disease duration, EDSS, and increased disease activity ($p< 0.0001$). Progressive MS patients presented increased levels of IL-1B ($p=0.0168$) and IFNG ($p=0.0374$), and low levels of IL-12 ($p=0.0006$) than RRMS patients; and predominance of cytokines with Th1 profile on Th2 profile (IFNG/IL4, $p=0.0033$; IFNG/IL-10:$p< 0.0001$). The multivariate analysis showed that IFNG was independently associated with the progressive clinical forms ($p=0.0105$). Regarding the oxidative stress markers, patients with progressive clinical forms presented increased levels of hydroperoxide ($p=0.0408$), NOx ($p=0.0327$), AOPP ($p=0.0141$), and carbonyl protein ($p=0.0195$). Regarding the antioxidant defense, they presented decrease TRAP ($p=0.0489$).
Conclusions: This study revealed a complex cytokine network and redox state imbalance among patients, that was correlated with a progressive clinical course. Oxidative stress may be able to initiate a cascade of reactions affecting the function of several proteins that progressively become impaired and eventually culminate in relapse outburst. At this level, severe disability and inflammatory events occur and oxidative damage seems to affect inflammatory pathways. Given the central role of oxidative damage in the pathogenesis of MS, this mechanism might represent key targets for future neuroprotective therapies in patients with the disease.

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Neuropathological study of glucose and monocarboxylate transporters in multiple sclerosis

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Background: Recently, energy supply to axons via glial cells has received a lot of attention in the world. Nutritional substances

such as glucose and lactate are transferred from blood vessel to axons via connexins (Cxs) gap junction or glucose transporters (GLUTs) and monocarboxylate transporter (MCTs). We previously reported extensive loss of astrocytic and oligodendrocytic Cxs in active lesions of multiple sclerosis (MS), neuromyelitis optica (NMO) and Baló's disease (BD), suggesting early disruption of Cxs may cause the extensive energy failure and contribute to the pathogenesis in demyelinating disorders.

Objectives: To get more understanding of metabolic condition in demyelinating disorders, we studied the expression of GLUTs and MCTs in MS lesion.

Methods: We pathologically evaluated GLUT1, 3, 4, 5 and MCT1, 2, 4 expression relative to expressions of Cx43, glial fibrillary acidic protein (GFAP), extent of demyelination determined by MAG, MBP, MOG, and Nogo-A (oligodendrocyte marker) immunoreactivities, neurofilament and amyloid precursor protein (APP) (axonal markers), and lesion staging with CD68 staining for macrophages in six autopsied cases with MS including one case of Marburg's type and 20 with other neurological diseases.

Results: In myasthenia gravis case, GLUT1 and MCT1 were abundantly expressed in the microvascular endothelial cells and glial cells. GLUT3 and MCT2 were mainly expressed in axons. GLUT5 was specifically expressed in resting microglia. MCT4 was expressed in astrocytes and perivascular foot processes. In the active demyelinating lesion of MS, in spite of massive perivascular lymphocytic cuffing, endothelial MCT1 and GLUT1 were relatively preserved. On the other hand, immunoreactivity for MCT4 was diminished in the perivascular foot processes in active lesion. Microglial GLUT5 was up-regulated in activated microglia and foamy macrophages in active lesion. Immunoreactivity for APP was found in damaged axons and terminal axonal ovoids. Similar to APP, immunoreactivity for GLUT3 was also emphasized in damaged axons.

Conclusions: Our findings indicate that altered expression of GLUTs and MCTs could be seen in active MS lesion and may cause energy failure in early stage of MS. Especially, loss of MCT4 in astrocytic foot process may cause impaired transportation of energy to axons via glial cells. GLUT3 was accumulated in damaged axons of active MS lesions and immunostaining for GLUT3 may be useful as a novel potential marker for damaged axons.

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Is inflammation atherogenic in neurological diseases? A case-control study with migraine and multiple sclerosis patients

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Background: There has been some debate concerning the endothelial damage and cerebrovascular risk in Multiple Sclerosis, we analyze why the pathogenesis of the increases vascular risk disease in our patients.

Objectives: To determine the endothelial damage in patients suffering from multiple sclerosis (MS) and migraine measuring the carotid intima-media thickness (IMT) and the endothelial-dependant flow-mediated vasodilation (EDV), as subclinical biomarkers of atherosclerosis and predictors of cardiovascular events.

Methods: Subjects were recruited and matched for sex and age (mean age 37 y; range 20-55) according to McDonnald's 2010 criteria for MS diagnosis, and ICH-2004 and 2006 criteria for chronic migraine (CM). A control group were also recruited. Ultrasonografic images were obtained by a certified blind examiner. IMT was determined as the average of at least three different measurements. EDV was measured in the brachial artery with a non-invasive method. Other vascular parameters were also registered in the medial cerebral artery as of breath-holding index (BHI), sistolic velocity (SV), and pulsatility index (PI). Blood samples were drawn for nitric oxide, von Willebrand factor (vWF), ICAM-1 and VCAM-1 ELISA determination. Statitics were obtained with SPSS v.15 and included Student's t test, general lineal models with post-hoc Bonferroni correction with adjusted means, and Pearson regression test.

Results: We recruited 22 controls, 59 migraine patients (25 CM), 33 MS patients. IMT was thicker in MS patients than in controls (mean diference= 0.233 mm; p=5.4E-009), EM (mean differences= 0.150 mm; p=8.9E-006), and. CM patients (mean differences= 0.097 mm; p=0.008). CM had thicker IMTs than controls (mean defference=0.136 mm; p=0.001). There were no differences among groups regarding smoking, hypertension, BMI, and cholesterol levels. IMT strongly correlated with EDSS (r=0.464; p=0.011). IMT inversely correlated with EDV (r=-0.414; p=0.000013) and BHI (r=-0.300; p=0.015). BHI inversely correlated with vWF (r=-0.317; p=0.011). EDV was higher in controls than in MS (mean diference= 5.518 mm; p=5.6E-006), and CM (mean diference= 4.270 mm; p=0.001). MS and CM still predicted IMT and EDV under the model corrected for age and body mass index ($p< 0.001$).

Conclusions: Our findings suggest intrinsic endothelial vascular damage in MS patients so endothelial damage could be associated to the neuroinflammation status itself.

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CHI3L1 and SPP1 distribution in multiple sclerosis lesions

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Background: Chitinase-3-like protein 1 (CHI3L1) is a secreted glycoprotein that is up-regulated in inflammatory conditions, including multiple sclerosis (MS). The function of CHI3L1 expression has not been characterized. High cerebrospinal fluid (CSF) CHI3L1 levels are associated with increased risk of conversion of clinically isolated syndrome (CIS) to of clinically definite MS. In patients with progressive forms of MS, plasma CHI3L1 levels have been found to be significantly increased. A strong association between CHI3L1 and osteopontin (SPP1) expression levels in CSF has been reported.

Objectives: To study the distribution of CHI3L1 and osteopontin by immunohistochemistry in an autopsy material of brain tissue from 9 MS patients along with five healthy control samples.

Methods: Sections were immunostained for myelin proteolipid protein (PLP), human leucocyte antigen-DR (HLA-DR), glial

fibrillary acidic protein (GFAP), T cells (CD3), CHI3L1 and SPP1.
Results: 26 MS-lesions were detected: 2 active, 5 chronic active, 12 chronic inactive white mater lesions (WML) and 7 grey matter lesions (GML). CHI3L1 immunopositivity was detected on a subset of reactive astrocytes in most WMLs . A higher density of CHI3L1 immunopositive astrocytes were detected at the WML border, and at the lesion border in a subset of GMLs. A lower extent of immunopositivity was detected on phagocytic macrophages in active and chronic active white matter lesions and in a subset of neurons . Osteopontin immunopositivity was detected in activated macrophages, in subpopulations of neurons, and astrocytes, and in the extracellular matrix in all WMLs.
Conclusions: This finding indicates that there is increased expression of CHI3L1 and SPP1 in areas of inflammation in all stages of MS WML development, with a similar cellular distribution.

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Endothelial function in patients with multiple sclerosis
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Background: One of the first theories of pathogenesis of multiple sclerosis (MS) was a vascular theory. A key step in the pathogenesis of MS is the infiltration of activated leukocytes from the blood through the endothelium into the brain tissue.
Few studies confirm the presence of endothelial dysfunction (ED) in MS and its role in the pathogenesis of the disease.
Objectives: Identification in patients with MS ED, an assessment of its relation with the disease activity and disease modifying therapy (DMT) of MS, a comparative analysis of endothelial function in multiple sclerosis patients and healthy volunteers.
Methods: 33 patients (20 female, 13 male), age 40,8 (± 1,9), with definite diagnosis of MS (McDonald criteria, 2005), disease duration 7,55 (± 1,1) years. 49% - relapsing-remitting MS (RRMS), 3 of them there was exacerbation. 24% - secondary-progressive MS (SPMS), 5 of them - stage of progression. 27% - primary-progressive MS (PPMS), 3 of them - stage of progression. 21% (RRMS and SPMS) was naive for DMT, 79% use DMT for 3,87 (± 0,53) years.

The control group was represented by 30 healthy individuals.

Blood level of Von Willebrand factor (vWf) ELISA, desquamated endotheliocytes (DE) counting according to the method J. Hladovec (1978), statistical analysis - Mann-Whitney (U), Spearman (R).
Results: vWf values in patients with MS ranged from 0,8 to 2,2 U/mL (normal 0,5-1,5 U/mL), and was an average of 1,48 ± 0,07 U/ml. vWf values in healthy individuals ranged from 0,3 to 0,78 U/mL, and was an average of 0,6± 0,03 U/ml, p< 0,001. 22 patients (67%) has above normal level of vWf - 11 patients (33%) with RRMS, 6 (18%) patients with SPMS, 7 (21%) patients with PPMS. Its level was higher than normal in all cases of exacerbation and in 7 cases of progression (SPMS and PPMS).

Number of DE in MS patients was 8,82 (±2)x10^4/L (normal 0 -2 x10^4/L); in the control group - 0.5 (±0,06)x10^4/L, p < 0,001. 30 patients (91%) has above normal level of DE.

Found a significant positive correlation between the number of DE and vWf, R=0,37, p = 0,0027, the correlation with the period of disease and vWf, R=0,4 at p = 0,0007.
Conclusions: Almost all patients with MS have observe endothelial damage and dysfunction, which is obviously associated with disease activity.

Data obtained may be used as a evaluation of the activity of the disease and determine the effectiveness of therapy. In the future ED may be a separate target for therapeutic interventions in MS.

Neurophysiology

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Visual evoked potentials in neuromyelitis optica and its spectrum disorders
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Background: Optic neuritis (ON) is a key feature of neuromyelitis optica (NMO) and its spectrum disorders (NMOSD). It is responsible for the visual deficits of the patients. Visual evoked potentials (VEPs) can be used to assess the function of the visual pathway. After ON in classical multiple sclerosis (MS) VEPs typically show prolonged P100 latencies and often normal amplitudes.
Objectives: We aimed to analyse the pattern of VEP changes in NMO/NMOSD patients with and without preceding episodes of ON.
Methods: We analyzed fullfield pattern reversal VEPs in 43 patients with definite NMO, 18 with anti-aquaporin (AQP) 4 antibody-seropositive NMO spectrum disorders, and 61 matched healthy controls. Furthermore, longitudinal VEP measurements were performed on a subset of patients.
Results: Reduced amplitudes were found in 12.3%, prolonged latencies in 41.9%, and a lack of response in 14.0% of NMO/NMOSD eyes. Interestingly, P100 latencies were significantly prolonged not only after an ON but also in NMO/NMOSD eyes without a history of NMO. Aquaporin-4 antibody status did not influence the VEP patterns in our collective. Longitudinal evaluations revealed no short term changes of VEP patterns in the absence of ON.
Conclusions: Our data indicate a predominantly demyelinating nature of the damage in our NMO patients' eyes. Delayed P100 latencies in eyes without prior ON suggest subclinical affection. Longitudinal evaluations on larger collectives over a longer time are warranted to analyse these subclinical changes.

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Detecting cognitive impairment in MS based on a support vector machine classification of EEG P300 connectivity

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Background: Cognitive impairment affects half of the multiple sclerosis (MS) population, is difficult to detect and requires extensive neuropsychological testing. Due to its specific pathology, it is suggested that the use of brain connectivity based metrics to assess cognitive impairment may be highly beneficial.

Objectives: The aim of this research is to detect cognitive impairment in an MS population based on the trial averaged EEG response to the infrequent (target) stimuli and to assess the informative value provided by different connectivity metrics.

Methods: Cognitive impairment was defined as failing 2 or more tests included in the Neuropsychological Screening Battery for MS (NSBMS). Failing one test was defined as failing the 5th percentile of a normal population. A total of 167 patients resulted as cognitively preserved, 104 patients were cognitively intact.

The EEG signals were recorded on 21 electrodes following the standard 10/20-system. The infrequent stimuli evoke a large positive wave at about 300 ms after the stimuli (the P300). Two sets of features were selected, the first one included the more traditional P300 features like amplitude and latency of the P300 peak. The other set considered connectivity metrics.

Every connectivity metric results in a network in which the nodes are the electrodes and the edges are e.g. given by the correlation between every pair of electrodes. The information included in several frequently used connectivity measures (correlation, coherence, phase-lag-index, partial correlation, the imaginary part of coherency) is compared by entering all edges as input features of a support vector machine (SVM).

This SVM is subsequently optimized in a tenfold cross-validation scheme. A null-distribution of the accuracy is obtained by repeating this procedure 100 times with randomly reshuffled cognition labels.

Results: We show that correlation, correlation in the frequency domain and delta and theta-coherence bear significant information on a patient's cognitive status ($p < 0.01$). The obtained accuracies (around 70 %) are comparable to those found using the more traditional features and are not sufficient for clinical applications.

Conclusions: These result support the recent suggestion that cognitive impairment in MS might be caused by cerebral disconnection. Especially cerebral connectivity in theta and delta frequency bands seems to be implicated.

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Visual and auditory evoked potentials as related to fatigue in multiple sclerosis

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Background: The origin of fatigue in multiple sclerosis remains unclear. Disturbed neural conduction within the brain due to demyelination and axonal loss is considered as one of the possible mechanisms of fatigue, which encourages investigating neurophysiological methods in this field.

Objectives: The aim of the study was to evaluate visual and brainstem auditory evoked potentials (VEP, BAEP) in multiple sclerosis (MS) patients with regards to fatigue and disease-related variables.

Methods: The study comprised 86 MS patients and 40 controls. Duration of MS and degree of disability (Expanded Disability Status Scale - EDSS score) were determined. Fatigue was assessed using the Fatigue Severity Scale (FSS/FSS-5). Latencies and amplitudes of the P100 component of VEP and the I-V components of BAEP were analyzed. The results of EP were compared between non-fatigued, moderately and severely fatigued MS patients and controls, and referred to disease-related variables.

Results: P100 latency was increased and amplitude decreased in moderately and severely fatigued MS subjects. The latency of the V component of BAEP and interlatencies I-III-V were increased in severely fatigued patients. The amplitude of the V component was lowered in fatigued patients. VEP and BAEP abnormalities were usually one-sided. Interocular P100 latency difference tended to correlate with FSS/FSS-5. The parameters of the P100 and V components did not correlate with MS duration or EDSS.

Conclusions: Significant, usually asymmetrical VEP and BAEP abnormalities were found in fatigued MS patients, with no relationships to disease-related variables. EP may be considered an electrophysiological marker of fatigue in MS patients.

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Modulation of action tremor by repetitive transcranial magnetic stimulation in multiple sclerosis patients

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Background: Patients affected by Multiple Sclerosis (MS) can show a tremor of their upper limbs, mostly during the performing of finalised action (action tremor) or at the maintaining of a position against gravity (postural tremor), as key clinical feature of their disease.

Objectives: In order to reduce the tremor, patients underwent to repetitive transcranial magnetic stimulation (rTMS) of the primary motor cortex of left hemisphere.

Methods: Eight patients affected by a middle form of MS were enrolled into the study. A standard neurological examination was carried out and the individual degree of disability was established employing the Expanded Disability Status Score (EDSS). At EDSS patients scored from 1.5 to 6.5, mean 4.6 ±1.9 SD. Tremor was rated according to the Fahn-Tolosa Marin Rating Scale. rTMS was delivered at 1 Hz frequency, along ten subsequent, separate sessions lasting ten minutes each one (i.e. 600 pulses). All subjects were aware about the aim of the study and fulfilled a formal consent. They were free to interrupt the study at any time. Before the first session, at the fifth day and at the last day of stimulation, kinematics of upper limb movements were recorded. Patients were asked to perform a continuous pointing, moving back and

forth, as fast as possible, their right index finger between two black spots depicted on a working table, placed 15 cm apart. Task lasted six minutes divided in six parts, lasting one minute each one, divided by a resting period of 30 sec. Movements were recorded by means of a optoelectronic device (ELITE system) working at the sampling rate of 100 Hz. At the end of the last session a new clinical assessment was carried out.
Results: Movement time, peak of velocity and acceleration and deceleration were analysed. Number of peaks of deceleration during the targeting phase was considered as the key marker of the tremor. Results of kinematics showed an overall improvement of symptoms including a decrease of time of movement execution and time of the deceleration phase as consequence of the reduction of number of peak of deceleration (i.e. tremor).
Conclusions: Reduction of tremor became apparent at the clinical evaluation. Moreover, tremor was effectively influenced by rTMS and patients reported a subjective improvement of their symptoms. We concluded that this technique could be useful in order to reduce disability in MS patients.

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Heart rate variability analysis in recently diagnosed patients with multiple sclerosis
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Background: Multiple sclerosis (MS) can cause cardiovascular autonomic dysfunction. Previous studies have determined this with using tilt table test, heart rate responses to Valsalva maneuver and deep breathing, heart rate variability analysis with 24-hour Holter monitoring.
Objectives: The aim of the study is comparison of the heart rate variability (HRV) between recently diagnosed patients with relapsing-remitting MS and healthy controls by using 24-hour Holter monitoring. The heart rate variability analyses were performed before starting any immunomodulatory or immunosuppressive treatment. Also we intended to investigate relationship between Expanded Disability Status Scale (EDSS) score, Multiple Sclerosis Functional Composite (MSFC) scores and cranial and spinal magnetic resonance imaging (MRI) findings and HRV.
Methods: Forty-one patients with newly diagnosed relapsing-remitting MS (RRMS) and 42 age- and sex-matched healthy controls were compared in this study. A patient with RRMS, who was already under immunomodulatory or immunosuppressive treatment, was excluded from the study. Echocardiography and HRV analysis with using 24-hour period Holter monitoring were performed in all of the subjects. Echocardiography was used to detect the presence of cardiac pathology. One MS patient with right ventricular dilatation and mobile intratrial septum was excluded from the study.
Results: Our results showed that HRV values were significantly lower in RRMS patients when compared with healthy controls: SDNN index (the mean of all the 5-minute standard deviations of NN (normal RR) intervals during the 24-hour period) (p=0,034), rMSSD (the root-mean-square successive difference) (p=0,044), spectral HRV power (p=0,029), spectral HRV VLF (very low frequency) (p=0,039), spectral HRV LF (low frequency) (p=0,013). However there was no significant relationship between HRV and EDSS score, MSFC scores or number of MS attack (p>0,05).
Conclusions: These findings suggest that cardiovascular autonomic dysfunction is evident even in the earliest stages of MS, before initiation of any immunomodulatory or immunosuppressive treatment.

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The VEMP score: a promising tool for evaluation of brainstem involvement in multiple sclerosis
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Background: Anatomical localization of brainstem lesions on the MRI does not correlate adequately with clinical signs of brainstem affection; approximately 60% of patients with brainstem signs have corresponding MRI lesions. On the other hand, several evoked potentials (EP) have been successfully used in determination of functional impairment in MS patients: somatosensory EP (SSEP), motor EP (MEP), visual EP (VEP) and brainstem EP (BAEP). As well, vestibular evoked myogenic potentials (VEMP) are recognized as fundamental in the assessment of brainstem involvement.
Objectives: Concerning the great importance of brainstem involvement in multiple sclerosis (MS), the aim of this study was to explore the role of newly developed VEMP score as a possible marker of brainstem involvement in MS patients.
Methods: This was a prospective, case control study, which included 100 MS patients divided into two groups (with and without clinical signs of brainstem involvement) and 50 healthy controls. Ocular (oVEMP) and cervical (cVEMP) were performed in all participants and analyzed for latencies, conduction block and amplitude asymmetry ratio. Based on this the VEMP score was calculated and compared to EDSS, disease duration and MRI data.
Results: MS patients with clinical signs of brainstem involvement (group 2) had statistically significant higher percentage of VEMP conduction blocks compared to patients without clinical signs of brainstem involvement (group 1) and healthy controls (p=0.027 and p< 0.0001, respectively). Similarly, the VEMP score was significantly higher in group 2 compared to group 1 (p=0.018) and correlated with EDSS and disease duration (p=0.011 and p=0.032, respectively). Multivariate linear regression analysis showed that the VEMP score have statistically significant influence on the EDSS score (p< 0.001, $R^2 = 0.239$).
Conclusions: Interpretation of the oVEMP and cVEMP results in the form of the VEMP score enables better evaluation of the brainstem involvement than either of these evoked potentials alone and correlates well with disability.

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Autonomic dysfunction and catecholamine levels in patients with clinicaly isolated syndrome
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Background: Signs of autonomic nervous system involvement in multiple sclerosis (MS) occur in 50-86% of cases. However, frequency of autonomic dysfunction was never investigated in patients with clinically isolated syndrome (CIS).
Objectives: To estimate the frequency of autonomic dysfunction in patients with CIS and to correlate clinical and MRI findings, as well as serum catecholamine levels with head-up tilt table test (HUTT) results.
Methods: This study involved 88 patients with CIS, 54 (61%) women and 34 (39%) men aged from 19 to 59 years (mean age 33,1 years). All the patients underwent HUTT and from all of them brain MRI was obtained, while spinal cord MRI was obtained from 76 patients. Blood samples in supine and upright position during HUTT were collected from 74 patients, while from one patient only the sample during supine position was obtained.
Results: Proportion of CIS patients with proven autonomic dysfunction was 65,9%. The most common type of HUTT result was vasovagal syncope (N=22, 25%), followed by POTS (N=16, 18%) and orthostatic hypotension (N=15, 17%), while two or more HUTT pathologies were found in 5 patients (6%). EDSS did not correlate with HUTT findings, while there was a statistically significant correlation between normal clinical findings concerning brainstem (bEDSS=0) and normal HUTT result (p=0,032). MRI findings did not show correlation with HUTT results, but significantly bigger difference between serum noradrenaline levels in supine and upright position were found in patients who had midbrain lesions evident on MRI (p=0,017). Serum levels of adrenaline in the upright position correlated with the type of CIS (p=0,013). When considering latter result highest levels of adrenaline in upright position were measured in patients with spinal cord syndromes and brainstem/cerebellar syndromes. Other adrenaline and noradrenaline values did not correlate with either HUTT results or type of CIS.
Conclusions: Autonomic dysfunction is common in patients with CIS. Since EDSS and bEDSS did not correlate with autonomic dysfunction in patients with CIS, adding new criteria for evaluation of autonomic nervous system, thus leading to better patient follow-up, should be considered.

~~**P702**~~

~~**Cervical and ocular vestibular-evoked myogenic potentials in patients with multiple sclerosis**~~

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~~**Background:** Vestibular evoked myogenic potential (VEMP) recorded at the sternocleido mastoid muscle (cervical VEMP) and the extraocular muscle (ocular VEMP) assesses the sacculo-spinal pathway and the vestibulo-ocular pathways.~~
~~**Objectives:** To clarify the usefulness for determining abnormality of cervical (cVEMP) and ocular (oVEMP) vestibular evoked myogenic potentials and to correlate with structural and functional abnormalities in patients with multiple sclerosis.~~
~~**Methods:** Twenty patients with multiple sclerosis were examined. Stimulated by air-conducted sound (ACS) (tone bursts, 1000 Hz, 5 ms) and bone-conducted vibration (BCV) using vibrator (Minishaker 4810, Bruel and Kjaer, Netheland) above the mastoid and forehead with bone-conducted tone bursts (500 Hz, 6 ms) at fixed levels above their individual vestibular evoked myogenic potential (VEMP) thresholds. Forty healthy subjects were included in the study as the control group.~~
~~**Results:** Response rate, amplitude and onset latency of vestibular evoked myogenic potentials (VEMPs) were analyzed. All healthy subjects showed clear cVEMPs and oVEMPs induced by ACS and BCV stimulation at the forehead and mastoid process. Eleven of the 20 MS patients showed abnormal recordings. In the MS subjects, the BCV-induced VEMPs showed a more frequently normal response than VEMPs induced by the ACS especially oVEMP. Subjects with MS lesions involving the brainstem showed significantly high abnormal response rate of cVEMP and oVEMP to ACS and BCV compared to healthy controls. Subjects with hearing loss showed nearly normal response of cVEMP to ACS and BCV, but they showed low response rate of oVEMPs especially to ACS. There was also a significant difference between MS and control groups with respect to latencies of P13 (cVEMP) and N10 (oVEMP) and amplitude. VEMP abnormalities were significantly related to the presence of brainstem demyelinative lesions.~~
~~**Conclusions:** VEMPs is the only electrophysiological method to detect dysfunction in central vestibular pathways. Our data revealed that VEMP abnormalities show the strongest correlation with demyelinative MRI lesions in the brainstem. These recently introduced methods provide a further tool to the clinician for the functional assessment of the brainstem in diseases such as MS.~~

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Measurement of visual evoked potentials (VEPs) from awake rats before and after introduction of gliotoxins into the optic chiasm

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Background: Visual evoked potentials (VEPs) are a biomarker used to track visual system function in MS and optic neuritis patients. Understanding how VEP measures relate to underlying pathology would benefit from a pre-clinical animal model, where VEP changes can be tracked over time following a controlled demyelinating lesion, and where histological analyses can be performed at defined time points.
Objectives: We aimed to develop an assay to measure VEPs chronically from awake non-behaving rats, before and after the introduction of demyelinating lesions into the optic chiasm.
Methods: Animals were chronically implanted with LED lights which independently stimulated the left and right eyes, and with electrodes measuring the electroencephalogram (EEG) signal from the left and right visual cortex. We additionally implanted a guide cannula targeting the optic chiasm. After obtaining baseline awake VEP recordings, we briefly anesthetized the animals and injected a gliotoxin (lysolecithin or ethidium bromide) into the optic chiasm via a needle inserted through the implanted cannula.

Additional VEP recordings were then obtained in awake animals in the days and weeks following injections.

Results: We found that VEPs could be repeatedly recorded on successive days for more than 2 months from individual animals, and that in non-injected animals the latency of the N1 component of the rat VEP was highly stable over this time period. VEP amplitudes were more variable, but such variability was largely accounted for by trial-to-trial changes in the spontaneous EEG state of the animal, and thus could be reduced by sorting trials based on this metric. We found that injections of gliotoxins could be specifically administered into the optic chiasm of implanted animals. Successful targeting of injections into the optic pathway white matter was verified in individual cases by including lidocaine (a reversible sodium channel blocker) in the injection solution and demonstrating a reversible blockade of VEPs during the procedure.

Conclusions: We demonstrate the possibility of measuring VEPs over days and weeks in individual awake animals following gliotoxin injections into the optic chiasm. This methodology will allow for dynamically tracking VEP measures in response to a controlled demyelinating lesion, and subsequently relating key physiological changes to histological observations.

P704

Visual evoked potentials and optic coherence tomography in monitoring involvement of visual pathways in multiple sclerosis

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Background: In the assessment the involvement of visual pathways in Multiple Sclerosis-MS, optical coherence tomography-OCT and visual evoked potentials-VEPs are the most common methods

Objectives: To investigate their value in longitudinal monitoring of visual pathways involvement.

Methods: Eighty people with MS (13 clinically isolated syndrome-CIS, 55 relapsing-remitting-RR, 9 secondary progressive-SP, 3 primary progressive-PP) underwent neurological and neurophysiological evaluation with OCT and VEPs, with repeated clinical assessment after a mean follow-up of one year, when 50 patients also repeated OCT-VEPs.

Results: VEPs were more sensitive vs OCT in eyes with recent (< 3 months) optic neuritis-ON at baseline (80.0% vs 6.7%, p=0.001), the two sensitivities were similar in chronic ON eyes (78.4 %). Comparing eyes with and without previous ON, VEP latency and RNFL thickness were respectively significantly higher (131.2 ms vs 118.8 ms, p=0.008) and lower (78,15 μm Vs 90,00 μm, p< 0.001) in the first subgroup. Significant longitudinal changes at follow-up, consisting in improved VEPs (-15,3 ms) and worsened RNFL thickness (-7,7 μm), were found only in eyes with baseline recent ON. No significant correlation was found between OCT-VEPs parameters and disease activity. Similar results were found when considering only RR and CIS patients.

Conclusions: These results would exclude recommending OCT and VEPs as surrogate biomarkers in short-medium term monitoring as in Phase II studies, with the exception of acute ON. However, these findings cannot exclude the usefulness of these techniques for longer follow-ups and/or large phase III studies.

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Motor evoked potentials from multiple recording sites of the lower limbs as a monitoring tool of central motor function in MS relapsing patients

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Background: Variability of Motor Evoked Potential (MEP) area hinders quantification of central motor conduction failure (CMCF), i.e. conduction block and axonal damage, in follow-up studies. MEP averaging, combined with objective control of voluntary facilitation, restrains intra-trial MEP variability (Di Sapio et al, 2013).

Objectives:

1. To develop a procedure to achieve maximum stability of MEP area in subsequent recording sessions.
2. To apply the procedure to relapsing multiple sclerosis (MS) patients to measure acute changes of CMCF to several muscle districts of lower limbs after high dose steroids therapy.

Methods: In 15 clinically stable MS patients and 12 healthy controls (HCs) MEPs to Transcranial Magnetic Stimulation with double cone coil and maximal Compound Muscle Action Potentials (CMAPs) to High Voltage Electrical Stimulation of lumbosacral roots (Troni et al, 2011) were recorded twice, 1-2 days apart, from Vastus Medialis and Lateralis (VM, VL), Tibialis Anterior (TA), Peroneus Longus (PL) and Flexor Hallucis Brevis (FHB) of both sides. The coil position, the vertebral stimulation point and all recording sites were marked with a dermographic pen. We measured intertrial variability of Central Motor Conduction Time (CMCT), Area and Area Ratio (MEP area/CMAP area, AR) by calculating the Coefficients of Variation (CV).

In 13 MS patients, presenting a lower limbs pyramidal relapse, we applied the described procedure twice, before and at the end of steroids therapy; in 8 of these cases the stimulation and the recording sites were marked with small tattoos and a third determination was obtained 30-40 days later.

Results: The use of unchanged stimulation and recording sites significantly reduced inter-trial MEP variability. The mean CMCT-CV ranged from 7.5%± 8.6% (PL) to 11,8 %± 10,9% (FHB) and the mean AR-CV from 11.2% ± 8.8% (FHB) to 16.6% ± 11.7% (PL). No significant differences were observed between MS and HC subjects.

In SM relapsing patients highly significant decrease of mean CMCTs was detected after treatment; abnormal AR values (< 0,2399) were observed in 27 out 119 muscle districts and 9 normalized after treatment. In 8 patients neurophysiological follow up was in agreement with clinical outcome.

Conclusions: The reduced inter-trial MEP variability makes the described technique suitable to quantify short-term changes of MEP parameters and a promising tool for long term monitoring of CMCF. Moreover trend analysis of several parameters (from 10 muscles) can sharpen the sensitivity of the method.

P706

Transient and steady-state visual evoked potentials during treatment with fampridine

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Background: Treatment with fampridine, is able to increase walking speed in some MS patients. Other symptoms as tremor, dysphagia and visual disturbances could be favourably influenced by this drug. However, the reason why only about one third of patients are ´responders´ is not known, as well as their clinical, radiological and neurophysiological profile. Visual evoked potentials (VEPs) are very sensitive to assess conduction in demyelinated optic nerves and have been used to document the effect of fampridine. Steady-state stimulation in addition to the classical transient stimulation may increase sensitivity disclosing frequency-dependent conduction blocks.

Objectives:

1) to assess if VEPs are influenced by fampridine treatment;
2) to compare the effects of treatment on transient and steady.state VEPs;
3) to test if clinical response as assessed by significant increase in walking speed is linked to neurophysiological changes
4) to test if clinical response can be predicted by basal clinical and neurophysiological data.

Methods: Twenty-five patients affected by MS with EDSS ranging from 3,5 to 6 in a stable clinical condition signed informed consent and were submitted, to: 25 feet walk test (25FWT), transient pattern reversal (2 rev/sec) VEP-tr by monocular 15´ and 30´ check stimulation; steday-state VEP-ss (8 rev/sec) with 15´ and 60´ checks. The procedures were performed basally and after 15 days on fampridine 10 mg twice a day. Patients were classified as responders if 25FWT showed a >20% improvement. P100 latency and N70-P100 amplitude were considered for VEP-tr. VEP-ss were cross-correlated with a sinusoid of equal frequency. Shift between VEP-ss and the sinusoid as well as total amplitude of the rectified signal were considered. Analysis was performed by repeated measures ANOVA (SPSS).

Results: Eight subjects could (32%) be classified as responders. VEP-tr were altered in 80% of eyes with 15´ checks and in 60% of eyes with 30´ checks. 15´ check VEP-tr latency significantly decreased after treatment irrespective of the clinical response. VEP-ss shift decreased and amplitude increased after treatment, the changes being greater in responders and 60´ checks. No clinical or neurophysiological basal parameter could predict clinical response.

Conclusions: VEPs are able to document the effect of fampridine in MS patients. VEP-tr seem to be less sensitive than VEP-ss whose changes are partially linked to clinical response. No clinical or neurophysiological basal parameter could predict clinical response.

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Longer QRS and QT interval duration and different QRS axis were found in relapsing remitting multiple sclerosis patients during remission

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Background: Autonomic nervous system dysfunction is present in multiple sclerosis (MS) patients, which may influence the electrocardiography (ECG) pattern.

Objectives: It was our aim to compare basic ECG parameters in relapsing-remitting MS patients during remission phase and healthy controls. The second aim was to examine correlation between MS features and ECG parameters.

Methods: Standard 12-leads ECG computer analysis of QRS duration, PR interval, QT interval, QTc interval, QRS axis and the frequency of right bundle branch block (RBBB) was compared between 101 patients with relapsing-remitting MS in remission and 101 age and sex matched healthy controls. All measured ECG parameters were correlate with age, duration of MS, disease activity relapse rate at 3 years) and the EDSS.

Results: There were 70 female and 31 male MS patients, average age was 37.4±8.2 average EDSS 2.55 ±1.37 average MS duration 12.3±7.4 average relapse rate 1.9± 0.7. All MS patients were in sinus rhythm, had longer QRS duration [92 (86-98 ms) vs 87 ms (81-92 ms), p< 0.001],longer QT interval [381 (363-403 ms) vs 259 ms (342-377 ms), p< 0.001], longer QTc interval [416 (399-431 ms) vs 399 ms (389-416 ms), p< 0.001] and different QRS axis ([7^{o} (0-66^{o}) vs 55^{o} (29-71^{o}), p< 0.001] than healthy controls. PR interval was similar. Some degree of RBBB was more common in MS patients (40.6% vs 19.8%, p=0.002). Male MS patients had significantly longer QRS and QTc interval compared to female MS patients. Age, MS duration, relapse rate and EDSS weren`t associated with the ECG parameters in MS patients.

Conclusions: Patients with MS have prolonged QRS duration and QT interval, more vertical QRS axis and more frequent presence of RBBB than healthy controls.Male MS patients have significantly longer QRS and QTc interval compared to females MS patients.

Neuroprotection and repair

P708

Q-space imaging is a marker of repair following spinal cord relapse in MS

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Background: Disabling MS relapses are associated with perivascular inflammation, demyelination and axonal injury. Resolution of inflammation and remyelination contribute to clinical recovery. High b-value q-space imaging (QSI) is a model free diffusion weighted imaging (DWI) technique which reflects change in axonal structure and myelin in animal models of dysmyelination and may be useful for studying repair in MS.
Objectives: We aimed to determine whether QSI indices reflect the tissue repair associated with clinical recovery following spinal cord (SC) relapse in MS.
Methods: 20 RRMS patients (14F; mean age 41years; baseline EDSS 4.5 (range 2.5-6.5); mean disease duration 8.3 years) presenting within 4 weeks of upper cervical SC relapse were recruited and assessed with the Expanded Disability Severity Scale (EDSS) at baseline, 1, 3 and 6 months. Patients and 22 healthy controls (17F; mean age 44years) underwent cervical SC imaging at baseline, 1, 3 and 6 months with a cardiac gated QSI protocol. 30 DWI volumes, covering 60mm of SC, centred on C2/3 were acquired in two directions perpendicular (x and y) and in one parallel (z) to the axis of the SC. The diffusion probability density function (dPDF) was computed and voxel-wise maps of the full width at half maximum (FWHM) (which represents the width of the dPDF) and zero displacement probability (P0) (representing the height of the dPDF), were derived for xy and z. A region of interest analysis was performed for the cervical SC at each time point. Patients were classed as "improvers" (EDSS change of ≤ -0.5 over 6 months), or "non-improvers" (no improvement on EDSS). Differences in QSI indices (FWHM and P0) at each time point between improvers and healthy controls as well as non-improvers and healthy controls were evaluated using multiple regression analyses, adjusting for age and gender.
Results: Ten (50%) patients clinically improved by 6 month follow up. After correcting for age and gender, perpendicular (xy) diffusivity was significantly higher at baseline in improvers ($P< 0.001$) and non-improvers ($P< 0.001$), compared to controls. By 1 month there was no statistical difference in perpendicular diffusivity between improvers and controls, however in non-improvers, perpendicular diffusivity remained significantly elevated at 6 months ($P < 0.001$).
Conclusions: We propose that normalisation of perpendicular diffusivity in patients who recover following SC relapse may reflect remyelination and that QSI may therefore be a potentially useful biomarker of repair in MS.

P709
NMDA receptor blockade is neuroprotective in experimental autoimmune optic neuritis
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Background: Optic neuritis can result in persistent visual impairment due to degeneration of optic nerve (ON) axons and apoptosis of retinal ganglion cells (RGCs). A major cause of neurodegeneration, is glutamate-mediated excitotoxicity, via α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptors.
Objectives: We assessed the effect of the NMDA receptor blockers memantine and MK-801, in a rat model of optic neuritis, inducing Experimental autoimmune encephalomyelitis (EAE) by myelin oligodendrocyte glycoprotein immunization.
Methods: Female BN rats were treatment with either vehicle, memantine hydrochloride 20 mg/kg (M20) or 60 mg/kg (M60), or MK-801 (0.15 mg/kg). RGCs were retrogradly labelled and quantified. ON histopathology was performed including Luxol-fast blue (LFB), Bielschowsky's silver impregnation, CD3 and ED1. Furthermore calcium imaging of RGCs and oligodendrocytes was performed.
Results: Disease severity was significantly lower in memantine treated groups. By immunohistochemistry demyelination (LFB: vehicle, 84.7%; M20 mg/kg, 41.4%; M60 mg/kg, 4.9% $p < 0.0001$), axonal damage (Bielschowsky: vehicle 70.4%; M 20, 33.9%; M60, 6.7%, $p < 0.0001$) and immune cell infiltration was reduced in the treated groups compared to vehicle (CD3-positive cells/mm2: vehicle, 41.9; M20, 21.1; M60, 5.5; $p< 0.05$).

In retinas of shamimmunised rats, mean RGC density was 2046 ± 120.2 cells/mm2. RGC count in vehicle dropped to 683.5/mm2 by day 8 of EAE whereas the treated rats had higher numbers of surviving RGCs (M20, 929/mm2; MK-801 907/mm2, $p < 0.001$). In retinas from day 7 after immunization, a timepoint preceding histopathological changes in the ON, higher RGC densities were found in M20 (1415/mm2) and MK-801-treated animals (1364 /mm2) compared to vehicle (1064/mm2; $p < 0.002$).

Upon adding 200 μM glutamate to isolated RGCs and oligodendrocytes, intracellular calcium rose rapidly in both cell types. However preincubation with 12 μM memantine significantly reduced the calcium response only in RGCs, confirmed by dose-response experiments to memantine. Conversely, oligodendrocytes were more responsive to blockade of AMPA and kainate receptors.
Conclusions: The results suggest that NMDAr blockade protects RGCs directly independent of an effect on oligodendrocytes and of an anti-inflammatory effect. This indicates an important pathophysiological role of NMDAr mediated glutamate toxicity, highlighting a potentially valid target for therapeutic neuroprotective strategies.

P710
NDC-1308, a gain of function estradiol analog for inducing remyelination in multiple sclerosis patients
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Background: Several estrogens have advanced to clinical trials, yet they impact only the neuroprotective aspects of multiple sclerosis (MS), rather than the elusive remyelination activity needed to repair the damaged myelin sheath. We previously reported that NDC-1308, a proprietary analog of 17-beta-estradiol (E2), activates known intracellular pathways for oligodendrocyte progenitor cell (OPC) differentiation. Unlike E2, NDC-1308 induces mouse OPCs to differentiate into mature oligodendrocytes *in vitro*. While NDC-1308 appears to have retained several qualities

of E2, these studies tested whether it has gained the ability to induce remyelination of axons *in vivo*.

Objectives: The main purpose of this study was to determine the efficacy of NDC-1308, an estrogen receptor (ER) agonist, to repair the damaged myelin sheath in a validated animal model of demyelination. Intended outcomes also include: i) an initial benefit-risk assessment for NDC-1308, ii) determining the dosing parameters for non-clinical IND-enabling studies, and iii) design of the Phase 1 clinical study. As such, NDC-1308 was formulated for injection and assessed for its ability to induce remyelination in the cuprizone mouse model of demyelination.

Methods: NDC-1308 was stably formulated for injection using an SBE-beta-cyclodextrin. Male mice were treated for 12-weeks with cuprizone and rapamycin to cause demyelination of white and gray matter regions of the brain. The demyelinated mice were administered NDC-1308 by intraperitoneal (60 mg/Kg, q.d) or subcutaneous (15-60 mg/Kg, b.i.d.) injections for 3 or 6 weeks. Blood was collected at termination for clinical chemistry analysis, along with reproductive tracts for pathology, and brain regions for assessing the level of NDC-1308 remyelination.

Results: A 3-week NDC-1308 treatment in cuprizone demyelinated mice resulted in greater than 20% ($P< 0.005$) and 16% ($P< 0.05$) remyelination of cortical and hippocampal regions, respectively. At 6 weeks of NDC-1308 treatment, remyelination in hippocampal regions increased to 30% ($P< 0.0001$). E2 did not induce remyelination. All animals tolerated the chronic NDC-1308 treatments well. Observed animal behavior and clinical chemistries were normal, and there was no evidence of aberrant mammary tissue.

Conclusions: These results suggest there may be a significant benefit for MS patients by using NDC-1308 to induce remyelination. Non-clinical IND-enabling studies and a first-in-human Phase 1 study are planned for 2015.

P711

Fingolimod effect on diffuse tissue damage is partly independent of its effect on focal damage in relapsing-remitting multiple sclerosis patients

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Background: Compelling evidence indicates both diffuse and focal tissue damage coexist in the brain of patients with relapsing-remitting multiple sclerosis (RRMS) and may occur independently. Diffuse damage underlies complex degenerative processes, including neuronal and axonal loss, and can be indirectly quantifiable via MRI-derived measures of global brain volume (BV). Focal damage may result from inflammation and manifest as MRI brain lesions and clinical relapses. In Phase 3 clinical trials, fingolimod showed significant reductions in both focal lesions and rate of BV loss in RRMS patients vs. placebo (pbo) and an active comparator.

Objectives: To investigate if the effects of fingolimod 0.5 mg on BV loss are mediated through its effects on focal damage or if fingolimod also acts independently in reducing diffuse damage.

Methods: In a pooled post-hoc analysis of the phase 3, 24-month (M), double-blind, randomized, FREEDOMS (N=1272) and FREEDOMS II (N=1083) studies, we assessed the percent brain volume change (PBVC, measured by the Structural Image Evaluation using Normalization of Atrophy, SIENA) at M12 and 24, in patients with no evidence of focal disease activity, namely, absence of both new active lesions (Gd+ T1 lesions and/or new/enlarging T2 lesions) and clinical relapses. A regression analysis was performed in the pooled intent-to-treat (ITT) population to quantify whether the extent of treatment effect seen with fingolimod vs. pbo in the overall population (unadjusted model), would be maintained after adjusting for new active lesions and on-study relapses (adjusted model).

Results: Of the 1383 patients included in this pooled analysis, 808 patients (pbo=142; fingolimod=666) had at M12 and 573 patients (pbo=79; fingolimod=494) at M24 showed no focal activity. Fingolimod significantly reduced PBVC by 52% vs. pbo over 12 M (-0.22 vs -0.46, p=0.002) and by 42% over 24 M (-0.50 vs -0.86, p=0.006). In pooled ITT population, an absolute difference in PBVC of -0.49% (< 0.001) in favor of fingolimod vs pbo over 24 M was observed and was still evident when adjusting for new active lesions and on-study relapse activity (-0.28%, p< 0.001). The regression model suggests that 57% (-0.28%/-0.49%) of the effects of fingolimod on PBVC are independent of its effects on focal damage.

Conclusions: The effect of fingolimod on diffuse damage is partly independent of its treatment effect on focal damage. This suggests that fingolimod has an effect on both inflammatory and neurodegenerative components of MS.

P712

Anti- SEMA4D antibody ameliorates pathogenic processes related to multiple sclerosis

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Background: Semaphorin 4D (SEMA4D/CD100) is expressed on neural and immune cells, and its high affinity receptor, Plexin B1 (PLXNB1), is expressed on dendritic, endothelial, and neuronal cells. SEMA4D signaling through PLXNB1 has been shown to modulate glial cell activation, inhibit differentiation and migration of oligodendrocyte precursor cells (OPCs), disrupt CNS endothelial tight junctions, and induce neuronal process collapse. Multiple sclerosis (MS) is a chronic neuroinflammatory disease characterized by CNS immune cell infiltration, blood-brain barrier (BBB) breakdown, myelin destruction, and neuronal degeneration. Antibody neutralization of SEMA4D could, therefore, ameliorate multiple sclerosis through multiple mechanisms.

Objectives: Blocking SEMA4D could promote differentiation of oligodendrocyte precursors and remyelination. In addition, preventing SEMA4D-mediated breakdown of the blood brain barrier (BBB) may suppress immune cell infiltration into the CNS and reduce inflammation and secondary immune responses to CNS antigens.

Methods: We generated a monoclonal antibody that binds with high affinity to mouse, rat, monkey, and human SEMA4D and blocks the binding of Sema4D to its cognate receptors.
Results: *In vitro*, anti-SEMA4D reverses the inhibitory effects of recombinant SEMA4D on OPC survival and differentiation. *In vivo*, anti-SEMA4D significantly attenuates experimental autoimmune encephalomyelitis in multiple rodent models by preserving BBB integrity and axonal myelination and can be shown to promote remyelination following chemically-induced demyelination. Current efforts are focused on elucidation of glia-specific SEMA4D signaling mechanisms and how they participate in pathogenic processes related to neuroinflammatory/neurodegenerative disease.
Conclusions: Collectively, these data suggest that antibody-mediated neutralization of SEMA4D represents a viable therapeutic strategy for multiple sclerosis. To this end, a randomized, placebo-controlled, double blind, single ascending dose Phase 1 study in MS patients was initiated using a humanized anti-SEMA4D antibody (VX15/2503). Safety, tolerability, and PK data from this trial are anticipated to be reported in early 2015.

P713

Exploration of spontaneous remyelination and its clinical relevance in MS: a longitudinal PET study with[11] C-PIB

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Background: Quantitative imaging of remyelination is crucially needed in MS, to understand the pathophysiology of the disease, as well as to evaluate the potential of remyelinating therapies.
Objectives: In this longitudinal study, we aimed to explore myelin dynamics in MS patients using positron emission tomography (PET) with [^{11}C]-PIB, which has been shown to bind myelin in the CNS.
Methods: Twenty patients with active MS were clinically assessed and underwent PET with [^{11}C]-PIB on a High Resolution Research Tomograph as well as conventional MRI at 3.0T at baseline and after either 2 or 4 months. Scan-rescan analysis was performed in 8 healthy controls (HC). Voxel-wise maps of [^{11}C]-PIB binding potential (BP), reflecting myelin content, were derived non invasively from PET images applying the Logan graphical method. Individual patient [11C]-PIB BP z-score maps were generated based on white matter values from HC at each time point, computed within previously delineated T2 lesion (T2L) masks, and registered onto a half-way space. [11C]-PIB BP changes over the follow-up period were calculated at the voxel level and considered significant only if below the 5th or above the 95th percentile of HC scan-rescan change. [11C]-PIB BP changes within lesions were then correlated with clinical scores using the Spearman's correlation coefficient.
Results: At study entry, severely demyelinated voxels (defined as having a z-score below 2SD) represented on average the 8,8% of lesional voxels (SD=4%), and were mainly localised in the central part of lesions on visual assessment. Over the follow-up period, 3.3±2.8% (mean±SD) of lesional voxels underwent significant decreases in [^{11}C]-PIB BP consistent with ongoing myelin loss, and 3.7±1.5% of lesional voxels (mean±SD) underwent a significant increase in [^{11}C]-PIB BP, consistent with myelin repair. Remyelinating voxels were mostly localised in the peripheral part of lesions. There was a significant negative correlation between the percentage of T2L voxels undergoing remyelination and EDSS (r=-0.6, p=0.004) and MS severity scale (r=-0.58, p=0.03) scores.
Conclusions: PET with [^{11}C]-PIB proved to be a valuable biomarker for assessing myelin dynamics in MS lesions. The remyelination index derived from PET images has the potential to predict disease severity, and could be of interest as an outcome measure in clinical trials testing promyelinating therapies.

P714

Melanocortin receptor agonist ACTH 1-39 protects rat forebrain neurons from apoptotic, excitotoxic and inflammation-related damage

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Background: Patients with relapsing remitting multiple sclerosis (RRMS) are commonly treated with high doses of intravenous corticosteroids (CS). ACTH 1-39, a member of the melanocortin family, stimulates production of CS by the adrenals, but melanocortin receptors are also found in the central nervous system (CNS) and on immune cells. ACTH is produced within the CNS and may have direct protective effects on glia and neurons independent of CS. Neurons are the most vulnerable cells in the CNS. They are terminally differentiated, and sensitive to inflammatory and excitotoxic insults. We previously found that ACTH 1-39 protected oligodendroglia and their progenitors from a panel of excitotoxic and inflammation-related agents (Benjamins et al., 2013). For therapeutic protection of gray matter in MS, it is important to extend these studies to the effects of ACTH on neurons.
Objectives: To investigate protective effects of ACTH 1-39 on neurons *in vitro*.
Methods: Cultures highly enriched in neurons were isolated from 2-3 day old rat brain (Eide and McMurray, 2005), with the addition of 1 μg/ml nerve growth factor (NGF) (Lisak et al., 2011). Neurons, immunostained for NeuN or neurofilament- H (NF-H), represented 85-90% of the cells, with less than 10% astrocytes and 4% microglia, as determined by double label immunofluorescence. Cultures were treated for 1 day with selected toxic agents with or without ACTH. Neuronal death was assessed by trypan blue uptake; apoptosis was measured by Apoptag staining.
Results: ACTH (200 nM) protected neurons from death induced by 20 nM staurosporine, a classic inducer of apoptosis, by a mechanism including generation of reactive oxygen species. Neuronal death was 12% in untreated control cultures; by 24 hours, staurosporine caused 80% neuronal death, which was reduced to 55%

by ACTH ($p < 0.01$). Apoptag staining also demonstrated protection by ACTH from staurosporine. Immunostaining for NF-H showed that ACTH preserved neuronal processes in the presence of staurosporine. ACTH similarly protected neurons from 100 μM glutamate, 1 mM NMDA, 50 μM AMPA, 150 μM kainate and 25 μM quinolinic acid, a kynurenine pathway metabolite that can produce oxidative damage dependent on or independent of NMDA receptors.

Conclusions: ACTH 1-39 protects neurons *in vitro* from several excitotoxic and inflammation-related insults.

P715

KB3944, a selective estrogen receptor beta agonist, in preclinical development for neuroprotective and regenerative therapy of multiple sclerosis

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Background: There is growing evidence that estrogens can promote remyelination and neuroprotection. Selective estrogen receptor (ER) beta agonists, avoiding the ERalfa mediated increased risk of cancer and thrombosis, represent a novel neuroregenerative therapy of Multiple Sclerosis. Karo Bio has discovered and developed a selective, non-steroidal, small-molecule ER beta agonist, KB3944 that is highly selective over ERalfa. We have recently demonstrated increased myelination and improved electrophysiological function after KB3944 administration in the Cuprizone mouse model. In addition, we have also demonstrated KB3944-induced increased proliferation of oligodendrocytes *in vitro*.

Objectives: The present study was conducted to confirm neuroprotective effects of KB3944 on the neurological disease symptoms in a rat model of relapsing-remitting experimental autoimmune encephalomyelitis (RR-EAE), and an *in vitro* neuroprotection assay.

Methods: RR-EAE was induced in female Dark Agouti rats by subcutaneous injection of an emulsion consisting of homologous spinal cord homogenate. KB3944 was tested at 3, 9 and, 18 mg/kg p.o. twice daily from disease onset until 25 days post-immunization. Neurological disease symptoms were scored daily and histological analyses were carried out. A neuroprotection assay was performed on rat fetal cortical neuronal cultures. Cultures were incubated with KB3944 and were exposed to a brief excitotoxic glutamate pulse. Microtubuli-associated-protein-2 were immunostained for neuronal evaluation 48h after the glutamate insult.

Results: KB3944 significantly reduced disease symptoms over the total treatment period following 9 mg/kg treatment in the RR-EAE experiment. In the *in vitro* neuroprotection assay, low nM concentrations of KB3944 significantly increased the survival of neurons and was effective at stimulating neurite network following glutamate insult.

Conclusions: The selective ERbeta agonist KB3944 reduce neurological scorings in a relapsing-remitting EAE rat model and demonstrates neuroprotective effects after glutamate excitotoxicity *in vitro*, warranting further development of KB3944 as a neuroprotective and regenerative therapy of MS.

P716

The role of vitamin D and gender in optic neuritis recovery

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Background: Optic neuritis is a common manifestation of demyelinating disease. The optic nerve can serve as a model of the central nervous system, allowing for evaluation of inflammation and degeneration using optical coherence tomography (OCT) to measure retinal nerve fiber layer (RNFL) thickness and other afferent pathway markers. Vitamin D insufficiency is a risk factor for multiple sclerosis while vitamin D ameliorates inflammation. Assessment of vitamin D status in optic neuritis may support a neuroprotective role as well.

Objectives: We hypothesize that vitamin D sufficiency (25(OH) D > 80 nmol/L) is associated with better OCT outcomes and axonal preservation/recovery after optic neuritis. Outcomes include RNFL thickness, ganglion cell layer (GCL) thickness and inter-eye difference (IED) in both at 6 months and baseline between vitamin D sufficient and insufficient groups.

Methods: In this prospective cohort study, a total target of 50 patients with acute optic neuritis undergo OCT to assess RNFL GCL, macular volume (MV) and serum 25(OH)D testing at baseline and month 6.

Results: Fifty-four patients were screened and 50 enrolled, although that number dropped to 49 with a change in diagnosis. Of the 49 patients studied, 68% were vitamin D insufficient at baseline, which was associated with greater baseline edema in RNFL (131 vs. 106 μm, $p=0.14$) and MV (10.2 vs. 9.8 mm^3, $p=0.036$). At month 6, while RNFL edema persisted and the higher RNFL IED in vitamin D insufficient patients did not reach statistical significance (12 vs. 8 μm), the higher GCL IED in insufficient versus sufficient patients (14 vs. 6 μm, $p=0.067$) was a more robust finding. This is presumably related to the absence of edema in the GCL. Regardless of baseline RNFL or vitamin D status, men had significantly lower 6-month RNFL (70 vs. 81 μm, $p=0.018$), lower 6-month GCL (60 vs 67 μm, $p=0.040$) and greater IED in both the RNFL and GCL (21 vs. 7 μm, $p=0.003$ and 21 vs. 8 μm, $p=0.003$ respectively) versus women.

Conclusions: Not surprisingly, vitamin D insufficiency at optic neuritis onset is associated with greater baseline edema. At 6 months, there is also evidence that vitamin D insufficiency is associated with possible neuronal/axonal loss in the optic nerve. Furthermore, we have shown that male gender is an independent risk factor for poorer OCT outcomes at 6 months. Thus, despite residual edema at 6 months, OCT data suggests vitamin D and female gender may confer neuroprotection and/or improved recovery of the optic nerve after optic neuritis.

P717

Tissue plasminogen activator (tPA) influences recovery after white matter damage by acting on astrocytes and oligodendrocyte progenitors

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Background: Multiple sclerosis (MS) is a chronic disease of the central nervous system (CNS) characterized by successive cycles of demyelination and remyelinisation. Some studies suggest that tissue plasminogen activator (tPA), a serine protease expressed in the CNS, could be involved in MS.
Objectives: The aim of this study is to define the role of tPA in the processes of demyelination and remyelination in animal models of MS.
Methods: First, we have shown that the outcome of experimental autoimmune encephalomyelitis (a classical model of MS) is worse in tPA knockout mice than in wild type mice. We hypothesized that this result could be due to an effect of tPA on demyelination and/or remyelination. To test this hypothesis, we used a model of focal demyelination, in which we compared tPA knockout mice to wild type mice.
Results: We observed that the size of white matter lesions was enhanced and that recovery was slower in tPA knockout mice. We observed by histological analysis that this effect was associated to changes in astrocyte and oligodendrocyte maturation, proliferation and migration into the injured area.
Conclusions: In conclusion, this study suggests positive actions of tPA on remyelination, suggesting potential benefit in white matter injury occurring in CNS disease such as MS.

P718
Laquinimod treatment prevents cuprizone-induced demyelination independent of Toll-like receptor signaling via MyD88 and TRIF
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Background: Laquinimod (LAQ) is a small, orally active and well-tolerated molecule that has been shown to reduce disability progression, brain atrophy and relapse rate in patients with relapsing-remitting multiple sclerosis (MS). LAQ reduces demyelination and axonal damage in mice with cuprizone-induced demyelination. These effects were shown to be mediated via down-regulation of astrocytic NFkB activation.
Objectives: To test whether Toll-like receptor signaling plays a key role for LAQ-mediated effects on cuprizone-induced demyelination, inflammation, axonal damage and astrogliosis.
Methods: 10-week-old male C57BL/6J and MyD88$^{-/-}$ and TRIF$^{-/-}$ mice received 0.25% cuprizone for 6 weeks. They were treated daily with 0 or 25 mg/kg LAQ. After 6 weeks of cuprizone induced demyelination axonal damage, macrophage infiltration and astrogliosis were analyzed in the corpus callosum.
Results: Untreated control as well as MyD88$^{-/-}$ and TRIF$^{-/-}$ mice displayed extensive callosal demyelination as well as microglial and astrocyte activation. Control as well as MyD88$^{-/-}$ and TRIF$^{-/-}$ mice treated with 25 mg/kg LAQ in contrast showed mainly intact callosal myelin. The demyelination score was significantly higher in all untreated mice groups (control as well as MyD88$^{-/-}$ and TRIF$^{-/-}$) compared to mice treated with LAQ. There were significantly fewer APP-positive axonal spheroids, MAC3-positive macrophages/microglia and less astrogliosis in the corpus callosum of LAQ-treated mice in comparison to untreated controls.
Conclusions: Our results show that LAQ has a preventive effect in the cuprizone model of toxic demyelination. The present data suggest that effects of LAQ may not involve Toll-like receptor signaling. Furthermore, these findings indicate that LAQ may have a role in the future treatment of MS.

P719
Promoting re-myelination in MS via the GPR17 receptor, a new key actor in oligodendrogenesis
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Background: We have recently proposed GPR17, a key regulator of Oligodendrocyte Precursor Cells (OPCs), as a new target for re-myelination in MS. GPR17 is expressed early in OPCs, is needed to start differentiation but is turned down before cells reach functional maturation. Of note, GPR17 is upregulated *in vivo* under demyelinating conditions like focal cerebral ischemia or lysolecithin-induced demyelination, suggesting that its alterations may contribute to myelin dysfunction.
Objectives: Here, we aimed at (i) analysing GPR17 expression in the experimental autoimmune encephalomyelitis (EAE) MS rodent model, and (ii) assessing the functional consequences of persistent GPR17 up-regulation in cultured OPCs.
Methods: EAE was induced on C57BL/6 female mice and immunohistochemical analysis performed 21 days after immunization. Cultured OPCs were transfected with a fluorescent reporter plasmid in which GPR17 coding sequence had been fused with Green Fluorescence Protein (GPR17-GFP). As a control, the same GFP empty vector was used. Cells taking up these plasmids become green and their final destiny can be traced by fluorescence microscopy.
Results: In EAE spinal cord, increased numbers of GPR17^{+} OPCs accumulated around areas of tissue infiltration by blood-derived cells. To confirm that this really reflected receptor up-regulation, GPR17 mRNA was increased in EAE compared to Control (qRT-PCR, $p<0.0001$). *In vitro*, at variance from OPCs transfected with the control GFP empty plasmid that underwent spontaneous differentiation in culture, cells incorporating the GFP-GPR17 vector for forced GPR17 over-expression maintained an immature phenotype and never expressed the mature marker CNPase, confirming that interferences with GPR17 physiological silencing prevents terminal maturation.
Conclusions: Our *in vitro* data show that failure to downregulate GPR17 in late stage OPCs impairs progression to myelinating phenotypes and blocks cells at immature stages. Important, in EAE mice, GPR17 is upregulated at demyelinated sites: we propose this alteration to contribute to insufficient re-myelination in MS. We also propose that pharmacological agents reverting GPR17 upregulation may induce OPCs to overcome this maturation block and to resume myelination. In this respect, we are currently developing new diverse chemical entities acting as selective GPR17 ligands.
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P720

The role of CDP-choline in CNS remyelination

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Background: Remyelination is the natural repair mechanism of CNS demyelinated lesions in multiple sclerosis (MS), but it is often incomplete or even fails. Since demyelinated axons are vulnerable to atrophy, remyelination might protect against progressive axonal damage and thus long-term disability in MS patients. Still, therapeutic approaches to increase myelin repair are not available.

Objectives: We analyzed the effects of cytidine-5`-diphospho (CDP)-choline on CNS remyelination in mice after cuprizone induced demyelination.

Methods: To induce demyelination C57BL/6 male mice were fed with 0.2% cuprizone for up to 5 weeks. Animals received CDP-choline, beginning on the day of cuprizone feeding, for the subsequent days until the animals were killed. Mice were sacrificed at different time points to analyze the effects of CDP-choline on de- and remyelination (for demyelination weeks 4, 4.5 and 5; for remyelination weeks 5.5 and 6). Mouse brains were investigated using histological and immunohistological staining methods and ultrastructural analyses. Additional *in vitro* studies with CDP-choline in oligodendrocyte precursor cell and microglia cell cultures were also performed.

Results: CDP-choline did not exert any effects on cuprizone induced demyelination. During remyelination CDP-choline effectively increased myelin repair. Mice treated with CDP-choline presented high densities of new myelin proteins throughout the complete corpus callosum and increased numbers of myelinated axons as shown by electronmicroscopy. The increased remyelination arose from an increase in the numbers of proliferating oligodendrocyte precursor cells and oligodendrocytes. *In vitro* studies suggest that this process is regulated by protein kinase C.

Conclusions: We have identified CDP-choline as a new substance to enhance CNS remyelination. Due to its regenerative action combined with a known excellent safety profile, CDP-choline could become a promising substance for MS patients as add-on therapy.

P721

Small molecule inducers of oligodendrocyte differentiation

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Background: A primary pathological feature of multiple sclerosis is the loss of myelin-producing oligodendrocytes (OL) and myelin sheaths, resulting in axonal loss and irreversible neurological decline. Recent animal studies have shown that the most effective way to protect axons is restoration of myelin. Although there are plenty of oligodendrocyte progenitor cells (OPCs) within MS lesions, the efficiency of spontaneous generation of OL declines dramatically with every episode of demyelination. Therapeutic intervention to stimulate differentiation of OL and remyelination has the potential to reverse function decline in MS patients.

Objectives: To identify new small molecule compounds that stimulate differentiation of OL, using a highly pure population of mouse primary OPCs and high-content screening system in a medium throughput 96-well plate format.

Methods: OPCs were derived from E14.5 mouse embryos expressing *PLP*-EGFP. Small molecules were used at 10 μM and media was changed once at 48 hrs. Cells were fixed and stained with nuclear dye Hoechst. Plates were imaged in a Cellomics Arrayscan and algorithms were used to distinguish pyknotic cells, viable cells and EGFP+ OL.

Results: With thyroid hormone T3 as a control, we optimized the screening system to achieve a consistently positive Z′ score and repeatable results. Using this system, we identified 45 hits with EC50 lower than 1uM after screening a library containing 20,000 small molecules. The effect of these hits was confirmed in secondary screening using oligodendrocyte markers O4 and MBP. These hits belong to at least six distinct chemical structural groups, with a EC50 of ~0.02uM for the lead compound. Western blotting analysis further confirmed that protein expression levels of oligodendrocyte differentiation markers, including PLP, CNPase, and MBP, were stimulated as early as 24hr at levels similar to or higher than our positive control T3. These compounds do not contain obvious toxicophores and have low toxicity, as revealed by MTT assay. The molecular pathways stimulated by these compounds are being investigated.

Conclusions: Using our optimized high-content screening system, we have identified several small molecule leads that can induce differentiation of oligodendrocytes with high efficacy and low toxicity. These leads have the potentials to be developed into remyelination therapeutics to fill unmet needs of MS patients.

P722

TGFβ signaling drives oligodendrocyte development and regeneration

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Background: Research on myelination has focused on identifying molecules capable of inducing oligodendrocyte progenitors (OP) differentiation in an effort to develop strategies that promote oligodendrocyte (OL) regeneration and functional myelin regeneration in demyelinating disorders.

Objectives: We studied the role of TGFbeta signaling in oligodendrocyte development and regeneration after demyelination.

Methods: We used conditional genetic mouse models and pharmacological approaches combined with developmental studies and animal models of demyelination.

Results: Here, we show that TGFβ signaling is crucial for allowing OP cell cycle withdrawal, and therefore, for oligodendrogenesis and postnatal CNS myelination. Selective deletion of TGFβ-RII in OP prevents OL differentiation and causes deficits in myelination during postnatal development and delays

remyelination in animal models of demyelination. Conversely, TGFβ signaling pharmacological activation induces OPs differentiation, accelerating CNS postnatal myelination and functional remyelination after demyelination.
Conclusions: These studies reveal a pivotal role of TGFβ signaling in OL development and regeneration during postnatal development and remyelination that may provide new therapeutic avenues for the treatment of demyelinating disorders.

P723
Lineage tracing reveals dynamic changes in PDGF alpha receptor-derived cells following cuprizone-induced demyelination
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Background: Oligodendrogenesis is essential for successful remyelination and repair in demyelinating diseases such as multiple sclerosis (MS). Understanding the time course of the oligodendrocyte progenitor cell (OPC) response to demyelination in the cuprizone model may help determine the kinetics of the remyelinating response and could allow further modeling of why the process does not efficiently occur in MS patients.
Objectives: To observe the accumulation and maturation of OPCs over time in a mouse model of demyelination/remyelination.
Methods: Fate mapping of OPCs was performed using *PDGFαR-CreER;*Rosa26-eYFP mice that conditionally express YFP in OPCs after induction of recombination with 4-hydroxytamoxifen (4-HT). Mice were fed a diet of 0.2% cuprizone, a demyelinating neurotoxin, or regular chow for 6 weeks. Both groups then received regular chow for an additional 4 weeks. Recombination was induced with 4-HT at week 2. Mice were sacrificed weekly, beginning 2 weeks after recombination. The fate of the OPCs was determined imunohistochemically via colocalization of eYFP with multiple cellular makers including PDGFαR (OPCs), CC1 (oligodendrocytes), GFAP (astrocytes), and NeuN (neurons).
Results: At 4 weeks the density of YFP^+ $PDGF\alpha R^+$ cells (OPCs) was 3 fold higher in the corpus callosum of cuprizone treated animals as opposed to controls, while the density of YFP^+CC1^+ cells (mature oligodendrocytes) was low in both groups. This was followed by a 6 fold increase in the density of YFP^+CC1^+ cells at the 6 week time point and a return of YFP^+ $PDGF\alpha R^+$ cell density to control levels. Interestingly, the kinetics of OPC differentiation and maturation in gray matter areas appeared to be quite different with much less expression of recombined cells at these time points. Additionally, we observed no deviation of OPCs into astrocytes (YFP^+GFAP^+) or neurons (YFP^+NeuN^+) in any brain regions. Analysis at other time points is ongoing.
Conclusions: Overlaying data from OPC fate mapping in the cuprizone model with knowledge of key demyelination/remyelination time points in white matter vs gray matter may provide unique insight into the capacity and mechanisms of remyelination in different parts of the brain. The weekly time course of these events will allow us to better understand OPC proliferation, accumulation, maturation, and remyelination, and could provide insight into how this may occur in MS.

P724
Effects of vitamin D on axonal loss during de- and remyelination
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Background: Axonal injury and degeneration are considered the major cause of neurological disability in multiple sclerosis (MS). Vitamin D has been shown to modulate relapse risk and MRI disease activity in a favorable manner, through immunomodulatory pathways.
Objectives: To study the effects of vitamin D on axonal damage and axonal regeneration in the cuprizone model for de- and remyelination.
Methods: To study the effects of vitamin D on prevention of axonal loss during demyelination, 48 female c57Bl/6 mice were exposed to 0.2% (w/w) cuprizone and supplemented with either low dose cholecalciferol (< 50 IU/kg or 500 IU/kg) or high dose cholecalciferol (6200 IU/kg or 12500 IU/kg). The mice were euthanized after six weeks of cuprizone exposure.

To study the effects of vitamin D on axonal regeneration, 42 female c57Bl/6 mice were exposed to 0.2 % (w/w) cuprizone for five weeks, and then treated with either 0.2µg calcitriol or placebo intraperitoneally twice weekly. Mice were sacrificed after seven weeks of cuprizone exposure, or after one or three weeks of remyelination.

Paraffin embedded coronal sections from the bregma were stained immunohistochemically for axonal transection by anti-amyloid precursor protein (APP), and for axonal loss by phosphorylated (neurofilament light chain, NFL) and non-phosphorylated neurofilament (SMI-32) antibodies. Axonal transection was quantified as the density of APP-immunopositive bulbs in the midline of the corpus callosum. Phosphorylated and non-phosphorylated neurofilament was quantified by calculating the area of immunopositive staining, in digital images from the midline of the corpus callosum.
Results: After six weeks of cuprizone exposure, there was a significant effect of high dose cholecalciferol on the extent of axonal loss, as measured by the area of NFL immunopositivity, 78.5% ± SD 9.27 (high dose) vs. 50.3% ± SD 26.6 (low dose, p=0.006). There was a trend towards a significant difference in the density of transected axons, 74.9 bulbs/0.0625mm2 ± SD 57.2 (high dose) vs. 109.7 ± SD 38.9 (low dose, p=0.06). There were no significant differences between the calcitriol- and placebo-treated groups on either axonal transection or loss during remyelination.
Conclusions: High dose cholecalciferol, given during cuprizone exposure, seems to have a protective effect on axonal loss. High dose cholecalcitriol, given after the demyelination phase, seems not to influence axonal regeneration.

P725

Non-steroidal anti-inflammatory drug promotes remyelination

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Background: Inflammation and permanent demyelination contribute to axonal damage and loss, the cause for progressive neurological deficit observed in MS patients. Differentiation of oligodendroglial precursor cells (OPCs) to myelinating oligodendrocytes is often impaired, resulting in limited remyelination, especially in chronic MS lesions. Multiple pathways, among them the Wnt/beta-catenin signaling cascade has been implicated to contribute to remyelination failure in MS. In earlier studies we could show that a non-steroidal anti-inflammatory drug (NSAID) is able to promote significantly the differentiation of murine oligodendrocytes.
Objectives: Aim of our study was 1. to determine whether the NSAID promotes also the differentiation of human oligodendrocytes 2. to study the remyelination promoting capacity of the NSAID and 3. to dissect the mechanisms by which the NSAID promotes oligodendroglial differentiation.
Methods: Human and murine oligodendroglial cell culture, qRT-PCR, reporter assays, Western blots, cuprizone model, immunohistochemistry, EM
Results: We observed a significantly increased number of mature oligodendrocytes after addition of the compound to human oligodendroglial cell cultures. In the cuprizone model daily injections of the NSAID during the remyelinating phase resulted in higher numbers of mature oligodendrocytes, increased expression levels of myelin associated genes and accelerated remyelination using immunohistochemistry, qRT-PCR and electron microscopic studies. Exposure of murine oligodendrocytes to the compound increased phosphorylation of beta-catenin at serines 33, 35 und 37 as shown in Western blots. The positive effect of the NSAID on oligodendroglial differentiation was abrogated using inhibitors of glycogen synthetase 3 beta (GSK3b) that is part of the beta-catenin degradation complex. Transfection of a murine oligodendroglial cell line with a reporter assay confirmed the downregulation of the Wnt/beta-catenin signaling pathway after addition of the NSAID. Furthermore, in genetically modified oligodendrocytes in which beta-catenin cannot be phosphorylated and degraded, no effect of the NSAID on differentiation was observed.
Conclusions: The NSAID promotes differentiation of murine and human oligodendrocytes *in vitro* and enhances remyelination; furthermore our results suggest that these effects are at least partly mediated by activation of GSK3beta and increased degradation of beta-catenin.

P726

The effects of GSK239512 on lesion remyelination in a relapsing remitting MS population: design of a phase 2a imaging study

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Background: GSK239512 is a potent, selective, orally bioavailable and brain penetrant histamine 3 receptor (H3R) antagonist/inverse agonist. In MS, evidence supports a failure of oligodendrocyte precursor cells (OPC) differentiation as the root cause of remyelination failure. Preclinical studies demonstrating H3R expression and activity on OPCs have indicated the potential for development of GSK239512 in MS.
Objectives: To present the design and preliminary subject disposition of a GSK-funded phase 2a study investigating the effect and safety of GSK239512 in promoting remyelination in patients with relapsing remitting MS (RRMS), using brain MRI assessments of magnetization transfer ratio (MTR) to detect lesion remyelination.
Methods: GSK study H3M116477 is a randomized, parallel group, placebo-controlled study (CT.gov identifier: NCT01772199; EudraCT identifier: 2012-003627-38). Subjects with RRMS able to maintain stable background treatment (≥1 year prior to enrollment) with either Avonex (Interferon-beta1a) or Copaxone (Glatiramer Acetate) for the duration of the study were randomized in a 1:1 ratio between placebo and GSK239512 for a total treatment period of 48 weeks. The primary endpoint is the evaluation of mean changes in lesion myelination using two MTR endpoints comparing placebo to GSK239512 treated subjects:

1) New Gadolinium enhanced (GdE) lesion MTR differences (calibrated to reference scan) from before enhancement to stable recovery (≥3 months post new GdE lesion), and
2) New Delta MTR lesion MTR differences (calibrated to reference scan) from before lesion appearance to stable recovery (≥3 months post lesion appearance). Secondary endpoints are designed to further evaluate the impact of GSK239512 on standard MRI, MS Clinical, and Safety measures.

Results: The study enrolled 131 patients, 18-50 years of age, with RRMS (2010 revised McDonald criteria), an Expanded Disability Status Scale (EDSS) score of 1.0-4.5, disease duration ≤10 years and evidence of recent clinical disease activity (i.e., relapse or lesion activity within the prior year).
Conclusions: Using changes in MTR, this study is designed to establish the ability of GSK239512 to promote lesion remyelination. Secondary MRI and clinical endpoints will assist in data interpretation and in the design of future studies to interrogate the clinical benefit of lesion remyelination in RRMS patients.

P727

Dimethyl fumarate enhances glutathione recycling by increasing expression and function of glutathione reductase

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Background: Chronic disability in multiple sclerosis (MS) is due to neuronal degeneration, which is not or only incompletely amenable to immunomodulatory therapy. The underlying mechanisms

remain elusive, but there is accumulating evidence that oxidative stress may play a key role. Dimethylfumarate (DMF) is a novel oral therapeutic, which reduces disease activity and progression in patients with relapsing-remitting MS. These effects are presumed to originate from a combination of immunomodulatory and neuroprotective effects.

Objectives: In this work, we determined time- and concentration-dependant effects of DMF on viability in a model of endogenous neuronal oxidative stress.

Methods: By measuring the intracellular gluthathion content as well as its recycling, the nuclear translocation of transcription factors and the expression of cyto-protective genes we were able to show the protective effect of dimethylfumarate.

Results: Previous work from our laboratory demonstrated that DMF protects from oxidative stress by enhancing glutathione (GSH) synthesis and recycling. Recycling is mediated by glutathione reductase (GSR), a homodimeric flavoprotein that catalyzes GSSG reduction to GSH by using NADPH as a reducing cofactor. Protection was still present after inhibition of GSH synthesis in medium lacking cystine, the limiting amino acid of the tripeptide GSH. Protective concentrations of DMF induced GSR at the protein level. Knock down of GSR by siRNA abolished the protective effect of DMF in conditions with inhibited glutathione synthesis.

Conclusions: We conclude that DMF enhances glutathione recycling by increased expression and function of glutathione reductase.

P728

A high throughput flow cytometry based approach to assess the differentiation of oligodendrocyte precursor cells into mature oligodendrocytes

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Background: Oligodendrocytes are specialised cells in the brain responsible for myelinating neurons. In multiple sclerosis (MS), myelin becomes damaged, and over time, repair of demyelinated lesions fails. Oligodendrocyte precursor cells (OPCs) are present in MS brain, but appear trapped in an immature form, unable to differentiate and remyelinate lesions. Thus, promoting the differentiation of OPC's into mature, myelinating oligodendrocytes is an attractive regenerative strategy with potential therapeutic efficacy. The maturation of OPCs is immunocytochemically well characterised and is typically quantified by manual counting and applying a scoring system based on the percentage of cells that have a mature phenotype compared to total cells. Such a method is labour intensive and time consuming.

Objectives: The aim of our work was to apply flow cytometry to develop a sensitive, high throughput assay to detect subtle changes in OPC differentiation by investigating multiple cellular markers simultaneously.

Methods: Flow cytometry was used to investigate OPC differentiation in cells obtained from P2 Wistar rat brains. Mixed glial cell preparations were grown for 10 days, shaken at 200rpm to obtain OPCs and pre-plated to remove microglia before seeding. Cells were treated with fibroblast growth factor (FGF)/platelet derived growth factor (PDGF) for 3 days and then maintained in differentiation conditions for a further 3 days. Expression of GLAST and CD11b were used to exclude astrocytes and microglia respectively and oligodendrocyte markers A2B5, O4 and O1 were used to assess and quantify differentiation.

Results: Astrocytes and microglia represented approximately 10-15% of total cells. OPCs maintained in FGF/PDGF remained in an immature state with no O1 expression. O1 expression was induced by growth factor withdrawal and by triiodothyronine (T3). Other, previously described, promoters of differentiation such as 9-cis retinoic acid also induced O1 expression. Simultaneous analysis of A2B5 and O4 showed de-differentiation of the OPCs 1 day post FGF/PDGF treatment compared with OPCs stained directly post shake off.

Conclusions: Flow cytometry is a robust, reproducible, data rich and a novel platform to investigate and quantify OPC maturation on a high throughput scale.

P729

Serial individual lesion MTR follow-up for studies of potentially remyelinating therapies in MS

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Background: Magnetisation Transfer Ratio (MTR) decreases with demyelination, and increases with remyelination. A trial of autosomal mesenchymal stem cells in secondary progressive MS analysed mean MTR of all lesions serially to calculate rate of change of MTR before and after intervention and showed a non-significant trend for MTR increase during the post-treatment phase. This technique, however, is not sensitive to changes within individual lesions.

Objectives: To develop a lesion analysis pathway allowing individual lesion MTR to be measured serially, investigating lesion myelination before and after a treatment intervention.

Methods: 10 patients had 3 MRI scans prior to infusion (6, 3, 0 months before), and 2 scans post infusion (3, 6 months after). Lesions were marked on $T2_w$ and $T1_w$ scans obtained at baseline, and follow-up scans were registered to the baseline. Baseline lesion masks were registered to the baseline scan, then divided into individual lesion masks. Registrations were visually checked by two assessors. Individual lesion masks were applied to the MTR maps, to calculate the MTR of each $T2_w$ and $T1_w$ lesion at each time-point. A piecewise linear mixed model was used to model the MTR gradients of individual lesions before and after infusion; a similar model was used in the previous analysis of average lesion MTR level gradients.

Results: All results are in percent units per month. For mean $T2_w$ lesion MTR, rate of MTR change was -0.1738 before infusion, and +0.3859 after infusion. The difference in rate of change after infusion was +0.5597 (p=0.186, 95% CI -0.2703, +1.3896). For individual lesion MTR, rate of change of T2w lesions was -0.052 before infusion, and +0.045 after infusion. The difference in rate

of change after infusion was +0.097 (p< 0.001, 95% CI +0.071, +0.124). For mean $T1_w$ lesion MTR, rate of MTR change was -0.1867 before infusion, and + 0.5791 after infusion. The difference in rate of change after infusion was +0.7659 (p=0.097, 95% CI -0.1389, +1.6706). For individual lesion MTR, rate of change of T1w lesions was -0.065 before infusion, and -0.016 after infusion. The difference in rate of change after infusion was +-0.049 (p=0.052 95% CI -0.001, +0.099).

Conclusions: Serial follow-up of individual lesion MTR showed significantly different gradients of MTR change during the pre- and post-treatment phases for $T2_w$ lesions, and may provide a more sensitive indicator of myelination in clinical trials of potential remyelinating therapies.

P730

Neuroprotective effects of hesperidin in a C57BL/6 mouse model of multiple sclerosis

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Background: Experimental allergic encephalomyelitis (EAE) is a T cell-mediated inflammatory demyelinating autoimmune disease that serves as an animal model for multiple sclerosis (MS). Destruction of myelin sheaths and oligodendrocytes as well as neurodegeneration in MS are associated with massive oxidative stress and mitochondrial injury.

Objectives: It was thought that antioxidant and anti-inflammatory compounds such as flavonoids may protects nervous system against autoimmune disease such as MS. Hesperidin (HP) is a bioflavonoid found abundantly in Citrus species, such as lemon and orange, that has been reported to possess pharmacological activities mainly antioxidant and anti-inflammatory effects. We evaluate the effects of hesperidin in experimental MS model.

Methods: To explore the therapeutic potential of HP totally 40 C57Bl/6 mice were equally divided into four groups:

(1) Control,
(2) EAE,
(3) HP, and
(4) HP+EAE.

After induction of EAE with (MOG_{35-55}) and pertussis toxin, the mice treated with HP at the doses of 50 mg/kg/day for 7 days subcutaneously.

Results: To our results, induced oxidative stress in EAE group via an increase in lipid peroxidations (TBARS) and decrease in elements of the antioxidant defense systems (SOD, CAT, GSH and GPx) in brain tissue significantly prevents with HP treatment. Also, HP led to a decrease in high level immunstaining of IL-17 (cause express in pro-inflammatory cytokines) and caspase-3 (show apoptosis) and histopathological damage in brain due to EAE. Besides, TNF-α and IL-1β levels were decreased with HP administration in mice of EAE+HP group.

Conclusions: In conclusion, the current study demonstrated that HP treatment effectively prevents oxidative, immunological and histological damage in the brain caused by EAE. It was thought that the beneficial effects of HP are likely a result of its strong antioxidant and anti-inflammatory properties. Therefore, HP may be clinically useful for the treatment of MS.

P731

A phase II study of the anti-LINGO-1 monoclonal antibody, BIIB033, in subjects with acute optic neuritis: baseline data

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Background: Acute optic neuritis (AON) is characterized by inflammatory demyelinating and axonal injury to the optic nerve and is frequently the first MS manifestation. AON slows nerve conduction velocity (NCV) and compromises visual function. LINGO-1 is a CNS-specific cell surface glycoprotein that may suppress remyelination and axonal regeneration in CNS diseases. In rodent models of optic nerve injury, LINGO-1 blockade leads to axonal protection and improved retinal ganglion cell survival. An anti-LINGO-1 monoclonal antibody, BIIB033, blocks LINGO-1 and was well tolerated in Phase I studies in healthy volunteers and MS subjects.

Objectives: To evaluate efficacy, safety, and pharmacokinetics of BIIB033 in a Phase II study of subjects with AON (RENEW) based on positive findings from preclinical and Phase I studies.

Methods: RENEW is being conducted in 11 countries in subjects with a first, unilateral episode of AON. Eligible individuals (N=82; aged 18-55) were randomized to receive intravenous infusions of BIIB033 (100 mg/kg) or placebo every 4 weeks for 20 weeks. The primary efficacy endpoint is change in optic NCV at Week 24 for the affected eye from the baseline value for the unaffected eye, using full-field visual evoked potentials. Secondary endpoints include change in thickness of the retinal nerve fiber layer (RNFL) and retinal ganglion cell layer/inner plexiform layer (RGCL/IPL), using spectral domain optical coherence tomography and change in low-contrast letter acuity (LCLA) and high-contrast visual acuity (HCVA) using Sloan charts. Vision-related quality of life is being measured using the 25-item National Eye Institute Visual Functioning Questionnaire (NEI-VFQ-25) and 10-item neuro-ophthalmic supplement. Safety and tolerability is being monitored, and population pharmacokinetics of BIIB033 will be assessed (ClinicalTrials.gov #NCT01721161).

Results: 81 patients were enrolled and dosed (mean [SD] age, 32.1 [8.1] years; female, n=57; white, n=78; black, n= 3). Full baseline demographic/clinical characteristics (e.g., time from onset to randomization, AON diagnostic criteria, optic NCV,

RNFL and RGCL/IPL thickness, and LCLA/HCVA) will be described.

Conclusions: Baseline data from RENEW will identify potential treatment group differences and establish pre-treatment reference points for assessing potential effects of BIIB033 on visual function and paraclinical markers of demyelination/axonal loss among participants with a first episode of AON.

P732

Production of differential screening-selected gene aberrative in neuroblastoma (DAN) in the CNS may support neurogenesis/ oligodendrogenesis in MS

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Background: The inadequate tissue repair of multiple sclerosis (MS) lesions is assumed to be, in part, due to dysregulated expression of signaling molecules that induce oligodendrogenesis and neurogenesis

Several bone morphogenic proteins (BMP) stimulate the differentiation of neuronal stem cells (NSCs) towards astrogliogenesis at the expense of oligodendrogenesis and neurogenesis. BMP-2,4,5 are upregulated in immune cells of multiple sclerosis patients, while their antagonists, noggin and follistatin, are down regulated in these cells.

Differential screening-selected gene aberrative in neuroblastoma (DAN) is a member of the DAN family of secreted glycoproteins that are putative BMP antagonists. Its role in multiple sclerosis has not yet been studied.

Objectives: To study the expression of DAN levels in the sera, CSF and supernatants of immune cells of patients with relapsing-remitting MS (RRMS).

Methods: Twenty four untreated RRMS patients, 25 interferon b (IFNb) treated RRMS patients and 26 matched healthy controls (HC) participated in the study. The control group of CSF donors were patients with non-infectious and non-inflammatory disorders. DAN levels in the sera, CSF and the supernatants of peripheral blood mononuclear cells (PBMCs), that were either not stimulated or stimulated with anti-CD3/CD28 mAb, or LPS, were measured by ELISA with a sensitivity of 125 pg/ml.

Results: In the CSF, DAN levels were detected only in the RR-MS patients group (mean±S.D= 591.9±147.3 pg/ml, p=0.031) as compared to CSF from control group. There were no significant differences in the sera levels of DAN between untreated patients with RRMS (720.7±486.1 pg/ml) vs. HC (898.1±615.2 pg/ml) and vs. IFN-b treated RRMS patients (975.1±432.6 pg/ml). DAN levels were below the detection threshold in supernatants of all studied group and stimulatory conditions.

Conclusions: Reduced production of BMP antagonists from PBMCs of MS patients was previously demonstrated and led to the suggestion that immune mediated neuroregenesis/oligidendrogenesis is defective in patients with MS. Our results show that DAN, a BMP antagonist, is not expressed by immune cells. Moreover, the increased DAN levels in the CSF of RR-MS patients, with no differences in sera levels between RRMS patients and HC, suggest a new and unreported aspect of the central nervous system attempt to induce neurogenesis/ oligodendrogenesis mediated by DAN in MS.

Neuropsychiatric aspects

P733

Predictors of fear of sexual rejection in individuals with multiple sclerosis

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Background: Multiple Sclerosis (MS) is an autoimmune disease of the central nervous system that is often associated with a myriad of physical and cognitive impairments. Sexual dysfunction (SD) is a common yet often overlooked symptom in MS.

Objectives: The present study aimed to explore one type of SD, namely perceived fear of sexual rejection among individuals with MS. Predictor variables, including gender, age, employment status, bladder/bowel symptoms, mental health status, and disease severity were explored.

Methods: The sample comprised of 5979 respondents to the spring 2006 North American Research Committee on Multiple Sclerosis questionnaire. Respondents who answered the fear of sexual rejection item on the Multiple Sclerosis Intimacy and Sexuality Questionnaire-19 in the MS Intimacy and Sexuality Section were included in the analysis. The Patient Determined Disease Steps was used to measure disease severity. The 12-Item Short-Form Health Survey, Version 2, Mental Health Scale was used to assess mental health. Demographic information was also collected.

Results: A logistic regression analysis was performed. The full model containing all predictors was statistically significant, c^2 (7, N = 5979) = 1426.01, $p < .001$, indicating that the model was able to distinguish between respondents who reported fear of sexual rejection and those who did not. The model as a whole explained between 21.2% and 29.0% of the variance in fear of sexual rejection, and correctly classified 72.9% of cases. Five of the independent variables made a unique statistically significant contribution to the model, including gender, bladder symptoms, bowel symptoms, disability, and mental health. The strongest predictor of reporting fear of sexual rejection was gender.

Conclusions: Findings indicate that SD, particularly fear of sexual rejection, is a complex and multi-faceted phenomenon. Overall, this study highlights the need for interventions designed to help people with disabilities work through their fears and build their sexual confidence to help facilitate healthy sexual activity. Women with disabilities may be particularly disadvantaged in terms of their sexual health and esteem and would likely benefit from gender-specific interventions for women with physical impairments to help address their individual needs regarding the fear of sexual rejection.

P734

Development of the neurological coping index for multiple sclerosis (NCI-MS)

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Background: Coping in MS refers to cognitive and behavioural efforts to manage stresses imposed by the illness. Several coping strategies may be utilised, such as Problem-focused, Emotion-focused, and Avoidance. There are generic measures of coping such as the COPE, but an MS-specific measure of coping has not been published.
Objectives: Our aim was to develop the first MS-specific measure of coping.
Methods: Following qualitative interviews with MS patients and item extraction, a draft Coping Index and the COPE were given to MS patients attending hospital clinics in the UK. Data from the Index were fit to the Rasch model. Fit was judged by a non-significant chi-square, item and person fit residual SD < 1.4; and both the assumptions of local independence of items and unidimensionality upheld. Differential Item Functioning (DIF) was tested for age, gender, marital status, disease duration and type, and EDSS level. Concurrent validity was ascertained against the COPE.
Results: 254 people with MS participated, their mean age was 50.4 years (SD12.1) and mean time since diagnosis 13.2 years (SD9.6). 68.6% were female. 48.0% had relapsing-remitting MS; 25.4% secondary progressive MS; 15.5% rapidly evolving relapsing-remitting MS and 11.1% primary progressive MS.

Data from the 43 draft items were fit to the Rasch model. Initial fit was poor (Chi-Square 649.1; $p < 0.001$) with strong indications of multidimensionality. Consequently two domains were considered, after adjustments for locally dependency and misfit; one 17-item domain associated with a positive 'Approach' such as acceptance and planning, and another of 12 items associated with a negative 'Avoidant' approach such as disengagement and denial. Both had adequate fit to the Rasch model (Approach = Chi-Square 66.3 (df 51); p=0.07; mean item fit residual -0.164; SD 1.21; PSI 0.83; Avoidant = Chi-Square 43.0 (df 36); p=0.20; mean item fit residual 0.154; SD 0.85; PSI 0.65). Both domains showed positive correlations with their respective comparator domains on the COPE.

A bi-factor solution, based upon both 'Approach' and 'Avoidant' domains, accounted for 83% of the non-error variance.
Conclusions: A self-completed index of coping for those with MS, consistent with Moos' theory of coping, built from the patient experience and satisfying Rasch measurement model standards, provides a disease-specific scale for this important mediating construct. The scale is free for use in not-for-profit settings.

P735

Psychiatric diagnoses, medication and risk for disability pension in multiple sclerosis patients; a population-based register study

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Background: Psychiatric comorbidity is common among multiple sclerosis (MS) patients. The majority of MS patients of working ages are on disability pension.
Objectives: The aims of this study were to chart the prevalences of psychiatric diagnoses and medication among MS patients of working ages, and to investigate their association with the risk for future disability pension.
Methods: This nationwide, population-based prospective cohort study includes 10,750 MS patients and 5,553,141 non-MS individuals who in 2005 were aged 17-64 years. Psychiatric diagnoses and medication were identified using nationwide registersries. Odds ratios (ORs) with 95% confidence intervals (CI) were calculated adjusting for socio-demographics. Furthermore, a survival analysis with five-year follow-up was performed among the 4,571 MS patients not on disability pension in 2005 with psychiatric diagnoses and medication as risk factors, and disability pension as the outcome.
Results: Among MS patients, 35% had been prescribed psychiatric medication compared to 10% of non-MS individuals, adjusted OR 3.72 (95% CI 3.57 to 3.88). Ten percent of MS patients had received a psychiatric diagnosis, compared to 5.7% of non-MS individuals, OR 1.82 (95% CI 1.71 to 1.94). Serotonin reuptake inhibitors (SSRIs, 17%), were the most commonly prescribed drugs (17%) among MS patients, while depression (4.8%) was the most common psychiatric diagnosis. In the survival analysis, MS patients with any psychiatric diagnosis had a hazard ratio (HR) of 1.83 (95% CI 1.53 to 2.18) for disability pension compared to other MS patients. MS patients with any psychiatric drug prescription had a HR for disability pension of 2.09 (95% CI 1.84 to 2.33).
Conclusions: Psychiatric diagnoses and medication are common among MS patients and adversely affect risk for disability pension. This highlights the importance of correct diagnosis and management of psychiatric comorbidity, in a clinical as well as in a societal perspective.

P736

Depression and multiple sclerosis in the CombiRx study

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Background: Depression is common in persons with multiple sclerosis (MS). Severity can change overtime and be related to worsening outcomes. Understanding these issues is important for improving the quality of life of MS patients.
Objectives: To assess prevalence and severity of depression in relapsing remitting MS and the association with the EDSS, self-report Mental Health Inventory (MHI) and Depression Subscale (MHD) - part of the MSQLI.
Methods: The CombiRx trial was a multi-center, double-blind, three-arm trial that randomized 1008 participants to combination therapy [interferon (IFN) and glatiramer acetate (GA)] versus each agent alone plus placebo. The core study was 3 years with up to 7 years of follow up in the extension phase. History of depression (HD) was assessed at baseline using medical history or report of feeling depressed within 4 weeks prior to enrollment. The EDSS was measured quarterly, the MSQLI annually. Study Exclusion criteria included history of suicidal ideation or an

episode of severe depression within 12 weeks prior to randomization.

Results: HD was reported by 720 (71.4%) of participants, with no differences by age, race, gender, diagnosis criteria, duration of disease, or treatment arm. HD was more prevalent in single persons (p=0.005), those with more relapses in the prior 12 months (p=0.034) and higher among those with an EDSS > 2 (p=0.003). When adjusted for BL EDSS score, follow up time, and treatment, HD saw more EDSS worsening over time (0.3 vs 0.05, p=0.008), with no difference in the likelihood of relapses. There was no difference in core study completion by HD at baseline (p=0.09) but when adjusting for treatment, those reporting more severe depression by both the MHI (p=0.003) and MDH (p=0.005) at baseline were more likely to terminate prior to 3 years.

Conclusions: Those with a history of depression prior to enrollment saw increase worsening in both clinical and patient reported outcomes. While history of depression alone was not predictive of early termination, those with worse depression related measures at baseline were more likely to terminate early.

P737

Personality traits are associated with the quality of patient-provider relationships in multiple sclerosis

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Background: Low conscientiousness and high neuroticism are associated with poor medication adherence. The quality of provider-patient relationships, including greater perceived autonomy support, is associated with better medication adherence. No studies have examined whether personality influences Multiple Sclerosis (MS) patients' perceptions regarding the quality of their relationships with their providers.

Objectives: The purpose of this investigation was to examine the association between non-adherent MS patients' personality traits and their perceived relationships with their treatment providers.

Methods: Participants (n = 62) were recruited via direct contact, patient mailings, internet advertising, and advertising through an MS Newsletter as a part of a larger study designed to facilitate medical decision making among non-adherent MS patients. The Big Five personality traits were assessed using the Ten-Item Personality Inventory (TIPI) and the perceived quality of their relationship with their provider was assessed with respect to perceived autonomy support using the Health Care Climate Questionnaire (HCCQ).

Results: Non-adherent relapsing-remitting MS patients with higher rates of agreeableness ($r_{s=}$ .39, p < .01), openness ($r_{s=}$ .31, p < .05), and extraversion ($r_{s=}$ .29, p < .05) indicated greater perceived autonomy support from their treatment providers. In contrast, the quality of provider-patient relationships was not associated with mental health, overall disability, intelligence, education level, or disease duration.

Conclusions: Findings suggest that several of the Big Five personality traits are associated with the quality of MS patients' relationships with their healthcare providers. Increased understanding of MS patients´ personality traits may aid with preventative medical and psychiatric treatment by tailoring provider styles to best fit patients' personality traits.

P738

Disease management and perceived self-efficacy: how does one's personality contribute?

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Background: It is well appreciated that personality traits play a role on one's perceptions and management of their illness. For instance, individuals high on neuroticism are more prone to somatization, are less adherent to medication, and are more likely to engage in negative health behaviors (e.g., substance use). In contrast, individuals high on conscientiousness are more adherent, engage in more positive health related behaviors, and demonstrate greater self-management of their illness.

Objectives: The purpose of the present study was to examine the influence of personality on individuals' perception of their self-efficacy in managing their illness and overall self-management, including health maintenance behaviors, MS knowledge, patient-provider relations, treatment barriers, adherence, and substance use as a means of coping.

Methods: Seventy-seven individuals with multiple sclerosis (MS) were administered the NEO Five Factor Inventory, the MS Self-management Scale, the Disability Management Self-efficacy Scale, the General Self-efficacy Scale, and the Morisky Adherence Questionnaire.

Results: Higher levels of neuroticism was associated with lower self-management (r=.33,p=.003), lower general and MS self-efficacy (r=-.52,p< .001; r=-.39,p< .001, respectively), less knowledge of MS (r=-.28,p=.014), poor adherence (r=.39,p=.001), treatment barriers (r=-.24,p=.036), and greater substance use as a means of coping (r=.33,p=.004). In contrast, high levels of conscientiousness was associated with greater self-management (r=.28,p=.013), general and MS self efficacy (r=.43,p< .001; r=.37,p=.001, respectively), and adherence (r=.41,p< .001), and less use of drugs/alcohol as a means of coping (r=-.29,p=.011). Openness was associated with greater MS knowledge (r=.23,p=.045), health maintenance behaviors (r=.24,p=.040), and general self-efficacy (r=.30,p=.009). Extraversion was shown to be related to greater patient-provider relations (r=.23,p=.045), MS knowledge (r=.24,p=.039), and general and MS self efficacy (r=.44,p< .001; r=.35,p=.002, respectively).

Conclusions: Given the importance of effective management of MS, including adherence and maintenance of health behaviors, consideration and assessment of personality traits appears warranted in hopes of assuring optimal outcomes and tailoring one's interventions.

P739

Depression correlate with disability and clinical course in multiple sclerosis patients: an Italian multicenters study

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Background: Multiple Sclerosis (MS) is an inflammatory, demyelinating disease of the Central Nervous System (CNS) and this results in a number of consequences, including psychological and psychiatric diseases. The frequency of depression is reported in about 50% of patients with MS (pwMS).
Objectives: The aims of this study are to investigate the prevalence of depression in a wide multicenter MS population using Beck Depression Inventory II, and to find possible connections between psychopathologic symptoms and demographic and clinical variables.
Methods: Data was collected in a multi-centre, cross-sectional study involving 6 italian MS centres using a face-to-face structured questionnaire compiled by a neurologist in subjects with a diagnosis of MS according to recognized criteria or clinically isolated syndrome (CIS) over a period of 6 month. The questionnaire included demographic data, year of symptom onset and diagnosis, Expanded Disability Status Scale (EDSS), clinical course, Beck Scale, medication for MS.
Results: 1011 MS patients participated at the study and clinical and demographic characteristics were shown in Table 1. Briefly 676 (66.9%) patients were females, with mean age 34 years (SD 10.8), a mean EDSS of 3.3 (0 - 9.5) and mean disease duration of 10.3 years (1 - 50 years). Most of the patients had a relapsing remitting (RR) (n° 708, 70%) while 236 (23.3%) a Secondary Progressive (SP) and 44 (4.4%) primary progressive (PP) course. Based on BDI score 668 subjects (66.1%) have a score lower than 14 and 343 subjects (33.9%) have a score greater than 14, i.e clinically depressed values. Considering disease course, SP patients were significantly different both from RR patients ($p < 0.001$) and from PP patients ($p < 0.001$). Particularly among PP patients 10/44 (22.7%) had clinical depression vs 188/708 (26.6%) in RR and 142/236 (60.2%) in SP patients.
Conclusions: Due to the high frequency of depression in this population, the possibility to successfully treat depression and its implication on an individual's quality of life, we suggest that the BDI be used in clinical practice as a screening tool for depression in people with MS.

P740

Does anxiety moderate the relationship between bowel dysfunction and illness intrusiveness in multiple sclerosis?

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Background: Patients with MS often experience bowel dysfunction, with fecal incontinence and constipation the two most prevalent symptoms. Patients may experience bowel symptoms or abdominal distress when they become anxious. Bowel dysfunction can negatively affect patients' quality of life (QoL). Additionally, symptoms may be under-reported to their healthcare providers due to embarrassment. A determinant of QoL is illness intrusiveness, in which the disease and related treatments interfere in aspects of patients' lives, such as intimacy, instrumental activities of daily living, and relationships. Previously, we showed a trend with depression ($p = .0514$), as measured by the Beck Depression Inventory-II (BDI-II), moderating the relationship between bowel dysfunction and illness intrusiveness.
Objectives: To examine the relationship between anxiety and bowel dysfunction, and how that relationship contributes to perceived illness intrusiveness.
Methods: Participants ($N = 208$) were MS patients at an outpatient clinic at Holy Name Medical Center in Teaneck, NJ who signed research consent forms. Bowel dysfunction was measured by the Incapacity Status Scale (ISS). Moderation analyses were run with bowel dysfunction as the predictor, anxiety as measured by the Hospital Anxiety and Depression Scale (HADS) as the moderator, and Illness Intrusiveness Rating Scale (IIRS) Total Score as the outcome measure.
Results: Bowel dysfunction ranged from severe (4) to none (0), with the majority having mild to none. The mean HADS anxiety score was 7.93 ± 3.75 (range: 0-20). The interaction between bowel dysfunction and anxiety was significant, $F(3, 204) = 19.34$, $p = .03$, as was each variable separately (anxiety: $p = .005$; bowel dysfunction: $p = .01$).
Conclusions: Bowel dysfunction and anxiety were found to affect perceived illness intrusiveness individually. Anxiety also acted as a significant moderator between bowel dysfunction and illness intrusiveness. This finding highlights the need to consider patients' mental as well as physical well-being, emphasizing the importance of a multi-disciplinary approach to patient-centered care.

P741

The Penn State worry questionnaire provides a valid measure of worry across a broad range of disability in multiple sclerosis

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Background: Chronic worry is a construct that is distinct from a multidimensional generalised anxiety disorder. Although generalised anxiety disorder is known to be common in multiple sclerosis (MS), chronic worry has not been extensively studied in this population. In other conditions, chronic worry has been associated with features which are also common in MS such as chronic pain, poor sleep, and fatigue.
Objectives: We aimed to validate the Penn State Worry Questionnaire (PSWQ) in an MS population by testing it for fit against the Rasch model.
Methods: The scale was completed by 254 MS patients (68.6% female, mean age 50.4y, sd 12.1y). The Hospital Anxiety and Depression scale and several other measures were included as part of the Trajectories of Outcomes in Neurological Conditions (TONiC) study investigating quality of life in chronic

neurological disease. Respondents had a wide range of disabilities (37.8% EDSS 0-4, 47.6% EDSS 4.5-6.5, 14.2% EDSS>7.0), disease durations (mean 13.2y, sd 9.6y) and disease subtypes (48.0% relapsing-remitting MS; 25.4% secondary progressive MS; 15.5% rapidly evolving MS and 11.1% primary progressive MS). Data were analysed in an iterative procedure using RUMM 2030 software.

Results: The original scale showed excellent reliability (α=0.92) but did not fit the Rasch model (p< 0.000) due to significant misfit of 7 of the 16 items. The scale breached the assumptions of the model due to evidence of widespread local dependency and multidimensionality (t-test=22.5%). Combining dependent items into testlets eliminated local dependency and multidimensionality (t-test lower confidence interval=0.045) but did not improve fit to the model (p< 0.000) due to significant under discrimination of the testlet incorporating items 1, 3, 8 and 11. Removing these 4 items along with item 10 provided a reliable solution which met the assumptions and fitted the Rasch model (α=0.94 p=0.144, t-test=1.98%). The modified version of the scale showed only moderate correlation with the HADS-anxiety scale (Pearson Correlation=0.585).

Conclusions: The modified PSWQ is a reliable, valid and unidimensional measure of worry in MS and can be used to study this construct across a broad range of disabilities in all disease subtypes. The PSWQ and HADS-anxiety scales measure distinct but related constructs.

P742

Stress burden and satisfaction with treatment in caregivers and patients with multiple sclerosis. MS-feeling study

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Background: In chronic illnesses such as relapsing-remitting multiple sclerosis (RRMS), caregiver burden is associated with the disease control rate perceived by patients and their caregivers, and may influence treatment effectiveness by decreasing medication adherence. Very little is known about the degree of distress in caregivers of Spanish RRMS patients. In addition, the availability of new disease-modifying drugs may have changed the perception of disease by caregivers and patients, and thus it is important to investigate their satisfaction with current treatments.

Objectives: The main objective of the study is to evaluate the burden of distress in caregivers and patients suffering from RRMS, and to describe their satisfaction with their current treatment.

Methods: The MS-Feeling is a multicenter, observational, cross-sectional study which will include approximately 200 patients (and their informal caregivers) aged≥18 years with RRMS, treated with a disease-modifying drug for at least 1 year. The primary endpoint will be the caregiver burden, assessed by the Zarit Caregiver Burden Interview. Secondary endpoints will include: family stress perceived by caregivers and patients (Family adaptation, partnership, growth, affection, resolve -AGPAR-Questionnaire); degree of social support perceived by caregivers (Duke-University of North Carolina Functional Social Support Questionnaire); depressive symptoms in caregivers and patients (Center for Epidemiologic Studies Depression Scale, short form); and satisfaction of caregivers (Caregiver Satisfaction with Treatment of Multiple Sclerosis Questionnaire) and patients (Treatment Satisfaction Questionnaire for Medication) with treatment. The expected sample size will be a representative sample of approximately 1.3% of the eligible population.

Results: By 5th March 2014, 21 centers participate in the study recruiting 171 patients. Further details of the study results will be presented at the congress.

Conclusions: Multiple sclerosis is a chronic, disabling disease that, if not adequately controlled, can generate important caregiver distress. The MS-Feeling study will provide useful information about the burden of RRMS in Spanish caregivers and patients, and their satisfaction with current management practices. This knowledge will allow physicians to improve patient care and adherence to treatment.

P743

Neuropsychiatric features and fatigue in a prospective population paediatric demyelinating disease longitudinal study

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Background: Little is known on childhood neuropsychiatric and fatigue manifestations in acquired demyelinating syndromes (ADS). The Paediatric UK Demyelinating Disease Longitudinal prospective Study aims to describe outcomes after first episode of acute disseminated encephalomyelitis (ADEM); transverse myelitis (TM); optic neuritis (ON); other clinically isolated syndrome (CIS); neuromyelitis optica (NMO).

Objectives: To test the hypothesis that children with MS/NMO/TM have a higher neuropsychiatric and fatigue symptom burden than ADEM/ON 6 months after onset; and to determine if neuropsychiatric symptoms predict those with higher fatigue morbidity.

Methods: Children were recruited within 3 months of first ADS presentation. Six months after diagnosis parents were asked to complete the PedsQL multidimensional fatigue scale (lower scores represent worse fatigue; 0-100) and the Profile of Neuropsychiatric Symptoms (PONS), a scale measuring frequency and impact of neuropsychiatric symptoms (33 items). Results were analysed (SPSS version 22) using statistical tests appropriate to distribution of data.

Results: Data from 33 patients (19 male, median age 8; range 1-16) with ADEM (n=11); ON (n=7); TM (n=8); NMO (n=2); MS (n=5) was ascertained. All data given as median (range) and/or [mean (SD)]. Patients were followed up for 3.9 years (1.3-4.3). Six months after disease onset, multidimensional fatigue scores were

general 66 (17-100) [68 (26)], sleep 83 (33-100) [75 (20)], cognitive 70 (4-100) [66 (29)] and total fatigue score 73 (24-100) [70 (23)]. Scores for general fatigue and sleep fatigue were lower in the MS/NMO/TM group compared to the ADEM/ON group ($p< 0.05$). Median PONS scores for all patients revealed the most frequently reported symptoms as: sleep problems, inattention, eating problems, worries, low mood, oppositionality, explosive rage and fears; with trends ($p=0.059$) towards higher scores in the MS/TM/NMO group 62 (2-186) compared to the ADEM/ON group 35(9-127). The multivariate linear regression model revealed the following as strong predictors for fatigue: memory problems; low mood; sleep problems; hyperactivity; and impulsivity (r square 0.82).
Conclusions: At 6 months after onset of ADS there is significant impact of fatigue which is greater for patients with MS/NMO/TM compared to patients with ADEM/ON. Neuropsychiatric symptoms of memory problems, low mood, sleep problems, hyperactivity and impulsivity were all predictive of increased fatigue.

Pediatric MS

P744

Demographic and clinical features of children and adolescents with MS: from the US network of pediatric MS centers

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Background: Pediatric MS incidence is estimated at 0.2-0.6/100,000 persons; demographic and clinical features of US samples are incompletely characterized.
Objectives: Characterize pediatric MS patients from geographically diverse US regions.
Methods: Children and adolescents (< 18 years) were prospectively enrolled in a longitudinal observational study across 9 sites forming the US Network of Pediatric MS Centers.
Results:
Demographic: Of 340 cases, girls: boys = 2:1. Percentage of girls increased from 53% (≤ 11 yrs) to 71% (≥ 12 yrs). Mean age of onset was 13.7 (girls), 12.7 (boys). Race was self-identified as Caucasian (67%), African-American (20%), multi-racial (7%), other (6%). Race did not differ by gender ($p=0.59$) or age ($p=0.40$). Ethnicity was 69% non-Hispanic; 31% Hispanic. For 38% of the cases, one or both parents were born outside the continental US, most frequent: Mexico (29%), Puerto Rico (9%), Dominican Republic (8%). 5.3% of cases were foreign born.
Clinical: 31% of children had a prodrome prior to the first event: infectious (67%), closed head trauma (10%), vaccination (9%). Monofocal (60%) vs. polyfocal (40%) presentation, $p< 0.01$. Encephalopathy (5%) was more frequent among the youngest ≤11yrs (14%) vs ≥ 12 yrs (2%) $p< 0.01$. Optic neuritis (27%) was highest among those ≦11yrs (34%) vs ≥ 12 yrs (24%) $p=0.07$. At the initial visit, 77% had EDSS < 3. For those with ≥1yr follow-up (n=256) mean ARR=0.49.
Conclusions: Individuals from the US with pediatric MS vs. adult MS differ demographically with fewer Caucasians and many more first generation Americans. Overall, the female ratio increases with age; Encephalopathy is more common among the youngest.

P745

Cognitive impairment in pediatric multiple sclerosis patients is not related to cortical lesions

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Background: In adult MS patients, the number and volume of cortical lesions (CLs) detected with double inversion recovery (DIR) sequences correlate with the severity of cognitive impairment. A high proportion of patients with pediatric MS suffers from cognitive deficits. The contribution of CLs to these deficits has never been investigated, even if a previous study has suggested that CLs are rare in pediatric MS patients.
Objectives: Aim of this study was to investigate the contribution of CLs and tissue loss to cognitive impairment in pediatric MS patients.
Methods: Using a 3.0 T scanner, brain DIR, dual-echo, and 3D T1-weighted scans were acquired from 41 consecutive pediatric MS patients and 31 gender- and age-matched healthy controls (HC). Patients with abnormalities in ³ 2 neuropsychological tests were classified as cognitively impaired (CI). CLs and white matter (WM) lesions were identified, and their volumes measured. Brain GM and WM volumes were also calculated. Between-group comparisons were performed using chi-square, Mann-Whitney

and ANOVA tests. Negative binomial model was used to compare the number of lesions between the two groups of patients.
Results: Thirteen (32%) pediatric MS patients were CI. Compared to cognitively preserved (CP) patients, CI ones had similar disability (p=0.7) and longer disease duration (p=0.01). T2-hyperintense and T1-hypointense WM lesion volumes did not differ between CI and CP MS patients. CL number, CL volume and GM volume did not differ between CI and CP patients, whereas normalized WM volume was significantly lower in CI vs CP MS patients (p=0.007).
Conclusions: CLs do not contribute to cognitive impairment in pediatric MS patients, which is mainly related to damage to the WM.

P746
Young adults with pediatric-onset MS have a downward educational trajectory
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Background: Multiple studies suggest a relationship between educational attainment and employment in adults with multiple sclerosis (MS), with the underlying hypothesis that progressive MS pathology can impact cognition and, consequently, work capacity. Nonetheless, little is known about educational trajectories in pediatric-onset MS (POMS) patients as they reach adulthood.
Objectives: To examine changes in educational status over time in POMS, and the relationship between educational status and brain and white matter lesion volumes.
Methods: Longitudinal evaluation of prospectively collected data on individuals with POMS reaching adulthood (1999-2013) attending a Pediatric MS clinic. Information was collected at the time of disease onset, at one year of follow-up and at the most recent clinical evaluation. Data included: demographics, number of relapses, annualized relapse rate, disability, educational status, total brain volume, z-score (calculated using normative pediatric MRI data from the National Institutes of Health-funded MRI Study of Normal Brain Development), normalized brain volume, T1 and T2 lesion volumes. Spearman Correlation analysis was performed with a cutoff of $p < 0.05$ being considered significant. Ethics approval was received.
Results: 23 individuals (F=15) with POMS were included, age at onset 14.1±2.3 years. At onset, 100% attended school full-time, 91% had average or above average marks, 8.7% had an Individualized Education Plan (e.g. extra help) (IEP) or below average marks. At last visit (mean follow-up 5.4±2.6 years, annualized relapse rate of 0.79±0.76, mean age 19.5±2.0), 39% had discontinued school, had below average marks, or an IEP. MRI analysis (n=14) demonstrated age-expected brain volumes. Correlations were found between T2 lesion volume and educational performance (r=-0.49), discontinued school/had an IEP (r=0.610), graduated high school (r=-0.50) and proceeded with post-secondary education (r=-0.55).
Conclusions: Despite preserved brain volumes and low levels of disability, POMS patients demonstrated a decline in educational attainment. The results suggest a possible link between disease burden and decline in educational trajectory. Future studies will explore:

(1) cognitive analyses,
(2) tissue specific analysis using advanced brain imaging techniques to determine whether degenerative changes within individuals are partly responsible for this downward educational trajectory and
(3) the relationship between this downward trajectory/educational attainment and subsequent employment.

P747
Vitamin D status as a predictor of multiple sclerosis outcome in children with acute demyelinating syndromes: a prospective cohort study
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Background: Vitamin D may be a protective etiologic factor for multiple sclerosis (MS). Acute demyelinating syndromes (ADS) of the CNS represent the first clinical attack of MS for some and a monophasic illness for others.
Objectives: We sought to

(i) determine whether vitamin D status, as defined by serum 25-hydroxyvitamin D (25(OH)D) levels, at presentation with pediatric ADS and serially for up to 12 months post-ADS onset were associated with risk of MS and time to MS diagnosis and
(ii) assess factors influencing 25(OH)D at presentation.

Methods: Consecutively recruited participants (< 16 years old) were monitored prospectively from ADS onset at 23 sites participating in the Canadian National Paediatric Demyelinating Disease

Network. We used Cox proportional hazards (PH) to determine risk of MS and time to MS diagnosis as a function of serum 25(OH)D levels at ADS onset and in the following year, accounting for potential confounders such as age, body mass index (BMI), sex, season, and HLA-DRB1*15 status. We used multivariable regression models to evaluate factors influencing 25(OH)D levels at ADS onset.

Results: Of 225 eligible participants, 47 children (20.9%) were diagnosed with MS a median of 105 days (IQR 94-222 days) from ADS onset. Higher 25(OH)D levels at ADS onset were associated with a lower risk of MS (adjusted HR/10 nmol/L increase: 0.86; 95% CI: 0.77-0.97), but 25(OH)D levels in serial samples post-ADS were not (HR/10 nmol/L increase: 1.00; 95% CI: 0.89-1.12). Among children with MS, higher 25(OH)D levels at enrollment were associated with a lower hazard of a second clinical attack (adjusted HR per 10 nmol/L increase: 0.82; 95% CI: 0.67-0.99); that is, a longer time to a second attack. In multivariable analyses, higher 25(OH)D levels at ADS onset were associated with summer season, younger age at ADS onset, and intake of supplements containing vitamin D.

Conclusions: Higher serum 25(OH)D at pediatric ADS onset but not during the year thereafter is associated with a lower likelihood of MS outcome and longer time to second attack, suggesting that vitamin D contributes to the inciting biology of MS. Vitamin D supplement intake is a modifiable factor associated with higher 25(OH)D levels, indicating that vitamin D supplementation represents a strategy to improve vitamin D status and possibly reduce risk of MS.

P748

Puberty onset and pediatric multiple sclerosis course

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Background: Pregnancy is a major disease modifier for MS course. Another dramatic hormonal change is puberty. While pre-pubertal MS onset is rare, it affects males and females equally in contrast with the higher ratio of females in post-pubertal patients. How puberty influences clinical features and disease course in pediatric MS patients is unknown.

Objectives: To determine association of puberty with age of onset of pediatric MS and differences in clinical features of pre- versus post-pubertal MS in girls.

Methods: This is a longitudinal retrospective study using prospectively collected data from the University of California San Francisco Regional Pediatric MS Center database that includes 300 patients of which 100 are females with MS. Pediatric MS was defined as disease onset before the age of 18. As puberty onset is poorly defined, we used the year of menarches in female patients as a reliable proxy for puberty. Post-pubertal females with missing menarche data were not included. Poisson regression models were used for relapse analysis. Analyses were adjusted for ethnicity and body mass index (bmi).

Results: Preliminary results are available from girls of which 20 had pre-pubertal and 37 had post-pubertal disease onset. Of those with pre-pubertal disease onset, 5 have not yet reached menarche. Forty -six percent were Hispanic, and 44% non-white. Median age at disease onset was 14.1 years (IQ range 4.3 - 17.9). Duration of follow-up since disease onset was 4.7 ± 2.9 years (mean ± SD). For those who reached menarche, median disease onset was 2.2 years (IQ range -4.1 - 5.8) after menarches. Females with pre-pubertal disease onset had lower incidence of relapses (incidence rate ratio 0.14, 95% CI 0.1- 0.3, $p < 0.001$) compared to those with post-pubertal disease onset adjusting for ethnicity and bmi. Further analyses are in process to determine associations of pubertal status and anatomic localization and disease progression.

Conclusions: Pubertal status may influence MS course at least in female patients. Understanding how puberty influences MS clinical features may offer new insights in important factors regulating disease processes.

P749

Recurrent optic neuritis in children

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Background: Recurrent optic neuritis (ON) occurs in children without MS or NMO, yet is underreported in the literature.

Objectives: Our goal was to investigate the clinical characteristics of this patient subgroup.

Methods: Retrospective analysis of prospectively collected data from May 2011 to April 2014 on all patients in the US Network of Pediatric MS Centers database presenting with their first demyelinating attack before their 18th birthday who had more than one attack of ON but did not meet criteria for MS or NMO.

Results: 20 patients (8 girls, 12 boys) met inclusion criteria and were followed for a mean duration of 7 months. Mean age at first attack was 9.2 years (range 4.3-15.3). A total of 52 attacks of ON occurred in the cohort. The mean number of episodes of ON was 2.6 (range 2-4) and the median duration between attacks was 9 months (range 1-172 months). 9 patients had only idiopathic attacks of ON without other neurologic symptoms whereas 8 presented with ADEM followed by recurrent ON (ADEM-ON). 2 patients did not fit well in either group. 14 (27%) of attacks were preceded by prodromal symptoms including GI infection, respiratory illness and fever. Although, 31 (60%) of attacks were unilateral, over the follow-up period, 15 (75%) had an attack of bilateral ON. The median lowest recorded visual acuity from the most affected eye was between 20/70 and 20/200. At last follow-up, 14 (70%) recovered 20/20 vision and optic disc pallor was present in 11 (55%) of patients. 14 (70%) had lesions on brain MRI (5 idiopathic recurrent ON, 8 ADEM-ON) and 4 (20%) within the spinal cord (1 recurrent ON, 2 ADEM-ON). Oligoclonal bands were present in 1 idiopathic recurrent ON patient and 1 ADEM-ON patient. IgG index was elevated in one patient. No patient was positive for NMO IgG. 8 patients had evidence of infection with EBV (prior

or current infection). Treatments used in at least 2 patients included methylprednisolone, prednisone, and InterferonB-1a. 4 patients on treatment and 10 patients off treatment had been attack free for over 6 months at most recent follow up.
Conclusions: Of children with recurrent ON who do not meet diagnostic criteria for MS or NMO, most have a disease course consistent with idiopathic recurrent ON (45%) or ADEM-ON (40%). Some children with idiopathic recurrent ON may have Chronic Relapsing Inflammatory Optic Neuropathy (CRION), as described in adults. Increased awareness of this disease in children is necessary to facilitate better recognition, understanding and treatment of the disease.

P750

Behavioral ratings in pediatric multiple sclerosis (MS)

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Background: The behavioral correlates of pediatric MS remain unclear.
Objectives: To measure behavioral areas most frequently of clinical concern in pediatric MS.
Methods: A total of 129 outpatients consecutively evaluated at the Lourie Center for Pediatric MS were administered the Behavioral Assessment System for Children, second edition (BASC-2), self- and parent-report forms as part of their routine clinical visit which included a neuropsychological evaluation. The BASC-2 includes clinical and adaptive functioning scales, with scores falling greater than two standard deviations from the normative mean considered to be clinically significant.
Results: Participants ranged in age from 4 to 18 years with a mean of 14.74 ± 2.8; were 50% female; 69% Caucasian and 26% Hispanic. Diagnoses were pediatric MS (75%), clinically isolated syndrome (20%) and radiologically isolated syndrome (5%). Mean disease duration was 1.82 ± 1.92 years; participants had a median EDSS of 1.0 (range 0 to 6.5). On the BASC-2, all mean scores for self- and parent-reported clinical and adaptive scales were within the average range, with mean t scores between 45 and 55. Parents most frequently rated the participants to be in the clinically significant range for the scales measuring somatization (34.9%), social skills (28.7%) and attention problems (26.7%). Participants self-report ratings fell in the clinically significant range most frequently for attention problems (25.6%), relationship with parents (24.0%) and somatization (21.7%). Parent ratings of atypicality (unusual or odd thoughts, behaviors, mood swings) were significantly related with EDSS (r=0.30, p=0.002). Otherwise no disease features were strongly related to any other parent- or child-reported behavioral ratings.
Conclusions: Most individuals with pediatric MS and their parents do not report clinically significant behavioral problems. The most common areas of clinical concern include depression, attention problems, social skills and relationship with parents.

P751

Treatment type and EDSS outcome of paediatric acute disseminated encephalomyelitis: a retrospective analysis of children from a US Network

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Background: Acute disseminated encephalomyelitis (ADEM) is an inflammatory demyelinating disease of the CNS occurring more often in children than adults. Corticosteroids are usually first line therapy and outcome is usually good.
Objectives: To describe treatment type and EDSS outcome of a cohort of children who had ADEM.
Methods: We performed a retrospective analysis of patients prospectively enrolled in a database by the US Network of Pediatric MS Centers. The database was queried for cases of ADEM prior to age 18. Treatment type and EDSS at last follow up were recorded.
Results: There were 94 patients with a diagnosis of ADEM in the database with a median follow up of 43.5 months. Initial course of treatment consisted of steroids in 75 patients (initial median EDSS 1), IVIG in 5 (initial median EDSS 1), and plasmapheresis in 1 (initial EDSS 3). Eventually a total of 5 patients required plasmapheresis (initial median EDSS 3) and 12 patients received IVIG (initial median EDSS 2). Eighty patients made a good recovery with an EDSS of 2 or less at last follow up. Those who required IVIG had a median EDSS at last follow up of 2 and plasmapheresis had an EDSS of 2.5.
Conclusions: ADEM is typically treated with steroids only. IVIG and plasmapheresis are usually reserved for more severe attacks. ADEM is usually associated with a good recovery in terms of disability measured by EDSS.

P752

Assessing long-term functional outcomes in children with acute disseminated encephalomyelitis

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Background: Acute Disseminated Encephalomyelitis (ADEM) is an inflammatory demyelinating disease of the CNS. Previous studies reveal that over 70% of patients experience full clinical

recovery. Critical recovery factors are physical status, speed of recovery, and potential risk of relapse. Little attention has been devoted to examining long-term clinical, behavioral, and neuropsychological outcomes, and their MRI correlates, in children with ADEM.

Objectives: To identify predictors for poor outcome in children diagnosed with ADEM: demographics, clinical manifestations, and degree and recovery time, as well as the relation to cognitive and academic status; and to compare baseline and follow-up MRI metrics in relation to functional outcomes.

Methods: Retrospectively reviewed 41 subjects ≤18 years of age (mean 5.7 years) diagnosed with ADEM at baseline and follow-up. Functional outcomes were assessed based on age of onset, sex (65.9% males), ethnicity (74.3 % Caucasians), symptoms, duration of illness (mean 26 days), speed of recovery (mean 272.3 days), cognitive measures, and EDSS. MRI scans were also reviewed and analyzed to determine the degree of functional outcomes.

Results: Paired sample t-tests comparing baseline and follow-up reveal significant improvements in EDSS[(t (df=40)=3.41, p=0.002] and MRI T2 lesion volume load [t (df = 29)=2.30, p=0.029]. About 29.3% of patients experienced clinical relapse (17.1% motor impairment) and 36.6% of patients developed seizures. Behavioral problems were reported in the following percentages of patients: 37% aggression, 22% impulsivity, 4.9% anxiety, 22% depression, 29.3% irritability, 24.4% behavioral regression. The proportion of patients receiving interventions are as follows: IEP (68.3%), PT (78%), OT (80.5%), and ST (29.3%). Neuropsychological testing in subset of patients (n=14) revealed that 21.4% scored ≤ -1.5 SD in their IQ tests.

Conclusions: Consistent with previous studies, our findings reveal that patients appear to improve overall in terms of their physical recovery and follow-up MRI scans. However, these patients tend to have behavioral/emotional problems and lower IQ scores as a consequence of their ADEM event. Further studies regarding neuropsychological outcomes and their MRI correlates are warranted.

P753

A case-control study for risk factors of pediatric multiple sclerosis in Iran: highlighting the role of puberty

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Background: Although several studies have assessed risk factors for multiple sclerosis (MS) in adult, there is a lack of data on pediatric MS (Ped-MS) population.

Objectives: To determine whether the age of puberty onset and the effect of lifestyle and environmental factors during pre- or post-pubertal period are risk factors for developing Ped-MS.

Methods: A case-control study, including 97 Ped-MS cases and 97 age and sex matched controls, was conducted in Isfahan MS Society-as a major referral center in Isfahan province, Iran. Information on age at puberty (pubertal onset define as the age at menarche in females and age at voice change and growing of pubic hair in males), lifestyles, environmental exposures, and past medical history was obtained from standard designed questionnaire by phone interview or during clinical surveillance.

Results: The age of puberty onset was significantly lower in Ped-MS group and there was approximately 4-fold increase risk of MS with decreasing age at puberty (odds ratio (OR): 4.072, 95% confidence interval (CI): 1.57-10.52). In conditional regression analysis, those reported sunlight exposure 45 minute per day or less before puberty compared with those reported sunlight exposure greater than 2.5 hours per day before puberty had 17.66-fold increased odds of having Ped-MS in future. The adjusted OR and 95% CI in children whose Mothers exposed to tobacco smoke during their pregnancy and those who was long-term passive smoking in their home during childhood was 2.667(1.240-5.737) and 2.333(1.274-4.272) respectively. Moreover, there was increased risk of MS in who had the positive medical history of rubella before puberty (OR= 5.206, 95% CI: 1.540-17.601). Finally, mode of delivery, past history of common surgical procedures, other childhood-disease, or exposure to pets and farm animals was not associated with MS risk.

Conclusions: Our findings suggest that earlier age at puberty and different modifiable lifestyles factors such as smoking and sunlight exposure in pre-puberty period were associated with higher MS risk in Ped-MS population.

P754

Sex related differences in T2 lesion load in pediatric multiple sclerosis patients

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Background: A preponderance of females affected by multiple sclerosis is well reviewed.

Several studies have shown an equal sex distribution before puberty.

So far no studies have been performed exploring possible differences in the MRI presentation of multiple sclerosis in boys and girls.

Objectives: To study sex-and age related differences in pediatric relapsing-remitting multiple sclerosis before and after puberty.

Methods: Single center retrospective study on T2 lesion load on cranial MRIs performed within the first six month after disease manifestation in 178 children with definite multiple sclerosis recruited by the Center for Multiple Sclerosis in Childhood and Adolescence at the University Medical Center Göttingen, Germany.

Results: Sex distribution in the whole cohort was 3:1 (124 girls and 54 boys) while the distribution in the pre-pubertal group was almost even (24 girls and 22 boys). Median number of T2 lesions in the whole cohort was 10 (range 0-145). In pre-pubertal boys median number of T2 lesions was 11.5 (mean 25.5, range 0-85), in pre-pubertal girls 7 (mean 12.2, range 0-68). In post-pubertal boys median number of T2 lesions was 16.5 (mean 18.8, range 0-64) and in girls 9 (mean 16.8, range 0-145).

In general boys (median 12, mean 21.5, range 0-85) had significantly higher lesion load than girls (median 9, mean 15.8, range 0-145), independent of the age group (p=0.018).

No significant differences in lesion distribution between the sex and age groups were observed.
Conclusions: In a large cohort of pediatric multiple patients the even sex distribution before puberty was confirmed. While no differences were seen regarding lesion distribution between the sex and age groups a significantly higher lesion load was seen in boys when compared to girls. Whether this group of affected boys shows a poorer clinical outcome has to be further investigated.

P755
A 3-year, longitudinal MRI study in pediatric patients with MS, CIS, ADEM and OND
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Background: MRI is one of the most important tools for the diagnosis and monitoring of patients with demyelinating diseases in the adult and pediatric population**.** However**,** longitudinal MRI studies in the pediatric population with demyelinating diseases are scarce.
Objectives: To investigate the accumulation of new/enlarging lesions and changes in brain volume in a cohort of pediatric patients with multiple sclerosis (MS), clinical isolated syndromes (CIS), acute disseminated encephalomyelitis (ADEM), and other neurological disorders (OND).
Methods: In this observational study, 62 patients (16 MS, 8 CIS, 19 ADEM and 19 OND) were enrolled and followed with clinical and 1.5T MRI examinations over 3 years. The OND group included 10 headache, 6 seizure and 3 CNS vasculitis patients. MRI was performed at baseline, with 2 and 3 years of follow-up. MRI analyses included estimation of T2, T1 and gadolinium (Gd) lesion outcomes and brain volume changes.
Results: The CIS and MS patients were slightly older compared to ADEM and OND patients (p=0.004), and the average age at baseline was 11.3 years in all study groups. No differences between the groups was found in disease duration, with an average of 1.9 years since disease onset. At baseline, there were significant differences in T2 and T1 lesion number and volume (LVs) between the 4 groups, with MS patients presenting the greatest lesion number and LVs ($p< 0.05$). No significant baseline differences between the groups were found for normalized volumetric measures of whole brain, lateral ventricle (LVV) and thalamus. Over 3 years, MS patients developed an average of 8.6 new/enlarging cumulative T2 lesions, compared to 1.8 in OND and 0 in CIS and ADEM groups (p=0.002). Among the brain volume changes, there was a significantly greater enlargement of LVV over 3 years in MS (+21.6%) and CIS (+11.2%) patients, compared to OND (+4.5%) or ADEM (-8.4%) (p=0.016 between all groups). In the MS group, baseline and follow-up changes in LVV were significantly related to accumulation of new/enlarging T2 and T1 lesions after 2 and 3 years ($p< 0.05$).
Conclusions: MS patients present with substantially greater accumulation of new/enlarging T2 lesions compared to CIS, ADEM or OND patients over 3 years of follow-up. The enlargement of the lateral ventricles provided the best volumetric outcome measure to distinguish MS and CIS patients from the ADEM and OND groups. The study findings may be used to guide future clinical trial design in pediatric MS.

P756
Oral disease modifying therapies in pediatric MS patients: a US network experience
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Background: New oral disease modifying therapies (DMTs) have been recently FDA approved for the treatment of adults with multiple sclerosis (MS).
Objectives: To evaluate safety, tolerability and preliminary efficacy of the new oral DMTs, FDA approved in pediatric MS patients.
Methods: Retrospective longitudinal study design within the US Network of Pediatric MS Centers. Pediatric MS patients who initiated one of the 3 new oral DMTs, FDA approved: fingolimod, dimethyl fumarate and teriflunomide for at least one dose before 18 years of age were included in this study.
Results: As of April 3, 2014, 28 patients (18 female, 10 male) with median age at disease onset of 14.2 years (range: 3-17) were identified across 7 participating sites. Ten patients were on fingolimod and 18 on dimethyl fumarate. Twenty-three patients were previously treated with other DMTs and 5 were treatment naive. The previous DMTs before switching to oral therapies were as follows: glatiramer acetate (n=2), interferon B-1a (IM n=6; SQ n=6), interferon B-1b (n=3), natalizumab (n=5); IVIG (n=1). The previous DMTs were discontinued due to ineffectiveness (n=7), various adverse effects (n=5) (i.e: anxiety, flu-like symptoms, GI symptoms, hair loss, headache, injection site reactions) or other reasons (n=6). The median follow-up prior to oral DMTs was 1.8 years (relapse rate of 0.73 relapses per patient-year), while the median follow-up on the new oral DMTs was 0.35 years. The reported side effects of the new oral DMTs include arrhythmia (n=1), GI symptoms (nausea, vomiting, abdominal pain) (n= 7), and rash (n=2). Three patients discontinued (D/C) the oral DMTs;

1 D/C fingolimod and switched to tecfidera due to efficacy concerns, 2 D/C tecfidera because of side effects (GI related).
Conclusions: Within the first 4 months of oral DMT medication onset, no safety concerns emerged in the pediatric MS population. Further longitudinal follow-up will be provided at the time of presentation.

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Neurological status, fatigue, motor performance and exercise capacity in children with multiple sclerose and acute disseminated encephalomeyelitis

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Background: Fatigue is one of the most common symptoms in children with multiple sclerosis (MS) and after acute disseminated encephalomeyelitis (ADEM). In children, motor performance and physical activity are related. We hypothesize, that fatigue and physical disability lead to poor physical activity and subsequently to reduced exercise capacity and poor motor development.
Objectives: We aimed to evaluate extent of disability, fatigue, motor performance and exercise capacity in children with MS and post-ADEM.
Methods: Sixteen children (MS n=9, post-ADEM n=7), aged 4-17 years attending our pediatric MS centre, were retrospectively studied. Extent of disability was measured with Expanded Disability Status Scale (EDSS), fatigue with Pediatric Quality of Life Inventory-Multidimensional Fatigue Scale (PedsQL MFS), motor performance with Movement-Assessment-Battery for Children second edition (MABCII), and exercise capacity with the Bruce protocol.
Results:
EDSS: mean(SD) extent of disability in the total group is: 0.66(0.83); 0.61(0.74) in children with MS and 0.71(0.99) in children post-ADEM.

PedsQL MFS: mean(SD) SDS total fatigue score was significantly below normal in the total group: -1.31(1.14); $p< 0.001$. Both children with MS [-0.94(1.05); p=0.03] as children post-ADEM [-1.86(1.12); p=0.01] experienced greater fatigue than healthy related pears.

MABCII: Only 8/16 children (84% expected based on reference values) had a normal overall percentile score, 2/16(12.5% vs 11%) had a borderline score, and 6/16(37.5% vs 5%) had a definite motor problem (distribution differed from reference population, $p< 0.001$). A normal score was present in 4/9(44.4%) children with MS and in 4/7(57.1%) post-ADEM.

Bruce: mean(SD) SDS endurance time was significantly below normal in the total group: -1.64(1.19); $p< 0.001$. Especially children with MS had a limited exercise capacity: [8/9(88.9%) scored below -1SD].

No correlation was found between EDSS and severity of fatigue, M-ABCII or Bruce.
Conclusions: Despite almost normal scores on the EDSS, children with MS and post-ADEM encounter greater fatigue than healthy pears. We also found impaired motor performance and decreased exercise capacity (mainly children with MS). We conclude that EDSS is not sensitive enough in children to detect problems in physical functioning. Prospective follow-up of more children with MS and ADEM is needed to determine which factors contribute to fatigue, impaired motor performance and decreased exercise capacity.

Prognostic factors

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Maximal lifetime brain growth (estimated with intracranial volume) is linked to level of disability on the multiple sclerosis functional composite

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Background: Physical disability is prevalent but variable across patients with multiple sclerosis (MS), even among patients with comparable disease burden. This makes it difficult to predict future disability. Consistent with the brain reserve hypothesis for cognitive decline, we show for the first time in a separate abstract that larger maximal lifetime brain growth (MLBG, estimated with intracranial volume) protects against physical disability measured with the Expanded Disability Status Scale (EDSS).
Objectives: We examined whether larger MLBG also protects against functional disability measured with the Multiple Sclerosis Functional Composite (MSFC), which has gained prominence as a primary measure of functional outcome in MS research, including clinical trials.
Methods: Seventy-two women with relapse-onset MS (independent from our EDSS abstract) were assessed with the MSFC, which evaluates gait (25-Foot-Walk; Ambulation Index) and fine motor dexterity (Nine Hole Peg Test), as well as cognitive status (Symbol Digit Modalities Test). MRI measured MLBG (estimated with intracranial volume) and disease burden (normalized volumes of gray, white, total brain, deep gray matter). Stepwise regression predicted MSFC with demographics, disease burden (above), and MLBG. We then investigated whether MLBG was related to each separate component of the MSFC (e.g., gait).
Results: Better MSFC performance was predicted by larger normalized deep gray matter volume (less atrophy; $R^2\Delta=.072$, p=.023) and larger MLBG ($R^2\Delta=.143$, p=.001). Controlling for normalized deep gray matter volume, larger MLBG was associated with better gait (Ambulation Index, $r_p = .263$, $p = .027$), better fine motor dexterity ($r_p = .331$, $p = .005$), and better cognitive status ($r_p = .392$, $p = .001$). In addition to Ambulation Index scores, larger MLBG was also linked to faster walking speed.
Conclusions: We show that larger MLBG protects against impairments in gait, fine motor dexterity, and cognitive status measured with the MSFC. Together with our finding that larger MLBG protects against disability on the EDSS (separate abstract), these results provide the first evidence in any population that larger MLBG protects against physical disability (extending the brain reserve hypothesis from cognitive to physical function). Consideration of a patient's MLBG (easily estimated with intracranial volume) may improve prediction of physical disability, with implications for research and clinical practice.

P759

Factors that determine disease course: the symptomatic lesion matters. 1000 CIS subgroup analysis

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Background: According to the 2010 McDonald criteria, "if a subject has a brainstem or spinal cord syndrome, the symptomatic lesions are excluded from the Criteria and do not contribute to lesion count". Therefore, it could be considered that CIS patients with one single symptomatic lesion in the brainstem behave as CIS patients with zero brain MRI lesions.
Objectives: To study the risk of developing MS and accumulation of disability of brainstem CIS patients presenting with one single symptomatic lesion in the brainstem.
Methods: From 1995 to 2013, 1015 CIS patients underwent prospective clinical and brain MRI follow-up. Patients were divided in three groups: patients with a normal brain MRI (0 brain lesions, n=261), patients with a brainstem syndrome and one single MRI lesion in the brainstem or cerebellum (n=20) and patients with an abnormal brain MRI (≥ 1 lesion, n=673). For each group, we have studied the risk for developing clinically definite MS (CDMS), 2005 McDonald MS and disability accumulation using uni (hazard ratio-HR) and multi (adjusted HR-aHR) cox regression models adjusted by age, gender, oligoclonal bands (OB), and disease modifying treatment (DMT). Same analysis was performed selecting only CIS patients with a brainstem syndrome.
Results: According to the three established categories (0 lesions/ one single symptomatic brainstem lesion and ≥ 1 lesion): OB were present in 20%, 44% and 70% respectively; CDMS occurred in 8%, 40% and 54%, McDonald MS in 10%, 50% and 68%; EDSS 3.0 in 4%, 15% and 16%. Compared to patients with 0 lesions, patients with a unique symptomatic brainstem lesion and patients with ≥ 1 lesion had an increased risk of: 1) CDMS: aHR of 4.4 [1.8 - 10.4] and aHR of 7.1 [4.2 - 12.0]), McDonald MS: aHR of 5.0 [2.3 - 10.8] and aHR of 7.8 [4.9 - 12.3] and EDSS 3.0: aHR of 2.5 [0.7-8.9] and aHR of 4.4 [1.8 - 10.4]). Similar results were found when selecting only CIS patients with a brainstem syndrome.
Conclusions: Brainstem syndromes with a unique symptomatic lesion in the brainstem or cerebellum have a higher risk of developing MS than patients with 0 lesions. Despite the recommendations of the 2010 McDonald criteria, the symptomatic lesion matters in terms of risk of developing MS and accumulation of disability.

P760

MRI predictors of time-to-second-attack and disability in children with MS: findings from a prospective cohort of children with CNS demyelination

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Background: MRI features of pediatric-onset multiple sclerosis (MS) are well understood, and recent work has focused on criteria that predict MS diagnosis in children with an acute demyelinating syndrome (ADS). The role of MRI in the prediction of outcome in children with MS has been less well studied. Early predictors of outcome in children with multiple sclerosis have implications for clinical care and for identifying eligible children for clinical trials.
Objectives: To determine MRI predictors of disability and time to second attack at first attack in children with MS.
Methods: Children younger than 16 years of age with an acute demyelinating syndrome (ADS) were enrolled at 23 sites into a national prospective study. Standardized clinical and MRI data were acquired at onset, 3, 6, and 12 months and annually after onset for 9 years. Children were diagnosed with MS according to McDonald criteria. Age at ADS onset, gender, date of second attack, Expanded Disability Status Scale (EDSS) score at most recent visit, and relapse count were extracted from the database. Baseline and serial MRI scans were evaluated using a standardized scoring tool. Clinical and MRI parameters that predict time to second attack and EDSS score were evaluated using Cox proportional hazards and linear regression models. Locally weighted scatterplot smoothing (LOWESS) was used to estimate best-fitting curves.
Results: Of 358 children enrolled, 72 (20%) have been diagnosed with MS (mean observation: 4.0±2.2 years, range: 0.04-8.1 years; 26 (65%) female). Of 72 children with MS, 51 (71%) had a second attack. Mean annualized relapse rate was 0.85±0.94 (mean total relapses: 2.0± 1.0). The presence of ≥1 persisting T1-hypointense lesion and ≥1 periventricular lesion at onset predicted time to second attack (HR 2.32, 95% confidence interval 1.04-5.18). Two of ≥1 periventricular lesion, ≥1 intracallosal lesion, and ≥1 T1-hypointense lesion predicted time to second attack (HR 2.49, 95% CI 0.99-6.31). In children followed ≥4 years, the presence of brainstem lesions (R=0.39, 95% CI 0.07-0.71) and new T2 lesion count over the first 12-months following the first attack (R=0.36, 95% CI 0.03-0.69) were associated with EDSS score at most recent visit.
Conclusions: Specific MRI parameters present at onset are associated with time to second attack. Early involvement of clinically eloquent brain regions (brainstem), as well as early MRI indices of disease activity (new T2 lesions) are associated with increased disability in children with MS.

P761

Clinical and molecular markers that predict severity of relapsing-remitting MS (RRMS) disease outcomes

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Background: Despite extensive research efforts exploring predictors of MS disease course, there has been no systematic investigation of demographic, clinical, radiographic and laboratory

data, which is possible through post-hoc analysis of clinical trials where such data was collected across the study population.
Objectives: To identify markers associated with on-study relapses and/or disability progression over 2-years, using data derived from the placebo arms of two large contemporaneous phase III studies (DEFINE and CONFIRM).
Methods: We investigated the association of baseline demographic, disease and molecular variables with clinical outcomes of 771 placebo-treated RRMS subjects. Molecular phenotypes were derived from whole blood mRNA profiling using Affymetrix Gene Chip®. Hypothesis-driven and hypothesis-free approaches were applied to identify baseline variables associated with clinical outcomes over 2 years. Baseline variables showing a statistically significant association with outcomes in a univariate model were subsequently tested for significance in a multivariate model.
Results: Using a hypothesis-driven approach, we identified several baseline demographic, clinical, or radiographic characteristics statistically significantly associated with relapse and/or disability progression outcomes.

A hypothesis-free approach identified relapse status as a major defining factor for the outcome subgroups. Amongst a number of laboratory test variables, several serum and blood biochemistry markers were found to be significantly associated with relapses and disability progression. Analysis of molecular profiling data suggested that signatures associated with immune cell signalling were associated with relapse rate and disability progression.
Conclusions: We have confirmed some previously identified factors associated with disease activity or progression such as age, pre-study relapses, baseline EDSS and Gd+ lesions, and in addition have identified putative laboratory markers of clinical outcome. A limitation of the current analysis was the paucity of relapse and disability progression events in the context of a two-year clinical trial. The current analysis will be expanded in the future with the addition of data from placebo subjects from other controlled trials, the scope of outcomes will be broadened using additional clinical and MRI data. Validation of these findings might enable stratification of patients according to disease severity and may eventually inform individualized treatment decisions.

P762
Possible prognostic factors for visual function after optic neuritis
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Background: Acute optic neuritis (ON) may lead to permanent visual impairment, but suitable markers for the visual prognosis are lacking.
Objectives: To investigate whether contrast sensitivity, Rayleigh match anomaloscopy or retinal nerve fiber layer thickness (RNFLT) can be used as prognostic factors for visual function after acute ON.
Methods: Twenty-nine patients (18 female, 11 male) with acute ON were examined. Best corrected visual acuity (BCVA), contrast sensitivity, Rayleigh match anomaloscopy and RNFLT, were determined at the time of diagnosis and after 6 months. Visual function was defined by BCVA.

For each possible prognostic marker, the patients were divided into a moderately to severely affected (MSA) group and a mildly affected to normal (MAN) group, depending on the baseline values. The MSA-group was defined by log contrast sensitivity values below 1,35, Rayleigh match setting range (SR) values higher than 16 and the overall RNFLT classified as "borderline" and "outside normal limits".

For statistical analysis, the logMAR of BCVA was used. Data was analyzed with non-parametric tests.
Results: For both contrast sensitivity and Rayleigh match anomaloscopy, the MSA-group had significantly worse BCVA at baseline, compared to the MAN-group (log contrast p-value (p)=0,0003, SR p=0,0001). There was a significant difference in the improvement of BCVA during the observation period between the two groups, for both contrast sensitivity (p=0,0008) and Rayleigh match (p=0,0003). The greatest improvement occurred in the MSA-group. At 6 months, there was no significant difference in BCVA when looking at contrast sensitivity (MSA-group mean -0,059, NMA-group mean -0,17, p=0,0948). However, BCVA was still significantly worse in the group with worst baseline SR, compared to the MAN-group (mean -0,037 vs -0,18, p=0,012).

For RNFLT, there was no significant difference in BCVA between the two groups, neither at baseline (p = 0,53) nor at 6 months (p=0,16). Both groups had a significant change of BCVA, but the improvement was not significantly different between the two groups (p=0,75).
Conclusions: Rayleigh match anomaloscopy could be a potential prognostic marker for BCVA 6 months after an episode of optic neuritis. Contrast sensitivity and the overall classification of RNFLT, does not seem to have this potential.
These results are from an ongoing study, and more extensive and detailed data will be presented in the future.

P763
Investigation of no evidence of disease activity (NEDA) and long-term disability prediction in a seven year longitudinal MS cohort
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Background: NEDA has become an important secondary outcome measure in MS clinical trials, but its predictive power for long-term disability status has not been explored.
Objectives: To investigate NEDA over 7 years as measured by relapses, disability progression, and yearly MRIs, and to determine the predictive value of NEDA annually for change in EDSS and timed 25 foot walk (T25FW) at 7 years.
Methods: Patients from the CLIMB Study at the Brigham and Women's Hospital were included if they had a diagnosis of CIS or RRMS and at least 7 years of prospective follow-up. NEDA was defined by the absence of relapses, sustained EDSS progression, and new T2 or T1 gadolinium-enhancing lesions at annual intervals. Patients were analyzed independent of disease modifying therapy. There were 219 subjects with yearly brain MRI and biannual clinical visits. Spinal cord imaging was available for 162 (74%) and T25FW data for 145 (66%). The positive and negative predictive values (PPV, NPV) of NEDA for EDSS change≤ 0.5 and no significant T25FW change at 7 years were calculated annually.

Significant change in T25FW was defined by an increase of at least 20% in the walk time relative to the baseline measurement.
Results: 46.5% met criteria for NEDA at year 1 and 27.0% at year 2, but only 7.9% maintained NEDA at 7 years. None of the subjects' baseline characteristics, including gender, race, age, disease duration and EDSS, significantly predicted NEDA at 2 and 7 years. For each year, assessment of spinal cord activity led to additional losses of 7-11% in the share of subjects free from MRI activity. The PPV of NEDA was 71.7% at year 1, and 79.3% at year 2 for EDSS change≤ 0.5 at 7 years; it was 82.3% at year 1 and 74.3% at year 2 for change in T25FW. The NPV of NEDA was 43.0% at year 1 and 43.3% at year 2 for EDSS change≤0.5 at 7 years; it was 27.8% at year 1 and 22.2% at year 2 for change in T25FW.
Conclusions: It is difficult to maintain NEDA long-term, but even when assessed during the first 2 years NEDA has good predictive power for long-term freedom from progression. However, loss of NEDA does not strongly predict a poor prognosis and thus should not be used to guide clinical decision making in favor of aggressive treatment. In this analysis, EDSS was a more stable measure of long-term disability status than T25FW changes. Further work is necessary to validate these findings using longitudinal cohort data with extended follow-up, ideally of >15 years.

P764

Early brain volume loss on interferon predicts disability progression after 4 years

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Background: At a trial level, the effect of MS therapies on brain volume loss, in addition to new lesion accrual, has been correlated with their effect on disability progression.
Objectives: To investigate the association between global and regional (gray and white matter) brain volume loss during the first year of interferon treatment and clinical outcome at 2 and 4 years.
Methods: We selected 105 naïve MS patients started on interferon-β (brain MRI scans performed in the 3 months prior and 12 months after therapy onset), and followed-up for at least 48 months. Presence of attacks and the Expanded Disability Status Scale (EDSS) were assessed every 6 months. The percentage brain volume change (PBVC) was assessed with SIENA (all patients); percentage changes in gray matter (GMVc%) and white matter (WMVc%) volumes were determined by SPM8 (due to segmentation errors data from 84 subjects were finally considered). Subjects were grouped into having (R+) or not (R-) further attacks and having (P+) or not sustained (P-) disability progression at 2 or 4 years of follow-up. Cutoff values for the presence of sustained disability progression were obtained for all brain volume measurements. Survival curves of time to sustained progression for each cutoff group were compared, and 2 models of Cox regression were applied to adjust the cutoff values for demographic and clinical parameters and number of new T2 lesions at follow-up scan (NL).
Results: No statistically significant differences for any global or tissue-specific measures were found between R-/R+ at 2 and 4 years of follow-up, nor in P-/P+ at 2 years of follow-up. However, P+ after 4 years of follow-up had statistically larger decreases in PBVC (P-, n 87, mean:-0.683%, SD: 1.030; P+, n 16, mean:-1.618%, SD:1.395; p=0.004) and WMVc% (P-, n 70, mean:0.126%, SD:2.505; P+, n 12, mean:-1.791, SD:2.776; p=0.032) than P-. Cutoff points for PBVC and WMVc% were -0.86% and -2.49%, respectively. Subjects below the cutoff were more prone to develop sustained disability progression (unadjusted HR: PBVC HR 3.875; p=0.005; WMVc% 4.246; p=0.004); PBVC and WMVc% were found to be independent predictors of sustained disability progression in the multivariate analysis (HR: PBVC 5.437; p=0.011; interaction WMVc%*NL 1.086; p=0.005).
Conclusions: At a patient-level, first-year global and white matter volume loss in interaction with NL are predictive of mid-term sustained disability progression in patients on interferon therapy.

P765

Assessing a predictive score for long-term disability progression in relapsing-remitting multiple sclerosis: 7/8-year follow-up in the PRISMS study

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Background: Early identification of patients with relapsing-remitting multiple sclerosis (RRMS) who are at risk of disease progression may facilitate therapy adjustment. The modified Rio score (MRS) stratifies by disease activity and may predict long-term therapeutic response.
Objectives: To determine if MRS predicts disability progression up to 8 years after treatment initiation in patients with RRMS treated with subcutaneous (sc) interferon (IFN) β-1a in the PRISMS study.
Methods: Patients who had received sc IFN β-1a for ≥1 year in the placebo-controlled PRISMS study were assessed at one long-term follow-up (LTFU) visit near the 7th or 8th anniversary of PRISMS Day 1. MRS at 1 year was calculated thus: 0 if ≤5 new T2 lesions + 0 relapses, 1 if ≤5 new T2 lesions + 1 relapse or >5 new T2 lesions + 0 relapses, 2 if ≤5 new T2 lesions + ≥2 relapses or >5 new T2 lesions + 1 relapse, 3 if >5 new T2 lesions + ≥2 relapses. Descriptive statistics assessed the relation between MRS and LTFU disability progression (defined as ≥1-point increase from baseline in Expanded Disability Status Scale [EDSS] score for baseline EDSS < 6.0, or ≥0.5-point increase for baseline EDSS ≥6.0) in the sc IFN β-1a arm.
Results: In total, 367 patients received sc IFN β-1a either 22 μg or 44 μg thrice weekly for ≥1 year. At 1 year, 146 (39.8%) had MRS 0, 108 (29.4%) MRS 1, 91 (24.8%) MRS 2, and 22 (6.0%) MRS 3. Baseline median (Q1, Q3) EDSS score was 2.5 (1.5, 3.5). EDSS progression up to and including LTFU was confirmed in 68/118 (57.6%) patients with MRS 0, 64/93 (68.8%) with MRS 1, and 75/98 (76.5%) with MRS 2-3. 58 patients (n=28 MRS 0, n=15 MRS 1, n=15 MRS 2-3) did not return for the LTFU visit and had

no EDSS progression in PRISMS. Unconfirmed EDSS progression at LTFU was seen in 47/104 (45.2%) patients with MRS 0, 45/78 (57.7%) with MRS 1, and 45/76 (59.2%) with MRS 2-3. The trend persisted when the patients who did not return for LTFU (n=42 MRS 0, n=30 MRS 1, n=37 MRS 2-3) were imputed as having progressed. EDSS increase at LTFU was least for MRS 0; median EDSS changes were 0.5, 1.0, and 1.5 for MRS 0, 1, and 2-3, respectively. Proportions of patients who reached EDSS ≥6 at LTFU were 19.2%, 21.8%, and 31.6% for MRS 0, 1, and 2-3, respectively.

Conclusions: MRS appeared to predict disability progression over 7-8 years. Fewer patients with MRS 0 had disability progression at LTFU compared with those with MRS ≥1.

P766

Assessing a predictive score for disease activity in secondary progressive multiple sclerosis: post-hoc analysis of data from the SPECTRIMS study

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Background: Timely identification of patients with multiple sclerosis (MS) who are at risk of poor outcomes facilitates therapy adjustment. The modified Rio score (MRS) is used to stratify patients by early disease activity and can predict response to treatment.

Objectives: To determine whether the MRS can predict later disease activity in patients with secondary progressive MS (SPMS) who had received subcutaneous (sc) interferon (IFN) β-1a in the SPECTRIMS study.

Methods: A post-hoc analysis was performed on data from patients who had received sc IFN β-1a thrice weekly for ≥1 year in the SPECTRIMS study. MRS at 1 year was calculated thus: 0 if ≤5 new T2 lesions and 0 relapses; 1 if ≤5 new T2 lesions and 1 relapse or >5 new T2 lesions and 0 relapses; 2 if ≤5 new T2 lesions and ≥2 relapses or >5 new T2 lesions and 1 relapse; 3 if >5 new T2 lesions and ≥2 relapses. Descriptive statistics were used to assess the relationship between MRS and disease status at 3 years, classified as clinical activity-free (CAF: no qualifying relapses or progression indicated by a ≥1-point increase in Expanded Disability Status Scale score sustained over two consecutive 6-month visits) or disease activity-free (DAF: no clinical activity, gadolinium-enhancing lesions, and new/enlarging T2 lesions). An identical analysis was performed on the subgroup of patients who had ≥1 relapse in the previous 2 years.

Results: The analysis included 399 patients, of whom 225 (56.4%) had an MRS of 0, 115 (28.8%) had MRS of 1, 54 (13.5%) had MRS of 2, and 5 (1.3%) had MRS of 3. At 3 years, 58/395 (14.7%) patients were CAF; of these, 57 (98.3%) had MRS of 0 and 1 (1.7%) had MRS of 1; 4 patients withdrew before Month 36. At the 3-year evaluation, 26/397 patients (6.5%) were DAF; all had MRS of 0. Two patients withdrew before Month 36. In the subgroup of patients who had ≥1 relapse in the 2 years prior to study entry (n=189), 89 (47.1%) had MRS of 0, 62 (32.8%) had MRS of 1, 35 (18.5%) had MRS of 2, and 3 (1.6%) had MRS of 3; 1 patient withdrew before Month 36. At 3 years, 20/188 (10.6%) relapsing patients were CAF and 13/189 (6.9%) were DAF. In the subgroup analysis, all patients who were CAF or DAF had MRS of 0.

Conclusions: In this SPMS population, few patients were free from clinical or overall disease activity after 3 years' treatment. Most CAF and DAF patients had an MRS of 0, demonstrating that the MRS may be a useful predictor of disease activity in patients with SPMS.

P767

Clinical and paraclinical parameters in newly diagnosed MS patients predictive of brain atrophy after 2 and 5-years

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Background: Conventional magnetic resonance imaging (MRI) has improved the diagnosis and monitoring of multiple sclerosis (MS). Parameters usually studied are weakly correlated with physical and cognitive impairment.

Objectives: To evaluate correlations between baseline clinical, neuropsychological and ophthalmological parameters and evolution of brain atrophy over 5 years.

Methods: 78 MS patients from 3 centres were prospectively evaluated at baseline, year 2 and year 5. Demographic (age), clinical (Multiple Sclerosis Functional Composite-MSFC, Expanded Disability Status Scale-EDSS) and neuropsychological (French adaptation of the Brief Repeatable Battery-BRB) variables were recorded. Optical coherence tomography (OCT) scans of the macula were performed. MRI 3D sequences were acquired on 1.5T machines, tissue-specific volumes were obtained (SIENAx) and volume changes calculated (SIENA).

Results: Baseline Nine-hole peg test (9HPT) and Go-no-Go test were correlated with global (p=0.006; p=0.008, respectively), grey matter (GM; p=0.012; p=0.008) and white matter (WM; p=0.003; p=0.003) atrophy. Only the learning phase of the Selective Reminding Test (SRT) was negatively associated with GM and WM volume (p=0.047). Temporal OCT atrophy was correlated with global, GM and WM atrophy (p=0.039). At year 2, EDSS score was correlated with global (p=0.015), GM (p=0.025) and WM (p=0.003) atrophy at the 2nd evaluation. 9HPT was also associated with global and GM (p=0.017; p=0.008) but not WM atrophy. SRT score was associated with WM atrophy (p=0.049). Addressing the correlation between baseline variables and volume changes over 2 years, Time to 25 Foot walk (T25FW, p=0.024)) and 9HPT(p=0.050) were associated with reduction of the global brain volume. Further data on focal GM atrophy at year 2 and 5 will be presented in September.

Conclusions: These first results show that only 3 baseline parameters are associated with brain atrophy: 9HPT,

Go-no-Go test and temporal OCT atrophy. After 2 years, EDSS is correlated with brain atrophy at the 2nd evaluation. Baseline predictors of brain atrophy at year 2 are the T25FW test and 9HPT.

P768

A clinical prediction model for definite multiple sclerosis in patients with clinically isolated syndrome

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Background: Clinically isolated syndrome (CIS) is often the first manifestation of multiple sclerosis (MS). However, not all patients with CIS will go on to develop multiple sclerosis (MS). The uncertainty about whether or not a CIS patient will develop MS is problematic both for the wellbeing of patients and for the decision to start treatment early in the course of the disease. With the most recent revisions to the diagnostic criteria for MS, the diagnosis of MS can already be made after one attack in a subgroup of patients with specific abnormalities on the brain MRI scan. However, this subgroup includes only a minority of CIS patients, leaving most patients still in the dark.
Objectives: To create a simple and reliable prediction model for MS in patients with CIS.
Methods: 431 CIS patients were included within 6 months after symptom onset. Potential predictors were chosen a priori based on existing literature and clinical experience. The outcome measure was clinically definite MS (CDMS) as defined by Poser and colleagues. A multivariate Cox regression model was created after univariate screening of candidate predictors and then further simplified using stepwise backward selection. A simple scoring system was then constructed giving equal weight to all predictors. The model was internally validated using bootstrapping techniques.
Results: The final model consisted of the following 5 predictors:

1. DIS+DIT2010 (the baseline scan fulfills criteria for dissemination in time and place according to the 2010 revised McDonald criteria),
2. corpus callosum lesion,
3. cerebrospinal fluid oligoclonal bands,
4. fatigue and
5. abnormal MRI.

Three risk groups were created: low risk (0-1 risk factor present), intermediate risk (2-3 risk factors) and high risk (4-5 risk factors). The 5-year risk for CDMS in the low-risk group was 19.4% versus 56.0% in the intermediate-risk group and 92.5% in the high-risk group. The final model had a reasonable discriminative ability with a c statistic of 0.71.
Conclusions: We created a simple clinical prediction model for CDMS in patients with CIS, distinguishing 3 risk groups based on widely available parameters. This can be a great practical tool to inform and provide support to CIS patients and to simplify the decision regarding the early start of immunomodulatory treatment.

P769

Predicting clinical course in multiple sclerosis using machine learning

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Background: Methods to predict disease course in MS are currently limited.
Objectives: To explore the utility of machine-learning techniques to reliably predict multiple sclerosis disease progression status at up to five years of follow-up.
Methods: 1693 patients from the Comprehensive Longitudinal Investigation in MS at Brigham and Women's Hospital (CLIMB), were included in the analysis. The main outcome was an increase EDSS≥1.5 (progressive) or not (non-progressive) at up to five years after baseline visit. Classification models used baseline features only or additional longitudinal clinical and brain MRI data. We used the machine learning technique, support vector machines (SVM), to build prediction models classifying MS patients into those with or without progression during the follow-up period. Bagging and undersampling of the larger non-progressive class and cost misclassification to reduce false negatives in the progressive group, were applied. Subgroups of patients, based on baseline EDSS were evaluated.
Results: Using baseline data alone, SVM models performed no better than random. In patients with baseline EDSS< 4, additional clinical observation for one year improved overall accuracy in predicting non-progressive cases (65-74%). MRI follow-up at 1 year further improved accuracy of predicting progressive cases (71-75%). When cost misclassification was applied, prediction of progressive cases improved (up to 87%). With further clinical follow-up over 2 years, the accuracy of predicting non-progressive MS improved (77-79%), with no benefit of adding MRI information (74-76%). Demographic features of race, ethnicity and family history of MS ranked highly as predictors of non-progressive MS. Brain parenchymal fraction, a surrogate of whole brain atrophy, ranked highly as a predictor of non-progressive cases, and global brain T2 lesion volume ranked highly as a predictor in progressive cases.
Conclusions: SVM incorporating short-term clinical and brain MRI follow-up data, class imbalance corrective measures, and classification costs may be a promising means to predict MS disease course.

P770

Early MRI predictors of clinical progression over 48 months in patients with clinically isolated syndrome

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Background: Accumulation of newly active lesions and progression of brain atrophy are the most relevant surrogate markers for conversion to clinically definite multiple sclerosis (CDMS) and development of sustained disability progression (SDP). Investigation of early MRI outcome changes after the first clinical event may identify clinically isolated syndrome (CIS) patients at higher risk for clinical progression.
Objectives: To investigate, in a prospective study, the predictive role of baseline and 6-month changes in MRI outcomes with respect the conversion to CDMS or development of SDP in CIS patients over 48 months.
Methods: This study examined 210 CIS patients treated with weekly intramuscular interferon beta-1a. All the CIS patients who entered the study showed ≥2 oligoclonal bands in cerebrospinal fluid and ≥2 hyperintense T2 lesions at disease onset. Multivariate Cox proportional hazard models were used to analyze predictors of CDMS and SDP (confirmed after 6 months) between months 6 and 48.
Results: Greater T2 lesion volume (HR 1.81; p =.005) and presence of contrast-enhanced (CE) lesions (HR 2.13; p < .001) at baseline were significantly associated with increased cumulative risk of conversion to CDMS over 48 months. Greater atrophy of the corpus callosum (CC) area (HR 2.74; p =.001) and greater increase of lateral ventricle volume (HR 2.43; p =.002) at 6 months relative to baseline were associated with increased cumulative risk of conversion to CDMS between months 6 and 48. In addition, increased risk of SDP over 48 months in patients with greater lateral ventricle enlargement at 6 months was found (HR 4.70; p =.001).
Conclusions: Greater T2 lesion volume, presence of contrast-enhanced lesions at baseline, atrophy of corpus callosum and enlargement of the lateral ventricles over the first 6 months of CIS may assist in identification of patients with the highest risk of conversion to CDMS. Enlargement of the lateral ventricles was also associated with SDP.

P771

Predictors of disability accrual in multiple sclerosis patients on first-line therapy

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Background: A principal objective when managing the clinical care of people with multiple sclerosis (MS) is to prevent the irreversible accumulation of neurological disability. Clinical trials provide evidence for clinical efficacy of disease modifying therapies (DMT) in reducing disease burden, however, the results of clinical trials are based on well-controlled environments, rigorous patient review, and do not necessarily reflect real-world patient characteristics or behaviours. Predictors of long-term EDSS score change in a cohort of patients treated with first-line disease modifying therapies in a real-world clinical setting is not known.
Objectives: To identify predictors of expanded disability status scale (EDSS) change in patients with relapsing-remitting multiple sclerosis (RRMS) treated with any interferon-beta (IFNβ) preparation or glatiramer acetate (GA) as a first therapy.
Methods: Data were extracted from the MSBase Registry. Median EDSS changes over 5 year (n=3367) and 8-year (n=1870) periods were determined. Predictors of EDSS change were analysed using adjusted quantile median regression. Continuation on first-line therapy was not a condition for remaining in the analysis.
Results: High relapse activity was the principal driver of 5 and 8-year post-baseline disability score increases. Duration of use of first-line DMTs was associated with decreased accrual of disability over the long-term. Annualised relapse rate (ARR) was highly predictive of increases in median EDSS score over both 5-year (coefficient (*coeff*) 0.63, p< 0.000) and 8-year (*coeff* 0.47, p< 0.000) periods. Additionally, on unadjusted analysis, on-treatment relapses in the first 5 years (*coeff* 0.53, p< 0.000), but not 8 years were predictive of an EDSS increase. Increasing proportion of the observation period on DMT therapy was associated with a significant decrease in EDSS change at 5 and 8 years (*coeff* -0.67, p< 0.000; -0.32, p=0.012), respectively. Additionally, pregnancy was shown to correlate with a decrease in EDSS score within the first 5 years of observation (p=0.001).
Conclusions: In this real-world cohort of patients observed for 5 and 8 years after first DMT initiation**,** high ARR, particularly early on-treatment relapse activity, is a poor prognostic indicator. Pregnancy and the use of first-line disease modifying therapy, however, are associated with a protective effect against 5 and 8 year EDSS increase.

PROs and QOL

P772

Psychosocial factors affecting quality of life in multiple sclerosis - a review of the current evidence base

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Background: Multiple sclerosis (MS) may have a significant impact on a patient's quality of life (QOL), those with MS report having a poorer quality of life than the general population. Several psychosocial factors may be possible contributors to the observed deterioration of QOL in MS.
Objectives: The aim was to analyse the current literature on the contribution of various psychosocial factors on QOL in MS.
Methods: Literature searches were conducted in Medline, Science Direct and Psych Info for articles on MS, quality of life, psychosocial factors and a series of psychosocial factors identified from the literature search. All primary studies published in English were eligible. Each database was searched up to 1 April 2014. Exclusion criteria included reviews, duplicate publications and papers on measurement of psychosocial factors or QOL, as opposed to the relationship *per se*.
Results: 137 papers yielded 30 studies on the potential influence of psychosocial factors on QOL in MS. Assessment of the relative importance of the factors is impaired because several factors are little studied, such as coping and self efficacy. The factors most frequently examined for effect on QOL were depression, employment status and cognitive impairment. Contributory factors ranked in order of number of papers examining the topic and finding a relationship to QOL were depression (15 out of 15 studies), employment (10 out of 11 studies), cognitive function (9 out of 10 studies), education (6 out of 7 studies), and fatigue (5 out of 5 studies). In addition, some factors may contribute to QOL in MS but appear to have been under-researched. These included anxiety (4 out of 4 studies) and obsessive compulsiveness disorders (1 study). Gender, age and marital status did not appear to influence QOL.
Conclusions: Current research determining factors affecting QOL in MS shows strong evidence in support of depression having a detrimental impact upon QOL in this population. However it is also evident that the literature focuses on a limited number of psychosocial factors. Future research needs to consider a wider range of psychosocial factors in order to gain a more holistic picture of what contributes to QOL in MS and hence determine possible ways in which this can be improved.

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Personality traits predict the perceived health-related quality of life in persons with multiple sclerosis

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Background: Neuropsychiatric disorders have been reported in persons with MS (PwMS) and found in association with brain morphostructural changes. Personality traits (eg., neuroticism) can affect the health related quality of life (HRQoL) in different disorders. Personality as defined by Eysenck is the sum of the actual or potential behavior patterns of the organism, as determined by heredity and environment, developing through the interaction of cognitive, conative, affective and somatic sectors. In MS, personality traits can determine patients' willingness to take on more risky treatment options, predispose to neuropsychiatric symptoms and affect coping strategies
Objectives: We aimed to investigate the role of personality traits as possible predictors of HRQoL in a large cohort of PwMS.
Methods: PwMS (Poser Committee criteria), consecutively recruited at the MS Center, Sassari University Hospital between January 1st 2004 and April 30th 2004, were asked consent to study participation. Raven Colored Progressive Matrices (RCPM) test and the STAI-X1 were used to screen for major intellectual deficits and state anxiety. PwMS' self-perceived mental and physical health status was measured with the 36-Item Short Form Health Survey (SF-36). The personality profile was assessed with the Eysenck Personality Questionnaire (EPQ-R) yielding scores for extraversion/introversion, neuroticism, psychoticism and lie. The correlation between HSQoL and personality traits was investigated by means of analysis of variance, with the SF-36 aggregated mental (MCS) and physical (PCS) scores as dependent variables and the EPQ-R subscales scores as independent variables, adjusting for possible confounders or effect modifiers (gender, age, trait anxiety, depression, degree of disability, education). The study was approved by local ethical committee.
Results: After screening with RCPM and STAI-X1/X3, 195 out of 250 MS patients (F:M=2.75), aged 41.7±10.2 years were included in the analysis. SF-36 MCS and PCS variance was largely explained by extraversion (39%,$p=0.017$; and 56%, $p< 0.0001$), neuroticism (61%, $p< 0.0001$; 51%, $p=0.0003$), psychosis (50%, $p< 0.0001$ for PCS only).
Conclusions: PwMS' HRQoL is largely influenced by personality traits which may act as predictors of better or worse perceived quality of life and should be included in clinical and experimental settings focusing on HRQoL.

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Predictors of quality of life in MS: relations with disability status, mental health, fatigue, and comorbid illnesses

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Background: Persons with multiple sclerosis (MS) report lower health-related quality of life (HRQoL) than those in the general population or in other chronic disease populations. Several factors have known associations with HRQoL in MS (e.g. sex, age, disability status). While comorbid medical conditions are common in MS little is known about their impact on quality of life.
Objectives: We investigated relationships among factors known to affect HRQoL in MS, and the presence of comorbid illnesses.
Methods: From July 2010 through March 2011, 949 consecutive adult patients with definite MS attending routine visits were recruited at 4 participating provincial MS Clinics across Canada

(British Columbia, Alberta, Manitoba and Nova Scotia). The Expanded Disability Status Scale (EDSS) score was recorded on the day of recruitment; all participants completed the Health Utilities Index Mark III (HUI) as a generic utility measure of HRQoL, the Hospital Anxiety and Depression Scale (HADS), the Daily Fatigue Impact Scale (D-FIS), and a previously validated questionnaire documenting lifetime diagnoses of 21 comorbidities.
Results: 75.2% of the sample was female and 72.4% had relapsing-remitting MS. The mean (SD) age was 48.6 (11.4) years, mean MS duration was 15.4 (10.2) years and median (IQR) EDSS was 2.5 (1.5-5.0). 56% reported at least1 physical comorbidity. The mean (SD) HUI score was 0.54 (0.32) with 63% of the sample falling into the category of severe disability (< 0.70). Mean (SD) HADS-Depression and HADS-Anxiety scores were 4.72 (3.57) and 6.56 (4.06); mean (SD) D-FIS score was 12.5 (8.24). Regression analyses showed that number of comorbidities, EDSS, self-rated anxiety, depression and fatigue were each associated with HUI scores. A multiple regression model including these factors as well as sex and age accounted for a large proportion of variance in HUI scores ($F=145.44$, $p< .0001$, adjusted $R^2=0.62$).
Conclusions: Neurologic disability, mental health symptoms, fatigue, and comorbidity were all associated with HRQoL in this representative clinic-attending sample of persons with MS. Together, these factors account for a large proportion of variance in self-rated HRQoL. Comorbid illnesses and symptoms of anxiety, depression and fatigue all represent potentially modifiable factors that may provide targets for improving HRQoL in persons with MS. A better understanding of both direct and indirect effects of these factors on HRQoL will help prioritize strategies for improving MS care.

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Validity of the Neuro-QOL lower extremity and upper-extremity scales in persons with MS with a range of cognitive disability as measured by the SDMT

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Background: Neuro-QOL is a set of self-reported measures that assess health-related quality of life (HRQOL) of adults and children with neurological disorders. The measures have undergone extensive psychometric testing but their performance in persons with multiple sclerosis (MS) with a range of cognitive impairment has not been fully assessed in 2 of the 13 Neuro-QOL measures, Upper Extremity Function (UEF) and Lower Extremity Function (LEF). These 2Neuro-QOL measures were included in a study to validate iPad analogs of the Timed-25-Foot Walk (T-25-FW), 9-Hole Peg Test (9-HPT), and Symbol Digit Modalities Test (SDMT). Non-computerized versions of the T-25FW, 9-HPT and SDMT were also administered and their results were used in the current analysis.
Objectives: To assess the relationships of the UEF and 9-HPT and the LEF and T-25-FW while controlling for cognitive processing speed as measured by the SDMT in this iPad validation sample. There were no cognitive exclusion or inclusion criteria for study entry but subjects, according to the MSFC protocol, had to complete the T-25-FW in 180 seconds and the 9-HPT in 300 seconds.
Methods: Descriptive statistics were analyzed. Pearson correlations were calculated for the UEF and 9-HPT and for the LEF and T-25-FW followed by partial correlations controlling for the SDMT.
Results: Data were available for 50 subjects. Most were female (80%), Caucasian (86%), with relapsing remitting disease (68%). Mean age=45.8 yrs (SD=10.1), mean education=15.0 yrs (SD=2.3) and mean disease duration=12.21 yrs (SD=9.1). Mean T-25-FW=7.1 (range 3.20-27.0), mean 9-HPT=23.0 sec. (range 13.8- 43.3), mean SDMT=54.3 (range 32- 78). Mean UEF raw score=37.6 (T-score=41.2), mean LEF raw score=34.0 (T-score=42.8). The 9-PHT and UEF correlation was r=- 0.43, p=0.002, while the partial correlation correcting for SDMT was r=-0.38, p=0.008. For the T-25-FW and LEF correlation was r=-0.55, $p< 0.0001$, while the partial was r=-0.518, $p< 0.0001$. There were no statistical differences between the r and partial r correlations for either set of comparisons.
Conclusions: The association between self-reported UEF and 9-HPT and self-reported LEF and T-25-FW are highly correlated and not affected by cognitive processing speed as measured by the SDMT. This provides further evidence of the utility of these two Neuro-QOL measures for those individuals with MS who have impaired processing speed.

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Living with multiple sclerosis: a quantitative exploration of a health-related quality of life battery of patient reported outcomes

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Background: Multiple sclerosis (MS) is a multifocal disease that impacts physical as well as psychosocial functioning. To fully assess the state of a patient, a number of patient reported outcome (PRO) measures are available. Although these measures have been validated separately, several constructs are measured in multiple questionnaires, and the optimal approach for combining information across questionnaires has not been considered.
Objectives: To explore the relationships among items from validated PRO scales and to determine if a smaller number of unobserved factors can be extracted.
Methods: A total of 401 patients enrolled in the Comprehensive Longitudinal Investigation of Multiple Sclerosis at the Brigham and Women's Hospital (CLIMB) had complete data on all items of the PRO scales. The mean (SD) age for these patients was 44.43 (11.18) and 73.57% were female. The PRO battery included The Center of for Epidemiologic Studies Depression Scale (CES-D, 20 items), MOS Modified Social Support Survey (MSSS, 18 items), Modified Fatigue Impact Scale (MFIS, 21 items) and Multiple Sclerosis Quality of Life - 54 (MSQOL-54, 54 items). Exploratory Factor Analysis (EFA) was used to identify latent constructs within the battery. We utilized the promax oblique rotation because the hypothetical constructs of the items were believed to be inter-related. Kaiser-Guttman's > 1.0 eigenvalue rule, proportion of common variance, and scree test were used to determine the ideal final factor model.

Results: The EFA yielded a 10-factor solution, and this accounted for 84.1% of the total variance in our data. Items from the MSSS loaded (>.8) on a single factor, without any items from other scales. Items on the MFIS did not load on a single factor, rather the items measuring cognitive fatigue loaded together with the cognitive functioning items from the MSQOL-54. The items measuring physical and psychosocial fatigue loaded together with the physical functioning items of the MSQOL-54. The five items from the Mental Health subscale of MSQOL-54 were found to have strong association to items in the CES-D scale. The remaining MSQOL-54 items loaded on separate factors as expected. Finally, all items from the Tangible Support subscale of MSSS also loaded (>.5) on a distinct factor.
Conclusions: These findings demonstrate that the PROs given to MS patients participating in CLIMB are interrelated and could be assessed utilizing a smaller number of items. Further psychometric evaluation is needed to confirm these findings.

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Correlations between patient-reported ambulatory function (MSWS-12) and objective disability measurements in SPMS: analysis of ASCEND baseline data

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Background: Ambulatory impairment is common in patients with secondary-progressive multiple sclerosis (SPMS). In the ASCEND study of patients with SPMS, ambulatory function is captured by several objective tools, as well as by the subjective patient-reported Multiple Sclerosis Walking Scale (MSWS-12).
Objectives: To evaluate the correlations between patient-reported ambulatory function and objective measures of disability at baseline in ASCEND.
Methods: ASCEND is an ongoing phase 3b, randomized, double-blind, placebo-controlled study to evaluate the efficacy of natalizumab on reducing disability in patients with SPMS. The primary endpoint is a composite of the Expanded Disability Status Scale (EDSS), Timed 25-Foot Walk (T25FW), and Nine-Hole Peg Test. In addition to EDSS and T25FW, another objective measure of ambulatory impairment in ASCEND is the 6-Minute Walk Test (6MWT). The MSWS-12 is also assessed to measure the impact of MS on walking from the patient's perspective (range: 0-100; higher score=more impairment). Patients with SPMS for ≥2 years, EDSS score 3.0-6.5, Multiple Sclerosis Severity Score ≥4.0, and documented evidence of disease progression independent of relapse within the prior year were eligible to enroll. Pairwise correlations between the MSWS-12 and the 3 objective measures were evaluated from pooled, blinded baseline data using Spearman's rank correlation coefficient.
Results: At baseline, 63% (n=556) and 37% (n=333) of patients had an EDSS score ≥6.0 and ≤5.5, respectively. Of the 889 patients enrolled, mean (median) T25FW was 13.5 (11.2) seconds, 6MWT was 215.9 (187.0) meters, and MSWS-12 score was 68.6 (72.9). Correlations were observed between objective disability measures (T25FW:6MWT *rho*=−0.80; 6MWT:EDSS *rho*=−0.72; T25FW:EDSS *rho*=0.68) in this SPMS population. MSWS-12 score positively correlated with EDSS (*rho*=0.48; n=889) and T25FW time (*rho*=0.38; n=888), and negatively correlated with 6MWT distance (rho=−0.42; n=861). Scatterplots to visually reveal these relationships will be shown.
Conclusions: In SPMS patients, subjective patient-perceived ambulatory function, measured by the MSWS-12 score, significantly correlated with the degree of overall disability and ambulatory disability demonstrated by higher EDSS, longer T25FW time, and shorter 6MWT distance.

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Longitudinal course of depression and fatigue in multiple sclerosis

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Background: Depression and fatigue are commonly reported in patients with multiple sclerosis (MS). Although there are numerous studies looking at the prevalence of depression and fatigue, few studies have examined the longitudinal course of depression and fatigue in MS.
Objectives: To estimate longitudinal changes in depression and fatigue in patients with MS.
Methods: A subgroup of subjects from the Comprehensive Longitudinal Investigation of Multiple Sclerosis at the Brigham and Women's Hospital (CLIMB) complete a battery of patient reported outcome (PRO) measures annually. The battery includesthe Center for Epidemiological Studies Depression Rating Scale (CES-D) and Modified Fatigue Impact Scale (MFIS). The MFIS was added to the PRO battery in 2006. For inclusion in this study, subjects were required to have completed PRO questionnaires for four consecutive years after the MFIS was added to the battery (n=351 for CES-D and n=343 for MFIS). The baseline mean (SD) of age of the subjects was 45.4 (10.7), the baseline mean (SD) disease duration was 12.3 (8.2) years, and 25.4% of the subjects were male. For the CES-D and MFIS, the sum of all available items was used for analysis. Longitudinal changes in the mean CES-D and MFIS scores were assessed using a linear mixed effects regression model with a categorical effect of time and random intercept to account for the within patient correlation.
Results: The estimated mean CES-D score at baseline was 29.6 (where the minimum CES-D score is 20). The estimated mean CES-D score decreased significantly after baseline ($p < 0.05$ for comparison of each time point to baseline). After baseline, the CES-D score remained generally constant (28.8 at year 1, 28.6 at year 2, 28.8 at year 3, and 28.4 at year 4). For the MFIS, the estimated mean at baseline was 26.4, and a significant decrease in the mean score between baseline and the subsequent time points was

observed ($p< 0.05$ for comparison of each time point to baseline). As with the CES-D, the estimated mean MFIS remained constant after baseline (24.4 at year 1, 24.7 at year 2, 25.0 at year 3, 24.8 at year 4). No significant interaction between length of time in the study and change with time was observed for either measure.
Conclusions: Declines in depression and fatigue were observed in CLIMB subjects after the baseline measurement, but no additional decline was observed. These results suggest that MS patients show no significant longitudinal change in depression or fatigue over four years.

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Cross-sectional analysis of patient-reported symptoms and impairment in relapsing-remitting and secondary progressive multiple sclerosis

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Background: Multiple sclerosis (MS) is a chronic and progressive neurological disease associated with substantial personal and economic burden.
Objectives: To evaluate patient-reported symptoms and impairment in a cross-sectional survey of patients with relapsing-remitting MS (RRMS) and secondary-progressive MS (SPMS) in the United States (US).
Methods: The National Health and Wellness Survey is an annual Internet-based survey of a nationally representative adult sample (age ≥18 years) in the US. Responses to the 2012 (March-August) and 2013 (April-August) surveys were evaluated; only the most recent response was used for patients responding to both surveys. The study included patients who reported a diagnosis of RRMS or SPMS. Proportions of patients with RRMS and SPMS reporting MS-related symptoms, employment status, impairment, and hospitalizations were compared using chi-square (categorical variables) and independent sample *t* (continuous variables) tests.
Results: Of the 810 respondents reporting a diagnosis of MS (mean age 49.6 years; 66.4% female), 458 (58.0%) had RRMS and 105 (13.3%) had SPMS. A greater proportion of SPMS than RRMS patients rated their MS as severe (21.9% vs 5.7%; $P< 0.001$). Symptoms reported by a higher proportion of SPMS than RRMS patients included difficulty balancing or walking (88.6% vs 69.9%; $P=0.001$); muscle spasms (74.3% vs 54.1%; $P=0.001$); sexual dysfunction (33.3% vs 22.7%; $P=0.023$); stiffness (56.2% vs 34.9%; $P< 0.001$); tremor (25.7% vs 15.3%; $P=0.011$); constipation (51.4% vs 28.2%; $P< 0.001$); and urinary incontinence or urgency (67.6% vs 41.7%; $P=0.001$). A lower proportion of patients with SPMS versus RRMS were employed (20.0% vs 39.7%; $P< 0.001$); although the number of working SPMS patients was small (n=21), patients with SPMS versus RRMS had a higher mean (SD) percentage of work impairment (47.6 [38.0] vs 31.2 [32.6]; $P=0.038$). Mean (SD) percentage of activity impairment was also significantly higher in SPMS versus RRMS (69.1 [24.0] vs 45.9 [31.2]; $P< 0.001$). The mean (SD) number of hospitalizations in the past 6 months was higher in SPMS than RRMS patients (0.30 [0.80] vs 0.17 [0.56]; $P=0.047$).
Conclusions: In this US survey, patients with SPMS described their disease as significantly more severe and reported a significantly higher burden of illness for multiple symptoms than patients with RRMS. Rates of work impairment, activity impairment, and hospitalizations were also significantly higher in patients with SPMS versus RRMS.

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P780

Cognition and fatigue in patients with relapsing multiple sclerosis treated by subcutaneous interferon beta-1a: an observational study SKORE

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Background: Cognitive impairment often occurs in multiple sclerosis (MS) patients, even in the early stages of the disease, and is believed to increase with worsening of physical disability, disease duration, onset of progressive disease, and also fatigue. Fatigue may occur with any severity in up to 90% of MS patients. Both cognitive impairment and fatigue can have a serious negative impact on patients' lives. Subcutaneous (SC) interferon (IFN) beta-1a can slow disability progression, and has also been reported to stabilize or delay cognitive impairment in most patients with mild relapsing MS.
Objectives: To evaluate cognitive function in patients with relapsing-remitting MS treated with SC IFN beta-1a, and its relationship to fatigue and neurological disability status.
Methods: This was an observational, prospective, multicenter study. Patients aged 18-65 years treated by SC IFN beta-1a were followed at 13 MS centers in the Czech Republic. Cognition was evaluated using the Paced Auditory Serial Addition Test (PASAT), fatigue by the Fatigue Descriptive Scale (FDS), and neurological disability by the Expanded Disability Status Scale (EDSS), all at baseline (BL) and Months 6, 12, and 24. Data were evaluated using descriptive statistics, and a relationship among the PASAT, FDS, and EDSS scores was assessed using Spearman's correlation coefficient.
Results: A total number of 300 patients entered the study; 272 completed 2-years´ follow-up. The proportion of patients with increased or stable PASAT score (no decline in cognition) versus BL was 61% at Month 24. The mean (standard deviation; SD) PASAT score was 48.49 (9.02) at BL and 49.98 (8.00) at Month 24. The proportion of patients with decreased or stable FDS score (no increase in fatigue) versus BL was 64% at Month 24. The mean (SD) FDS score was 3.58 (3.66) at BL and 3.68 (3.82) at Month 24. The proportion of patients with decreased and stable EDSS at Month 24 compared to BL was 31% and 33%, respectively. Using Spearman's correlation coefficient, no very high, high, or moderate correlation was found among EDSS, PASAT and FDS scores and its changes.
Conclusions: This study suggests stable or improved cognition, fatigue, and disability in the majority of patients treated with SC IFN beta-1a throughout 2 years´ follow-up, with about 30% of patients experiencing EDSS improvement. No correlation among cognition, fatigue, and disability level was found.

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Assessment of the patient's perspective in the European Register for Multiple Sclerosis (EUReMS): study protocol of the PRO study

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Background: The EUReMS (European Register for Multiple Sclerosis) project was designed as a platform to analyse and compare data of persons with MS (PwMS) within the EU member states. Herewith we report design and first results of a test study on patient-reported outcomes (PRO) that was developed to address one of the four EUReMS missions, namely the "assessment of PwMS' quality of life (QoL), burden of symptoms and socio-economic aspects from the patient's perspective".
Objectives: To show whether it is feasible to collect PRO data in a considerable number of PwMS on a European level, and to identify differences in QoL and employment between participating countries.
Methods: After identifying the existing registers in Europe, questionnaires and semi-structured interviews were conducted in order to assess the register's heterogeneity and their ability to participate. Based on these results, four registers (Germany, Poland, Sweden and UK) were identified to participate in the first stage of this study. A set of variables was identified in close cooperation between the registers and the Department of Medical Informatics at the University Medical Center in Göttingen, Germany, which represents the required information on demographics, basic disease characteristics, PRO data (i. e. EQ5d, MSIS-29), and data on employment.
Results: A EUReMS database was set up, and import frameworks were developed providing information on specifications and definitions for data items, guidance on data anonymization and data transfer, and supported export formats. Standard routines were developed to harmonize the heterogeneous datasets from different registers by mapping the national register data to the EUReMS PRO dataset and according metadata. Data transfer is being performed by a file transfer service. To refine procedures for data harmonization and data analyses, the registers were asked to transmit test data. The statistical models for comparing the register data between European countries were defined according to the hypotheses that have been formulated by the EUReMS group. Data are now analysed, first results will be presented during the meeting.
Conclusions: The test phase of this EUReMS study will enable the consortium to (1) improve processes and tools for the integration and comprehensive analyses of PRO data from different sources across Europe, (2) show whether it is feasible to collect PRO data in a considerable number of PwMS on a European level, and (3) compare QoL and employment of PwMS in selected European countries.

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How do lower urinary tract symptoms affect quality of life in multiple sclerosis: a systematic review of the literature

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Background: 70-80% of patients with multiple sclerosis (MS) will experience some lower urinary tract symptoms (LUTS) during the course of the disease. In light of this, there has been research interest over recent years regarding how LUTS in MS affect health related quality of life.
Objectives: We conducted a systematic literature review to determine how LUTS impact on quality of life (QoL) in MS patients, in preparation for the Trajectories of Outcome in Neurological Conditions (TONiC) study, a British multicentre study of QOL in MS.
Methods: Literature searches were conducted in Medline, Web of Science, Scopus, CINAHL and PsycInfo to identify studies, published from 1900 to March 2014 inclusive, primarily assessing how LUTS affect QoL in MS patients. In addition to this, the references sections of selected studies were reviewed to identify any further articles of value. Studies were excluded from analysis if their primary aim was to evaluate the effect of a therapy on QoL or to validate the use of a specific measure to evaluate QoL.
Results: 23 potential studies were identified; of these, 20 met exclusion criteria (reviews, case studies, diseases other than MS, outcomes other than QOL, not published in English). This left three studies meeting the eligibility criteria. Four additional articles were identified from references. These 7 studies examined the effect of LUTS on QOL in a total of 1459 MS patients. Six studies suggested that the presence of LUTS in MS patients significantly adversely affects QoL, all showed that the three domains most commonly affected were physical functioning, vitality/fatigue and social activity limitation. One study found no relation between LUTs and QOL, despite using the Short Form 36 (SF-36) which had shown a relation between LUTS and QOL in 3 other studies. The discrepancy may be due to under-estimation of the range of LUTS, as the negative study only assessed one urinary symptom (self-reported degree of incontinence).
Conclusions: Comparison of studies was challenging due to heterogeneity in study design and outcome measures, but there is preliminary evidence that LUTS do adversely impact on QoL for people with MS. Further studies are warranted to confirm findings and identify QoL domains most affected. These would be improved by utilising an MS-specific urinary symptom measure, such as the SF-Qualiveen, in order to sensitively assess the broad range of urinary symptoms encountered in this population.

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Identifying an important change threshold for the Multiple Sclerosis Walking Scale-12 (MSWS-12)

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Background: The 12-item Multiple Sclerosis (MS) Walking Scale (MSWS-12) is currently the only patient-reported outcome measure specifically assessing the impact of MS on walking. Estimating the magnitude of what is often referred to as the minimum clinically important difference (MCID) on the MSWS-12 is important for the appropriate interpretation of MSWS-12 results. More recently, the term responder definition (RD) is often applied to describe the individual patient score change over a predetermined time period that should be interpreted as a treatment benefit.

Objectives: Pre-specified summed score analyses estimated the MCID/RD threshold for interpreting individual change over time on the MSWS-12 in MS patients with walking impairment (defined as having as Expanded Disability Status Scale (EDSS) score of 4-7) participating in the 24-week double-blind, placebo-controlled, randomized MOBILE trial of fampridine-PR.
Methods: Both anchor- and distribution-based approaches were used to estimate the MCID/RD using the data collected at Week 2 and at subsequent monthly administrations of the MSWS-12 and other walking-related endpoints in MOBILE. Relevant improvements on: 1) the Patient Global Impression of Change and 2) the EuroQol-5D-5L mobility question were selected as anchors for estimating the MCID/RD of the MSWS-12. The distribution-based estimate of the MCID/RD was the standard error of measurement (SEM). Iterative comparisons (triangulation) of the anchor- and distribution-based estimates with observed breaks in blinded change score data from the MOBILE trial allowed for the estimation of a single best value for the MCID/RD.
Results: The anchor-based methods yielded median changes/reductions between 5.2 and 9.7 points on the MSWS-12 for small but important changes in walking ability. The distribution-based MCID/RD estimate was 6.8 points (one SEM). Through the triangulation process, a reduction of 8 points on the MSWS-12 was selected as the best estimate of the MCID/RD in these patients.
Conclusions: These findings indicate that an 8-point reduction on the MSWS-12 provides a reasonable threshold for estimating whether an MS subject with walking impairment (defined as having an EDSS between 4 and 7) enrolled in the MOBILE trial had experienced a meaningful improvement in their self-reported ability to walk over 24 weeks. The generalizability of this finding to other MS patients, such as those with milder walking disability, and other studies, deserves future investigation.

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Psychometric testing of the early mobility impairment questionnaire for multiple sclerosis

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Background: Nearly all subjects (93%) with multiple sclerosis (MS) experience some degree of mobility impairment—over half (58%) in the first year following diagnosis—which can significantly diminish health-related quality of life. Expanded Disability Status Scale (EDSS) is one measure but detects and quantifies late stage mobility impairment. A sizeable proportion of subjects (~40%) with MS "rarely or never" discuss walking difficulties with their healthcare providers (HCP). To facilitate discussion between subjects with MS and their HCPs, and improve identification of early mobility limitations, the Early Mobility Impairment Questionnaire (EMIQ) was developed based on qualitative research in patients with MS and in collaboration with key opinion leaders.
Objectives: To evaluate the psychometric properties of the 15-item EMIQ, including derivation of a scoring algorithm as well as clinical thresholds for subject screening.
Methods: The instrument's psychometric performance was evaluated in a multi-center, prospective, non-interventional observational study. Subjects with MS who were 18-65 years of age, spoke English, and had an EDSS score of 2.0 to 6.0 were eligible to participate. Subjects completed a series of questionnaires over two study visits one week apart. In addition to an item-level analysis and subsequent item reduction using discriminant validity, factor analysis, and item response theory, a scoring algorithm was developed and the instrument's psychometric properties were evaluated.
Results: In total, 124 subjects were included in the study [males = 23%; age (mean) = 52 years; and EDSS score (mean) = 4.2]. Six items were identified for elimination. The resulting 9-item scale had one strong underlying domain (first eigenvalue explained 60% of variance), and the remaining item set provided strong information across the whole range of the scale ($I>3.3$ for $-2.6\leq\theta\leq2.4$). The final EMIQ was found to perform well psychometrically, with strong evidence of test-retest (ICC=0.858) and internal consistency ($\alpha=0.893$) reliability, as well as construct (multi-trait: $r>0.4$; known groups: $p< 0.001$) and concurrent ($r>0.42$) validity.
Conclusions: The present study provides evidence supporting the EMIQ as a psychometrically sound instrument for the screening of subjects experiencing early mobility impairments due to MS, laying the groundwork for its use in clinical practice and a potential role in guiding clinical assessment and intervention.

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Evaluation of patient health status in the PR-fampridine ENABLE study using SF-36-derived utility scores

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Background: Prolonged-release (PR) fampridine tablet (dalfampridine extended release tablets in the US) improves walking in MS patients. ENABLE (observational study) evaluated the effects of 48 weeks' treatment with PR-fampridine (10 mg twice daily, taken 12 hours apart) on health-related quality of life (HRQoL) in MS patients with walking impairment. Composite HRQoL utility measures are used as an indication of the health status of an individual at a particular time point.
Objectives: This analysis evaluated the health state of MS patients who were treated/not treated with PR-fampridine over 48 weeks in ENABLE using SF-6D utility scores derived from the SF-36, a patient-reported outcome survey.
Methods: In ENABLE, MS patients (18-75 y) with walking impairment completed the Timed 25-Foot Walk (T25FW) at baseline and Weeks 2 and 4 and the MS Walking Scale (MSWS-12) at baseline and Week 4. Patients with any improvement in T25FW speed at Weeks 2 and 4 and in MSWS-12 score at Week 4 continued treatment. Patients who did not meet this criterion discontinued treatment but had the option to continue as part of the not treated control group. SF-36 (v2) was assessed at baseline and Weeks 12, 24, 36, and 48. The SF-6D preference-based algorithm[1] implemented in SF-6D scoring programmes was used to convert SF-36 data into SF-6D data which contains 6 score domains (physical functioning, role participation, social functioning, bodily pain, mental health, vitality) and health state utility scores (range 0 [dead] to 1.0 [perfect health]).

Results: Of 901 patients enrolled in ENABLE, 707 continued treatment with PR-fampridine (128 were not treated after Week 4). At baseline, the mean (SD) SF-6D utility score was 0.6 (0.09) for both patients treated and not treated. In patients treated with PR-fampridine, SF-6D utility scores improved by a mean(SD) of 0.05(0.09), 0.05(0.09), 0.04(0.09), 0.04(0.09) points from baseline at Weeks 12, 24, 36, and 48 (all $P< 0.0001$). No significant changes from baseline in SF-6D utility scores were observed at any study visit in patients who were not treated ($P>0.3$). Changes from baseline in SF-6D utility scores were significantly different between patients on treatment and those not treated at each study visit ($P< 0.001$).
Conclusions: Consistent health state improvements were observed in the SF-36-derived utility scores in MS patients treated with PR-fampridine (versus not treated). These improvements were maintained through 48 weeks of treatment.

1. Brazier, JE, et al. *Medical Care*, 2004;42(9):851-859

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Psychometric properties of the French version of the multiple sclerosis knowledge questionnaire
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Background: Improving patient information about multiple sclerosis (MS) requires reliable assessment of patient knowledge. The Multiple Sclerosis Knowledge Questionnaire (MSKQ) is a 25-item self-assessed instrument first developed and validated in Italian to test patient knowledge and understanding of MS. Further psychometric validation of the MSKQ in other populations is needed.
Objectives: To assess the psychometric properties of the French version of MSKQ in a large cohort of patients with MS, using data from patients enrolled in the ADOPTE study.
Methods: ADOPTE was an observational, prospective, longitudinal, multicenter study conducted in France by 99 neurologists. A total of 389 adult patients with MS initiating a treatment with intramuscular interferon beta-1a were enrolled. Psychometric properties of the MSKQ, including quality of completion, construct validity and internal consistency were assessed on 233 patients who completed the MSKQ at 6-month visit. MSKQ score is obtained by summing the number of correct answers (range: 0-25).
Results: Mean age of patients who completed the MSKQ was 39.2 (+/-10.1) years and 80.2% were female. Median time between MS diagnosis and MSKQ completion was 1.4 years (Min-Max: 0.5-35.0). Median Expanded Disability Status Scale (EDSS) score was 1.0 (Min-Max: 0.0-6.5). The number of missing responses per questionnaire ranged from 0 to 8, and 13.3% of MSKQ collected had ≥1 missing responses. For each of the 25 items, the percentage of correct answers ranged from 22.5% to 100.0%. Mean MSKQ score was 15.7 (+/-3.9, Min-Max: 3-24). Overall, 8 items had good discriminating properties (percentage of correct answers between 30-80% and correlation Item - Total score >=0.3). Internal consistency was acceptable (Cronbach's alpha: 0.74). The unidimensionality of the MSKQ, evaluated by principal component analysis, was not confirmed (only 15.8% of variance explained by the 1st axis). MSKQ score was significantly higher in women, in patients with higher educational levels and in patients with higher socio-professional categories.
Conclusions: Analyses of psychometric properties of the French version of the MSKQ showed satisfactory results. However, several items were poorly discriminant. Some of them were kept by MSKQ authors for their clinical and educational interest, others may be removed from the questionnaire. Moreover, the unidimensionality of the MSKQ should be further investigated.

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Rasch analysis of the Leeds Spasticity Scale in multiple sclerosis
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Background: Clinician-assessment of severity of spasticity correlates poorly with patient self-report and may not address aspects of spasticity that are important to patients. Furthermore, there are concerns regarding the validity and reliability of the most widely used measure for spasticity - the Ashworth scale. The Leeds Spasticity Scale (LSS) is an 8 item patient-reported, interval-level scale, which was developed in a population with post-stroke spasticity. It has not been previously validated in MS.
Objectives: To examine the construct validity of the LSS in an MS population using Rasch analysis.
Methods: The LSS was given to 401 patients with MS across three neuroscience centres in the UK. Three subscales (muscle stiffness, pain and muscle spasms) from the MS Spasticity Scale-88 (MSSS-88) and a numerical rating scale (NRS) for spasticity were co-administered.
Results: 213 records were available for analysis (53.1% response). 37.7% had progressive type of MS and 14.6% had EDSS>6.5. 56.1% had moderate (NRS 4-6) or severe (NRS 7-10) spasticity There was good fit to the Rasch model of the unmodified scale although low levels of item dependency were found between items 1 and 2, and 5, 6 and 7. This was remedied by testlet structuring of those item sets. The final scale had ordered category response thresholds, non-significant chi-square fit, a person separation index of 0.85 (meaning suitable for individual use) and was unidimensional according to post-hoc t-test. The scale was free from differential item functioning for a variety of important person factors (age, sex, Extended Disability Status Score, MS type). The concurrent validity was confirmed by moderate correlations between the LSS and MSSS-88 stiffness (rho=0.75), pain and discomfort (rho=0.67), spasms subscales (rho=0.66) and NRS spasticity (rho=0.65).
Conclusions: The LSS is a brief scale suitable for use in patients with MS and produces data which fit the Rasch model and has good correlation with longer form measures.

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Dalfampridine improves spasticity and fatigue in multiple sclerosis
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Background: Fatigue and spasticity are common symptoms in multiple sclerosis (MS) with significant impairment of quality of life.
Objectives: Evaluate the short and long-term (6 months) effects of Dalfampridine on spasticity and fatigue.
Methods: This is a retrospective study of prospectively collected data on patients with MS. The effect of Dalfampridine was assessed at 14 days (D14), 3 months (3M) and 6 months (6M) of treatment with the T25FWT as an objective measure of the walking speed. Fatigue was measured using the validated french questionnaire EMIF-SEP and spasticity with a validated numerical rating scale (NRS). A Friedman test was used for multiple comparisons and the Wilcoxon signed rank test was used for 2X2 comparisons. Differences were considered significant at an alpha level of $p< 0.05$.
Results: Between April 2013 and March 2014, 134 patients (age (mean ± sd): 54 ± 18.6 years; median EDSS: 6) having benefited from Dalfampridine were evaluated at D14. At 6 months, 104 patients were evaluated and continue Dalfampridine. The following results are from the 104 patients evaluated at 6 months. Between baseline and D14, The T25FWT (25.6 ± 42.8 vs 15.5 ± 23.2 sec; p < 0.0001), fatigue (57.6 ± 19.5% vs 37, 8 ± 19%; p < 0.0001) and spasticity (NRS) (5.7 ± 2.4 vs 3.9 ± 2; p < 0.0001) decreased significantly. For all assessment criteria, compared with baseline, the positive effects persisted after 3 month (T25FWT: p< 0.0001; fatigue: p< 0.0001 and spasticity: p< 0.0001) and 6 months (T25FWT: p< 0.0001; fatigue: p< 0.0001 and spasticity: p< 0.001) under Dalfampridine medication.
Conclusions: Dalfampridine reduces spasticity and fatigue in MS. Ruck et al., 2013 found a positive effect on fatigue (MFSC) after 14 days of treatment, which lasts 9 and 12 months. Prugger et.al, 2013 shows an effect on fatigue (FSS) only at 6 months. The effect on spasticity has been studied by Goodman et al., 2010 without significant treatment effect on the Ashworth score at 14 days.

Improvement in nerve conduction in demyelinated axons associated with decrease in conduction blocks may partially explain the improvement in fatigue and spasticity. Dalfampridine has a positive effect on walking ability, spasticity and fatigue and may participate in the improvement of quality of life.

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Evaluation of the health related quality of life in neuromyelitis optica spectrum disorder

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Background: Health-related quality of life (HRQoL) measurement are being considered increasingly important with regard to evaluating disease progression, treatment and the management of care provided to patients with chronic disease such as neuromyelitis optica spectrum disorder (NMOSD). However, there have been limited studies on evaluation of HRQoL in NMOSD.
Objectives: The purpose of this study was to evaluate HRQoL in NMOSD and to compare HRQoL in NMOSD with in MS.
Methods: Patients with NMOSD were recruited from June 2009 and February 2010 at the National Cancer Center in Korea. Patients were evaluated at the study entry via Korean validated self-questionnaires on HRQoL (MSIS-29, MusiQoL). Fatigue Severity Scale (FSS-9) and the depression Questionnaire (PHQ-9), expanded disability status scale (EDSS) and social characteristics were also implemented. We assessed the relationship of these parameters with age, gender, education level, disability and disease duration and also compared fifty-six patients with MS derived from our previous study.
Results: In total, 96 patients (female 89.6 %) with NMOSD were studied. The median age was 40.3±10.3 years, mean disease duration was 7.1±4.4 years and median EDSS score was 3±2. Twenty-six patients with NMOSD (27%) had depression and 50 (52%) had fatigue. EDSS were strongly associated with physical domain of MSIS-29 and MusiQoL (r=0.64~0.73, $P< 0.001$). PHQ-9 was significantly correlated with psychologic domain of MSIS-29 and MusiQoL (r=0.72~0.84, $P< 0.001$), followed by physical domain (r=0.57~0.59, $P< 0.001$). FSS-9 was moderately correlated with Symptom and Global index of MusiQoL (r=0.51~0.54, $P< 0.001$). Dimensions of MSIS-29 and MusiQoL were most impacted by financially dependence, followed by employment and education state. Marital and religion status did not influence, except for Sentimental and sexual life of MusiQoL. The comparison of demographic characteristics between MS and NMOSD demonstrated that age, EDSS score and ratio of female were significantly higher in NMOSD than in MS (p=0.037, $p< 0.001$, $p< 0.001$). No significant difference was noted between patients with NMOSD and MS for dimensions of HRQoL instruments, except for HRQOL related to physical disability (P =0.009~0.016) which was lower in NMOSD than in MS.
Conclusions: This study indicated that physical disability, depression are the main factors influencing HRQoL in NMOSD. Compared with MS patients, patients with NMOSD have tended to be lower-HRQOL associated with physical disability.

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An interim analysis of quality of life in patients with relapsing-remitting multiple sclerosis treated with delayed-release dimethyl fumarate

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Background: In the Phase 3 DEFINE and CONFIRM studies, patients treated with delayed-release dimethyl fumarate (DMF) showed better health-related quality of life (HRQoL) over 2 years as assessed by the Short Form-36 version 1 (SF-36v1), compared with placebo (PBO).
Objectives: To present a 2-year interim analysis of HRQoL data from ENDORSE, an ongoing, 5-year, dose-blind extension of DEFINE and CONFIRM.
Methods: Patients randomized to delayed-release DMF 240 mg twice (BID) or three times daily (TID) in DEFINE/CONFIRM continued the same dose regimen in ENDORSE. Patients receiving PBO (DEFINE/CONFIRM) and glatiramer acetate (GA) (CONFIRM) were randomized 1:1 to blinded treatment with delayed-release DMF BID or TID. The SF-36v1 was administered at baseline and every 48 weeks thereafter. Although data were collected for both the twice- and thrice-daily DMF regimens, results are presented only for those patients receiving BID, the approved dosage. Results are presented according to treatment received in the parent/extension studies, BID/BID and PBO/BID. Results for GA/BID are not shown due to the small sample size.
Results: The intent-to-treat population comprised 1,500 patients, including 501 (BID/BID) and 249 (PBO/BID). At 2 years (Week 96) of ENDORSE, mean change in physical component scale (PCS) scores relative to DEFINE/CONFIRM baseline were 1.03 (BID/BID, p=0.0102) and relative to ENDORSE baseline were 0.14 (BID/BID, p=0.7292) and -0.26 (PBO/BID, p=0.9483). In addition, mean change in mental component scale (MCS) scores were 0.49 (BID/BID, p=0.3885) relative to the DEFINE/CONFIRM baseline, and -0.16 (BID/BID, p=0.6278) and -1.81 (PBO/BID, p=0.0070) relative to the ENDORSE baseline. Changes relative to the DEFINE/CONFIRM baseline are considered most relevant for BID/BID, as these values convey the overall effect of delayed-release DMF dosing since treatment initiation.
Conclusions: Small, statistically significant changes from baseline in some of the PCS/MCS scores were observed, although these may reflect random variations. Updated data will be presented, including clinically relevant changes (≥5 points) in PCS/MCS scores.

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MS with versus without relapse - the patient perspective in the PEARL study

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Background: The PEARL study (**P**rosp**E**ctive ph**A**rmacoeconomic coho**R**t eva**L**uation) focusses on real world aspects of providing health care for MS patients in Germany.
Objectives: Here we focus on the patient perspective to treatment satisfaction, health state and quality of life. We compare patients with a relapse in the first study year (ACTIVE group) to patients without a relapse in the first study year (INACTIVE group).
Methods: PEARL is a 24-months, non-interventional study in 1705 patients with relapsing remitting multiple sclerosis (RRMS). Of 1705 patients, 1214 patients used injectable interferon and 491 patients injected glatiramer acetate.

In the first study year, 411 patients relapsed at least once (**ACTIVE group, "A"**); 1294 patients did not relapse (**INACTIVE group, "I"**). MS lasted 5.1±4.4 and 5.2±4.3 years (mean±SD) in ACTIVE and INACTIVE patients, respectively. The mean annual relapse rate was 1.4±1.0 and 0.1±0.2 relapses/year (mean±SD) over the two year period. ACTIVE patients switched drug and discontinued therapy more frequently (A: 30% and 35%, I: 12% and 18%).
Results: Comparable fractions of ACTIVE and INACTIVE patients were "very satisfied" or "extremely satisfied" with their medication at baseline as measured by the Treatment Satisfaction Questionnaire for Medication (TSQM-9). Over the 24 month study period, satisfaction declined continuously in ACTIVE patients, while rather increasing in INACTIVE patients (A: 54%→36%, I: 52%→55%).

Subjective general health state was worse in ACTIVE patients as measured by the EQ-5D scale, deteriorated and remained low over 24 months, while improving in INACTIVE patients (A: 69.9→68.0, I: 72.0→73.2). ACTIVE patients were worse off after 24 months in all five EQ-5D dimensions. E.g. at baseline, 52% ACTIVE and 49% INACTIVE patients had pain or discomfort and 55% ACTIVE and 41% INACTIVE patients after 24 months.

MS-specific subjective quality of life impairment declined in both groups over 24 months as measured by the PRIMUS QoL questionnaire. Impairment was stronger and declined less in ACTIVE patients (A: 8.7→7.8, I: 8.3→6.8). MS-specific subjective activity impairment tended to increase in both groups over 24 months as measured by the PRIMUS activity questionnaire. Activity impairment was stronger and declined more in ACTIVE patients (A: 4.7→5.0, I: 4.0→4.1).
Conclusions: Relapsing patients feel dissatisfied, and impaired in the year following a relapse. Strong relapse prevention may improve patient satisfaction, quality of life and activity.

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Symptoms and association with health outcomes in relapsing-remitting multiple sclerosis: results of a US patient survey

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Background: A variety of symptoms have been reported, but the prevalence of specific symptoms in relapsing-remitting multiple sclerosis (RRMS), how they are related to one another, and their impact on health outcomes is not well understood. While relapse rate is the regulatory endpoint for approval of therapies for RRMS, patient well-being and health-related quality of life (HRQoL) may be profoundly affected by experience of symptoms and as such be important to understand and measure in clinical trials.
Objectives: To describe how symptoms of RRMS co-occur and their impact on patient-reported outcomes.
Methods: Individuals who reported a physician diagnosis of RRMS in a large general health survey in the United States

indicated the symptoms they experience because of RRMS and completed validated scales, including the Work Productivity and Activity Impairment questionnaire and either the SF-12v2 or SF-36v2 health questionnaire. Symptom clusters were identified through hierarchical cluster analysis, and the relationship between clusters and outcomes was assessed through regression modelling incorporating the clusters along with age, length of MS diagnosis, sex, race, household income, cigarette smoking, alcohol use, and comorbidities. Clusters including multiple symptoms were included in the models according to whether the reported number of symptoms was above or below the median number of symptoms in the relevant cluster reported in the sample. Single-symptom clusters such as depression were included as the presence or absence of the symptom.

Results: A total of 447 respondents reported RRMS. Fatigue, difficulty walking, and numbness were the most commonly reported symptoms. Seven symptom clusters were identified, and several were significantly related to patient reported outcomes. Pain, muscle spasms, and stiffness formed a cluster strongly related to physical HRQoL, associated with a 5.8 point decrement on physical component summary scores and a .045 point decrement in the SF-6D preference-based health utility score; depression was strongly related to mental HRQoL, with an 8.9-point decrement on the mental health component summary score and .064 point decrement in SF-6D scores (all $p < 0.05$). Other clusters had smaller but still significant ($p < 0.05$) associations with reduced HRQoL.

Conclusions: Symptoms in RRMS show a strong relationship with HRQoL, and should be taken into consideration in treatment decisions and evaluation of treatment success.

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What is the influence of fatigue and depression on patients' perceived illness intrusiveness?

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Background: Up to 83% of patients with MS experience fatigue, which can affect their physical and/or mental energy. MS-related fatigue negatively impacts quality of life (QoL) and can interfere with employment. Depression, another symptom affecting QoL, is highly associated with fatigue. The Illness Intrusiveness Ratings Scale (IIRS), a determinant of QoL, is a 13-item questionnaire that measures several different domains, such as instrumental tasks of daily living and relationships.

Objectives: To investigate the relationship between depression and fatigue, and how that relationship contributes to perceived illness intrusiveness.

Methods: All participants were MS patients from Holy Name Medical Center who signed research consents. Correlations were run ($N = 98$) to determine which measure of fatigue (Fatigue Severity Scale (FSS), The Fatigue Scale for Motor and Cognitive Functions (FSMC), and Incapacity Status Scale (ISS) Fatigue item) shared the least amount of variance with our measure of depression (Beck Depression Index-II (BDI-II)). After choosing the fatigue measure, moderation analyses were run ($N = 203$) with depression as the moderator and the IIRS Total Score as the outcome measure, controlling for overall disability (ISS Total Score minus the Fatigue score).

Results: The ISS Fatigue score shared the least amount of variance with the BDI-II (r (96) = .428, $p < .005$) of all the fatigue measures. The interaction between fatigue and depression was significant, F (4, 198) = 42.00, $p < .05$, as was each variable separately ($p < .01$).

Conclusions: Fatigue and depression were moderately correlated and were found to affect perceived illness intrusiveness individually. In addition, depression was a significant moderator of the relation between fatigue and illness intrusiveness.

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Prevalence of adverse events with long-term disease-modifying therapy and their impact on quality of life in patients with multiple sclerosis

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Background: Glatiramer acetate (GA) and interferon-beta (IFN-β) are disease-modifying therapies (DMTs) for multiple sclerosis that are administered through subcutaneous (SC) or intramuscular (IM) injections. Several side effects associated with DMTs are common and may influence patient´s health-related quality of life (QoL).

Objectives: The aim of the study to determine the prevalence of adverse events associated with long-term DMT use, and to assess the impact of these adverse events on QoL.

Methods: It is a longutudinal study among patients with multiple sclerosis who had been treated with their first DMT for at least 2 years(Range 2-10 years). All the adverse events were assessed by retrospectively from the MS databases of two majör centers. Generic and dermatology-specific health-related QoL were assessed using validated patient-reported questionnaires and these databases and their change in prevalance over time is analysed.

Results: Datasets of 922 patients were analysed.The most frequently observed side effect was flue-like symptoms (70 %) especially in patients receiving (IFNβ1a) injections either weekly or 3 times a week ($p < 0.05$). The other side effects were anemia and leucopenia (60 %), 40% in patients using GA and 20% using (IFNβ1b) ($p < 0.05$), pain in different parts of the body (50%) distributed equally in all DMT's, dysfunction in liver tests (40%) all seen in patients using (IFNβ1b), depression and pyschiatric symptoms (30%) distrubuted evenly among the patients($p=0.05$), fatigue (20%) seen in patients using IFNβ1b and IFNβ1a consecutively.Among all these side effects, skin reactions and cutaneous adverse events were the most frequently encountered side effect (82%) seen more frequently over time and was higher for SC DMTs (65-82%) compared to IM DMT (41%)($p < 0.05$) and interefered with the patients adherence to treatment and the most frequent side effect interfering with the QoL,The prevalance

aforementioned other side effects decreased over time anf did not interfere with QoL.
Conclusions: The results of this longitudinal study revealed that the prevalance of cutaneous adverse events, above all was highest in long-term DMT's, interfered with QoL and patients adherence to treatment.The other side effects with long-term DMT decreased in prevalance over time and did not interfere with QoL and patients' adherence to treatment.

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A validation study of the FSMC: comparing the consistency of patient self-evaluation with objective fatigue measures

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Background: Fatigue is a complex and prominent symptom of Multiple Sclerosis (MS) affecting some 75-95% of MS patients. Further, research has shown that some 50-60% of MS patients identify fatigue among their most severe symptoms, interfering with activities of daily living and quality of life. Thus, as clearly denoted by the proliferation of research on the topic, reliable fatigue evaluation and assessment is imperative.
Objectives: The current study aims to add to the validity literature on the Fatigue Scale for Motor and Cognitive Functions (FSMC) by comparing the consistency of subjective patient self-evaluation with objective measures of cognitive fatigue and performance.
Methods: Data was collected from 90 patients referred for neuropsychological testing at the MS Center at Holy Name Medical Center. Cognitive fatigue was assessed using decreasing performance over time using modified scoring on the Symbol Digit Modalities Test (SDMT). This allowed for the discovery of within trial productivity changes. Greater decrease of within trial performance on the SDMT is indicative of greater cognitive fatigue. Participants were divided into four groups by fatigue severity using the established clinical staging cut-off values (no fatigue, mild, moderate, severe).
Results: Total SDMT score was not significantly correlated with FSMC total fatigue score [r= -.203, n= 90, p= .055] or FSMC cognitive fatigue subscale score [r= -.200, n= 90, p= .059]. A one-way between subjects ANOVA was conducted to compare the impact of within trial productivity change on level of total fatigue as measured by the FSMC. The result was not significant at the p< .05 level for the four fatigue severity groups [$F(3,85) = 2.08$, $p = .109$]. An additional one-way between subjects ANOVA was conducted to compare the impact of within trial productivity change on level of cognitive fatigue as measured by the FSMC. The result was not significant at the p< .05 level for the four fatigue severity groups [$F(3,85) = 2.28$, $p = .877$].
Conclusions: Results suggest that patient self-evaluation on the FSMC is not consistent with objective fatigue measures and suggests the need for further study concerning the validity of the FSMC.

P796

Development of the neurological hope index for multiple sclerosis (NHI-MS)

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Background: Hope can motivate patients to engage with treatment, facilitates coping with the unpredictable nature of Multiple Sclerosis (MS), and improves mood and quality of life. There is currently no validated measure of hope in MS.
Objectives: Our aim was to validate a new measure of hope for people with MS.
Methods: Following qualitative interviews and item extraction, a draft Hope Index and the Herth Hope Index were given to MS patients attending hospital clinics.

Data from the draft questionnaire were fit to the Rasch model. Fit was judged by a non-significant chi-square, item and person fit residual SD < 1.4; and both the assumptions of local independence of items and unidimensionality upheld. Invariance of the scale (Differential Item Functioning-DIF) was tested for age, gender, marital status, disease duration/type, EDSS level. All tests of fit and DIF were Bonferroni adjusted at 0.05. Reliability is reported as Peron Separation Index (PSI) and Cronbach's apha.
Results: 254 people with MS participated, with mean age 50.4 years (SD12.1) and mean time since diagnosis 13.2 years (SD9.6). 68.6% were female. 48.0% had relapsing-remitting (RR) MS; 25.4% secondary progressive MS; 15.5% rapidly evolving RRMS and 11.1% primary progressive MS.

Data from the 47 draft items were fit to the Rasch model. Initial fit was poor (Chi-Square 606.7; $p < 0.001$) with indications of multidimensionality. Clusters of locally dependent items were observed, suggesting redundancy in the item set. After removal of these items fit improved and an 18 items unidimensional scale emerged which satisfied Rasch model expectations (Chi-Square 99.7 (df 72); p=0.02; mean item fit residual -0.247; SD 1.39). Reliability (PSI) was high at 0.89 (alpha 0.92). No evidence of significant DIF was observed across all contextual factors.

The new scale correlated at 0.69 with the Herth Hope Index. A strong association between hopefulness and disease type was observed (Kruskall- Wallis p< 0.001) as with a subjective report of quality of life (Kruskall- Wallis p< 0.001). Thus those with progressive types of disease had a lower Hope Index, and those with higher self perceived quality of life reported a more hopeful disposition.
Conclusions: A simple self-completed Hope Index for those with MS, built from the patient experience and satisfying Rasch measurement model standards, provides a disease-specific scale for this important mediating construct. The scale is free for use in all not-for-profit settings.

P797

Final results of the Swiss post marketing surveillance monitoring quality of life and treatment satisfaction in patients with RR-MS (SWISSASCENT)

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Background: Treatment satisfaction and quality of life (QoL) are important disease management parameters in RR-MS. Fingolimod (Gilenya®), the first oral RR-MS treatment, is approved in Switzerland, since January 2011, to reduce frequency of relapses and delay disability progression.
Objectives: Provide real-world treatment satisfaction and QoL data for patients with RR-MS treated with fingolimod or other DMTs.
Methods: Patients with RR-MS considering a new DMT were interviewed at baseline (BL) and follow-up (FU, about 6 months after treatment initiation). Data collection was independent of treatment choice, did not require mandatory visits, and all assessments were done according to the Swiss label. Patients completed the MSIS-29 and TSQM-9 questionnaires to assess treatment satisfaction and QoL, respectively. 6h first dose monitoring (FDM) data were collected for fingolimod treated patients.
Results: 212 patients started treatment with fingolimod. 35% of patients were treatment-naïve. In patients with previous DMT (65%), reasons for switching therapy were needle fatigue, intolerance, lack of efficacy, poor QoL and JCV antibodies. 91% of patients were free of abnormal ECG findings or adverse events during the FDM. 99.5% of patients continued fingolimod treatment after the FDM. At FU (mean 8.3 ± 3.2 months after FDM), 96% of patients continued fingolimod treatment. All domains of the TSQM-9 (effectiveness, convenience, global satisfaction) showed significant improvement upon switching from other DMTs to fingolimod. The effectiveness and global satisfaction domains of TSQM-9 were comparable in both switch and in treatment-naïve patients, suggestive of high satisfaction with fingolimod treatment in both types of patients. QoL (total MSIS-29 score) remained constant in both switch and treatment-naïve patients during treatment with fingolimod, with a significant improvement of the mental subscore in patients switching from other DMTs to fingolimod. During FU, 12% of patients reported adverse events (all non-serious), including one patient (0.5%) with macular oedema. ARR decreased from 0.8 ± 1.0 to 0.2 ± 0.9.
Conclusions: In this real-world setting, efficacy, tolerability and safety of fingolimod were in line with data reported in Phase 3 trials. Treatment satisfaction with fingolimod was high in both treatment-naïve and switch patients, while QoL remained stable over the mean follow-up period of 8.3 months.

P798

Rasch analysis of the WHO Disability Assessment Schedule 2.0 for use in multiple sclerosis

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Background: The WHO Disability Assessment Schedule 2.0 (WHODAS-II) is a generic questionnaire that assesses disability across domains including cognition, self-care and participation.
Objectives: The current study evaluated the suitability of this measure for use in multiple sclerosis (MS) by applying scale data to the Rasch model, a modern psychometric technique.
Methods: Patients (N=401) were consecutively approached during routine clinic appointments across three sites in England (Liverpool, Preston, Salford). Patients were sent the WHODAS-II questionnaire alongside other measures as part of the Trajectories of Outcomes in Neurological Conditions (TONiC) research programme. The questionnaire was returned by 260 patients (68.4% response).

Data from each of the seven WHODAS-II subscales were individually applied to the polytomous Partial Credit Rasch Model. Fit to the Rasch model was assessed using the chi-square (χ^2) statistic. Other psychometric criteria including reliability, local dependency, differential item functioning, dimensionality and category threshold analysis. The bi-factor model was used to investigate the presence of a unidimensional higher-order factor.
Results: Rasch analysis confirmed the suitability of the 'Cognition' (p=0.29), 'Mobility' (p=0.24), 'Self-care' (p=0.26), 'Life activities' (p=0.66) and 'Participation' (p= 0.84) subscales for use in this population without modification. Reliability was acceptable for these subscales (PSI = 0.76-0.91). The subscales were free from local dependency and differential item functioning and were unidimensional.

The 'Getting along' subscale did not fit the Rasch model in its original form (p< 0.001). Misfit was driven by the item 4.5 'Sexual activities'. Model fit and other criteria were greatly improved following the removal of item 4.5 (p=0.18).

The bi-factor model was unable to confirm the suitability of a single unidimensional higher-order factor for the WHODAS-II (p< 0.001).
Conclusions: The WHODAS-II has good psychometric characteristics when completed by patients with MS. Item 4.5 'Sexual activities' caused misfit for the 'Getting along' subscale and should be removed to ensure accurate measurement of disability in this population. There was no evidence to support the use of total score that combined items from all of the seven domains WHODAS-II.

P799

Impact of treatment-related flu-like symptoms and injection site reactions on quality of life in patients with multiple sclerosis: ADVANCE study

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Background: Flu-like symptoms (FLS) and injection site reactions (ISR) are adverse events (AEs) associated with interferon beta treatments (IFN) in patients with multiple sclerosis (MS) and may have a negative effect on patients' health-related quality-of-life (HRQoL).
Objectives: To assess the impact of FLS and ISR on HRQoL using data from the phase 3 ADVANCE study.
Methods: ADVANCE, a randomized, double-blind, placebo-controlled study, assessed the efficacy of subcutaneous peginterferon beta-1a (PEG-IFN) 125 µg administered every 2 or 4 weeks versus placebo at 1 year in patients with relapsing-remitting MS (n=1512). HRQoL was assessed at baseline, and 12, 24, and 48 weeks using 3 instruments: the Multiple Sclerosis Impact

Scale (MSIS-29), 12-item Short Form Survey (SF-12), and EuroQoL 5-dimensions questionnaire (EQ-5D). The impact of FLS and ISR on HRQoL was assessed in separate mixed-effects regression models, with random intercepts and slopes. All 3 treatment arms were pooled in the analyses, and indicators for occurrence of AEs (i.e. ISR and FLS) were used as time-dependent covariates. ISR was coded as an "ongoing" event at the time of a HRQoL assessment (yes vs. no, including those never experiencing any ISR) based on the start and end dates of the event and FLS was coded as an event occurring "≤7 days (vs. >7 days, including those never experiencing any FLS)" prior to an assessment, due to a very small number of ongoing events identified at the time of HRQoL assessment. Various cut-off dates (e.g., ≤14 vs. 14+ days) for FLS, however, were tested in the analyses.

Results: A total of 35% (13% placebo; 47% PEG-IFN) and 46% (11% placebo; 63% PEG-IFN) of the study subjects reported FLS and ISR respectively, over 1 year. FLS and ISR did not have a statistically significant negative impact on HRQoL, regardless of the instruments used for assessment. For example, the mean differences in HRQoL scores for patients reporting FLS (≤7 vs. >7 days prior to an assessment) were 0.53 (p=0.32) and -0.24 (p=0.75) (MSIS-29 physical and psychological scales, respectively), and 0.10 (p=0.87) and -0.52 (p=0.48) for ISR (ongoing vs. not ongoing, respectively). Sensitivity analyses using different cutoff dates indicated similar results.

Conclusions: Although FLS and ISR are frequent AEs of IFN treatments, this analysis indicates that the occurrence of these AEs does not have a negative impact on HRQoL in patients treated with PEG-IFN.

P800

Multiple sclerosis-associated bladder dysfunction in the NARCOMS registry: a 5-year follow up study

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Background: Persons living with multiple sclerosis (MS) frequently experience bladder dysfunction which can vary in severity. Prior studies suggest bladder dysfunction is associated with ambulatory disability.

Objectives: In large MS population, to assess the relationship between self-reported bladder dysfunction and relapses and severity of disability over 5 years of follow up.

Methods: We included US residents, with MS who responded to the Fall 2005 NARCOMS bladder survey, did not report any surgical alteration to the bladder, and had completed ≥1 update survey annually from 2005-2010. In 2005 we classified respondents as: No overactive bladder (No-OAB), Dry-OAB, Urinary Incontinence (UI-Any). Disability status was measured using the Patient Determined Disease Steps (PDDS). Progression was defined as a 1-point increase in PDDS during the five year follow-up period. We compared the groups using chi-square or Wilcoxon tests, as applicable. Multivariable analysis employed logistic regression or ANOVA models.

Results: Of the 4870 eligible respondents, 24.6% reported No-OAB, 15.2% Dry-OAB, 60.2% with UI-Any. Of those with UI-Any: 50.8% reported slight UI, 30.6% moderate, 18.7% great. A higher proportion of Dry-OAB were male (34.1%) compared to No-OAB (21.9%), UI-Any (22.1%, p< 0.0001). UI-Any were older (52.9 years) compared to No-OAB (49.3), Dry-OAB (49.8, p< 0.0001); with no racial differences (94.1% Caucasian). There was no difference in the proportion reporting a relapse during the follow-up period (83.8%, p=0.35). However, UI-Any was more likely to report a higher number of relapses compared to No-OAB (p=0.011) and Dry-OAB (p=0.041), adjusted for follow up time. UI-Any were more likely to report progression compared to No-OAB (36.8% vs 28.0, p=0.004), adjusted for a relapse reported in 6 months prior to 2005. By 2010, a higher proportion of UI-Any in 2005 reported being severely bothered by: urinary/bladder problems (20.5%) compared to No-OAB (5.4%) and Dry-OAB (6.0%; p< 0.0001); bowel problems (UI-Any 11.0%) vs No-OAB (5.0%), Dry-OAB (5.1%, p< 0.0001); and sexual problems (UI-Any 25.3%), vs No-OAB (13.1%), Dry-OAB (19.9%, p< 0.0001).

Conclusions: Respondents with UI in 2005 reported a higher number of relapses over 2005-2010, though the proportion of participants reporting having a relapse did not differ. Also, those with UI in 2005 were more likely to report an increase in PDDS during the follow-up period and continued bowel, bladder and sexual problems in 2010.

P801

New sleep scales for multiple sclerosis

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Background: There is an intimate relationship between fatigue and sleep in multiple sclerosis (MS). During the construction of the Neurological Fatigue Index for MS (NFI-MS), two sleep related scales were identified but it was concluded that further work was required for their development. There is currently no MS-specific scale for measuring sleepiness.

Objectives: To develop patient reported outcome measures for sleep and sleepiness in MS by Rasch analysis.

Methods: New sleep related scale items were based on themes and phrases derived from forty, semi structured interviews on MS fatigue. Candidate scale items were screened by an expert panel of neurologists with interest in sleep disorders, rehabilitation specialists and MS neurologists and therapists. A four point, Likert response option was used. A cognitive debrief of the new items was performed by 10 MS patients. Scale items reflected the qualities of sleepiness and rest in a non situation-specific way. A 42 item scale was then posted to patients with clinically definite MS of any age, gender, disability and disease type in two centres in the UK. The Epworth Sleepiness Scale was co-administered.

Results: 251 records were available for analysis (62.7%response). Exploratory factor analysis revealed 2 dominant factors and a further 2 minor factors. The factor groupings guided the Rasch analysis which allowed item reduction until two 9-item scales were

generated with excellent fit to the Rasch model, and strict unidimensionality by post-hoc t-test. The scales were free from differential item functioning for a variety of important person factors. The first scale assessed diurnal sleepiness; it had moderate correlation (rho 0.6) with the Epworth Sleepiness Scale. The summed raw score cut point of 10 on the ESS was shown to equate to 17/27 in the diurnal sleepiness scale. The second scale measured the unrefreshing nature of nocturnal sleep.
Conclusions: This new scale development corroborated previous findings that there are latent traits relating to diurnal sleepiness and quality of nocturnal sleep which are meaningful to patients with MS. The resultant scales were shown to fit the Rasch model and therefore measure unidimensional constructs and generate interval level data. The diurnal sleepiness scale does not contain situation-specific items and hence provides an alternative to the Epworth Sleepiness Scale.

P802

Defining clinical meaning of patient-reported outcomes with disability assessment in multiple sclerosis: an analysis of the CARE-MS II study

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Background: Alemtuzumab is approved in over 30 countries for the treatment of relapsing-remitting multiple sclerosis (RRMS). In the 2-year phase 3 CARE-MS II study, alemtuzumab demonstrated superior benefits in relapse rate, disability, and patient-reported outcomes (PROs) over subcutaneous interferon beta-1a (SC IFNB-1a) in RRMS patients who relapsed on prior therapy. PROs have been routinely evaluated in clinical trials of RRMS. However, the clinical implications of PROs in reference to disability improvement or progression need further clarification.
Objectives: To evaluate the relationship between PROs and disability assessment in the CARE-MS II trial.
Methods: In CARE-MS II (NCT00548405), patients were randomized to receive 2 annual courses of alemtuzumab 12 mg (n=426) or SC IFNB-1a 44 μg 3 times weekly (n=202). Yearly changes in 36-Item Short-Form Survey (SF-36) physical component summary (PCS) and mental component summary (MCS), and Functional Assessment of MS (FAMS) scores were analyzed against change in Expanded Disability Status Scale (EDSS) score. Mixed-effects model for repeated measures was used to adjust for age, gender, race, randomization arm, and baseline PROs and EDSS scores.
Results: Mean age was 35 years at baseline, with 66% female and mean scores of 45 for PCS, 46 for MCS, 119 for FAMS, and 2.7 for EDSS. A 0.5-point worsening in baseline EDSS was associated with 1.0-point PCS worsening, 0.4-point MCS worsening, and 2.0-point FAMS worsening (all $p< 0.001$). PRO changes were more strongly associated with EDSS change than with baseline EDSS. Corresponding to a 0.5-point EDSS worsening over 12 months, we observed on average a 1.1-point PCS worsening, 0.8-point MCS worsening, and 3.0-point FAMS worsening (all $p< 0.001$). For baseline EDSS < 4, a 0.5-point EDSS worsening was associated with worsening of 3.6 points in FAMS and 1 point in MCS (both $p< 0.001$). For baseline EDSS ≥4, a 0.5-point EDSS worsening correlated with FAMS worsening (1.2 points; $p=0.04$), but had no correlation with MCS change ($p=0.82$).
Conclusions: PRO changes measured by SF-36 or FAMS correlated with EDSS at baseline, and were strongly related with change in EDSS score over the 2 years of CARE-MS II. If a half-point EDSS difference is considered clinically meaningful, a 1-point change in SF-36 PCS and MCS or 3-point change in FAMS may represent a minimum important difference in PRO for patients with MS. PRO appears to be more impacted by EDSS change when baseline EDSS is below 4.

P803

Physical disability, anxiety and depression in people with MS: an internet-based survey via the UK MS Register

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Background: People with Multiple Sclerosis are known to have a relatively high prevalence of both anxiety and depression. Studies of the relationship between physical disability and mental health in people with MS have reported mixed results, showing the need for further work. The relationship between the physical and psychological impacts of MS measured via the MSIS-29 has been documented and we have confirmed this in our cohort. However, the MSIS-29-PSYCH is not designed to distinguish between anxiety and depression.
Objectives: The aims of this study were to examine the impact of physical disability on anxiety and on depression in people with MS.
Methods: Between May 2011 and April 2012, 4516 people completed the MSIS-29 (v.1) and HADS scales via the UK MS Register within a 7 day time window. Chi squared and Kruskal-Wallis tests were used to test for differences in anxiety and depression across categories of physical disability (low, moderate and high). Multiple regression was used to model the relationships between anxiety and physical disability, gender, age, disease course and disease duration; and similarly for depression.
Results: The proportions of people experiencing anxiety or depression increased with physical disability such that 38.0% of respondents with low, and 66.7% with high disability reported at least mild anxiety, and 17.1% of people with low, and 71.7% with high disability experienced at least mild depression. The multiple regression model explained 18.4% of the variance in anxiety with MSIS-29-PHYS score being the strongest predictor of anxiety. The model for depression explained 37.8% of the variance with MSIS-29-PHYS score being the strongest predictor. Some of the other variables included showed negative associations with anxiety and depression, indicating that the influence of physical disability on mental wellbeing could be underestimated.
Conclusions: This study indicates that there is a positive relationship between physical disability and anxiety and depression, that physical disability impacts on anxiety and depression to differing

extents, and that the effects vary with gender, age, disease course and disease duration. We have shown that physical disability is a predictor of anxiety and depression, and that other factors may mask the extent of this effect. Whether the causes of anxiety and depression are reactive, organic or a combination, it is essential that mental wellbeing is given due attention in caring for people with MS so that all their health needs can be met.

P804

Relationship between relapses and quality of life in patients with relapsing remitting multiple sclerosis

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Background: Relapses affect the patients' psychological health and it would be useful evaluate their influence in quality of life. MusiQoL questionnaire constitutes a useful instrument to measure health-related quality of life in the clinical setting.
Objectives: To evaluate the influence of relapses in quality of life of patients with relapsing remitting multiple sclerosis.
Methods: Forty-nine MS patients with a relapse included in a Phase IV, multicenter, randomized, double blind trial that showed the no inferiority of oMP compared to ivMP to treat MS relapses, were analysed. The protocol variables were baseline disability (measured by EDSS), relapse severity (increasing of EDSS = or >1.0) and quality of life (measured by MusiQol at the time of the relapse (t=0) and at week 1 and 4 after MP treatment). The primary outcome was to analysed MusiQol at t=0, week 1 and week 4 after high-dose MP treatment. Secondary outcomes were to analysed the relationship between MusiQol with relapse severity and previous disability.
Results: There were no differences between oMP or ivMP in MusiQol scores because patients received both oMP or ivMP treatmens (active treatment or placebo).

MusiQol had lower scores at the time of relapse than at 4 weeks after MP treatment (62.8 vs 71.5, p=0.05). MusiQol score had no differences between patients with higher o lower relapse severity at any point anaysed (t=0: 62.2 vs 63.1, week 1: 64.4 vs 64.1, week 4: 71.1 vs 72.8). A positive correlation was found between MusiQol scores and baseline disability (R=0.37, p =0.02).
Conclusions: Relapses were related with a worsening of quality of life. Patients with greater disability had a worse quality of life regardless the intensity of relapse.

P805

Patient-reported physical functioning in relapsing-remitting and secondary progressive forms of multiple sclerosis: a cross-sectional survey

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Background: Patients with multiple sclerosis (MS) often report diminished health-related quality of life (HRQoL). Previous studies suggest that HRQoL may decrease with increasing physical impairment and disability.
Objectives: To evaluate patient-reported physical functioning in a cross-sectional survey of patients with relapsing-remitting MS (RRMS) and secondary-progressive MS (SPMS) in the United States (US).
Methods: Data were obtained from the 2012 (March- August) and 2013 (April- August) waves of the US National Health and Wellness Survey, an Internet-based survey of the healthcare attitudes and behaviors of a nationally representative adult sample (age ≥18 years). The study population included in this analysis comprised respondents who reported a diagnosis of RRMS or SPMS. The more recent survey was used for patients responding to both the 2012 and 2013 surveys. Physical aspects of HRQoL were assessed using the Physical Component Summary (PCS) score and the physical health domains from the Short Form-36 (SF-36v2); scores were compared between patients with RRMS and SPMS regardless of treatment status. Higher scores indicate better HRQoL. The mean (standard deviation [SD]) PCS score for the general US population is 50 (10). Respondent characteristics were compared using chi-square (categorical variables) and sample *t* (continuous variables) tests.
Results: A total of 810 (0.6%) survey respondents reported a diagnosis of MS; the mean (SD) age was 49.6 (12.9) years, and 66.4% were female. Of these 810 patients, 58.0% (n=458) had RRMS and 13.3% (n=105) had SPMS. Mean (SD) PCS scores were significantly higher in patients with RRMS than in those with SPMS (40.43 [10.34] vs 31.25 [7.45]; $P< 0.0001$). Mean (SD) scores were also significantly higher in patients with RRMS versus SPMS on the physical functioning (40.70 [11.48] vs 27.32 [8.20]; $P< 0.0001$), role limitations due to physical health (41.13 [11.27] vs 32.54 [9.88]; $P< 0.0001$), and general health (39.88 [10.70] vs 34.95 [9.27]; $P< 0.0001$) domains.
Conclusions: In this cross-sectional survey, SPMS patients compared with RRMS patients reported significantly worse physical aspects of HRQoL, including physical functioning, role limitations due to physical health, and general health; both groups of MS patients perceived their physical functioning as worse than the norm in the general US population.

P806

Italian validation of the 12-item multiple sclerosis walking scale (MSWS-12)

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Background: Gait impairment is commonly reported by people with MS (pwMS). The 12 item Multiple Sclerosis Walking Scale (MSWS-12) assesses patient rated measurement of walking quality.

Objectives: The aim of this study was to cross-culturally adapt and validated the MSWS-12 for the Italian population with MS.
Methods: This study included 321 patients with a diagnosis of MS according to recognized criteria. The steps of the adaptation pro-validation included translation, back translation, review by an expert committee and pretesting. A test and retest of MSWS-12/IT was made for validation, with comparison with another scale (FSS) and test (T25FW).
Results: Of 321 patients seen at the 6 participating centres between June 2013 and December 2013, 310 were enrolled.

Mean age was 47.55 years (range 18 - 76), 151 (48.70%) were men and 159 (50.78%) were women. Mean duration of MS was 13.8 years(range 1-42 years), and mean EDSS score was 4.46 (range 0 - 6.5). Most of the patients had a relapsing remitting (RR) (n=185, 57.6 %) while 92 (28.7%) a Secondary Progressive (SP), 43 (13.4%) primary progressive (PP) course and 1 (0.3%) had a clinically isolated syndrome (CIS). The Italian version of MSWS-12/IT was shown to be similar to the original. The results indicate that MSWS-12/IT is a reliable and reproducible scale.
Conclusions: MSWS-12/IT has been adapted and validated. It is a reliable tool for Italian population.

Rehabilitation and comprehensive care

P807

Cognitive motor interference in both upper and lower extremities in MS

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Background: Cognitive-motor inference (CMI) has been commonly reported in the lower limbs of people with multiple sclerosis (MS). CMI is typically documented based on performing a motor task alone (e.g., walking) and then whilst simultaneously performing a motor task and an attention demanding task (e.g., verbalising alternate letters of the alphabet). To date, researchers have not compared CMI in MS versus healthy controls sufficiently or examined the extent of CMI in both lower and upper limb tasks and across disability status.
Objectives: This study examined CMI in tasks involving the upper and lower limbs of persons with MS and compared the extent of CMI with healthy controls and across levels of neurological disability.
Methods: The sample included 62 people with MS and 20 healthy controls. The persons with MS were stratified into groups of mild (n=20, EDSS 0-3.5), moderate (n=22, EDSS 4.0-5.5), and severe (n=20, EDSS 6.0-6.5) disability. All participants completed the 9-hole peg test (9HPT) without a cognitive task (single task; ST) and then while reciting alternate letters of the alphabet (dual task; DT). The same paradigm was completed while walking over an electronic gaitmat. The data were analysed using ANOVA and bivariate correlations.
Results: The ANOVAs identified significant, large reductions in performance between the ST and DT trials for both walking (η_p^2=.411) and 9HPT (η_p^2= .384); there were no group (MS vs. controls) by task interactions for walking or 9HPT. Performance was decreased in the DT condition compared with ST as indicated by slower velocity (MS group= 12.5%, control group=17.5%) and longer time to complete the 9HPT (MS group= 36.2%, control group=33.2%). The degree of CMI for walking and 9HPT, based on DTC (100*((ST−DT)/ST)), was correlated in the overall sample and in MS and control samples separately (all *r's*>0.5). The ANOVAs further identified significant, large reductions in performance between the ST and DT trials for both walking (η_p^2=.387) and 9HPT (η_p^2= .478) in the MS sample alone; there were no disability group by task interactions for walking or 9HPT. The degree of CMI for walking and 9HPT, based on DTC, was correlated in those with mild, moderate and severe MS (all *r's*>0.5).
Conclusions: CMI occurs during performance of upper and lower extremity tasks in persons with MS, and the effect does not differ when compared with controls or across levels of disability. This indicates that CMI is not MS specific, restricted to walking, nor influenced by disability status.

P808

Corticospinal reserve predicts walking improvement in progressive multiple sclerosis patients undergoing neurorehabilitation and deep rTMS with H-coil

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Background: Walking impairment affect up to 85% of subjects with multiple sclerosis - MS - with a major impact on their quality of life. Transcranial repetitive magnetic stimulation - rTMS at high frequency enhances corticospinal and plasticity, potentially favouring the effects of neurorehabilitation. The H-coil allows deeper magnetic fields compared with traditional stimulators, reducing the limit to stimulating lower limb representation, deeply located under the skull. In a preliminary study, we found that improvement in walking speed and endurance after intensive neurorehabilitation was significantly enhanced by the association with deep rTMS.
Objectives: We aimed at replicating the results of our pilot study and at combining results of both studies in order to explore correlations between clinical improvement and baseline neurophysiological features.
Methods: We randomized 20 patients with progressive MS into real (n=10) and sham-placebo rTMS (n=10), who underwent 11 stimulation sessions (20 Hz, 90% resting motor threshold or 80% of maximal stimulator output in case of absent motor evoked responses at rest). Walking speed (10 meters test) and endurance (2 and 6 minutes Test) were assessed at baseline and at the end of treatment, as well as modified Ashworth Scale (MAS), visual analog scale-VAS for spasticity and pain, Fatigue Severity Scale, expanded disability score, MS walking scale-12, PASAT and nine hole peg test.
Results: Compared with sham, real rTMS group had a significant improvement in 10MWT and Ashworth, confirming data from a

previous pilot study on 21 patients. When pooling data with the latter study, a strong correlation between resting motor threshold and clinical improvement in walking tests was found exclusively in the real rTMS group.
Conclusions: Resting motor threshold results from the combination of corticospinal excitability and of the amount of corticomotor fibers available for conduction. While rTMS mainly acts on the former mechanism, both at the cortical and spinal level, the latter is a limiting factor in the presence of corticospinal damage, as in the case of progressive MS with lower limb motor involvement. In this condition, resting motor threshold could be considered an rTMS specific therapeutic reserve index, being predictive of therapeutic response to corticospinal neuromodulation.

P809

Efficacy of mental imagery to improve autobiographical memory in multiple sclerosis patients: a double approach in neuropsychology and neuroimaging

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Background: We previously showed autobiographical memory (AM) impairment in non-depressed relapsing-remitting multiple sclerosis (RR-MS) patients, very likely caused by a retrieval-strategy deficit. From that observation, a mental visual imagery (MVI)-based facilitation program was created.
Objectives: To probe the efficacy of this program on AM retrieval, by testing both clinical and cerebral network changes in pre- and post-facilitation.
Methods: All MS patients underwent a neuropsychological baseline and an AM assessment sessions. Then, the patients were allocated in three groups:

(i) experimental (EG; n = 10) who followed the MVI program,
(ii) placebo (PG; n = 10), who followed a sham verbal program. A post-facilitation reassessment of AM was conducted afterwards for these two groups.
(iii) The stability group (n = 13) underwent the AM test twice, with no intervention in between.

The EG and the PG completed also two fMRI sessions, during which they had to mentally evoke personal memories, within a pre-/post-facilitation study design. For each memory, a distinction between the initial retrieval and the further elaboration (search of additional details associated to the event) was made.
Results: For the first AM assessment, no significant difference was observed between the three groups. However, in post-facilitation, only the EG showed a significant improvement of their AM performances ($p < 0.001$). After facilitation, this AM improvement in the EG was accompanied with increased activations in the medial frontal regions during memories' retrieval and by decreased activations mainly in the lateral frontal regions during memories' elaboration ($p < 0.001$ unc; $k = 20$). Regarding the PG, after facilitation, we observed decreased activations in the medial frontal area during retrieval and increased activity in the superior frontal gyrus during elaboration ($p < 0.001$ unc; $k = 20$).
Conclusions: Clinically, this MVI-based program led to an AM improvement and was not attributable to a nursing or a test learning effects. Cerebral activation changes reflecting both an increase reliance on brain regions sustaining self-referential process and a decrease of those reflecting an effortful research process were displayed after the MVI program. While brain activity changes were also observed in the PG, they likely reflected an attempt to use the alternative sham strategy, but with no efficiency on AM functioning.

P810

Evaluation of gait abnormalities in MS patients with minimal disability

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Background: Gait abnormalities are a common feature of the multiple sclerosis (MS) patients. Even MS patients with minimal clinical disability (EDSS 0-1,5) show in kinetic and kinematic analysis decreased velocity of gait, shorter step length and impaired balance.
Objectives: To assess whether abnormalities of gait in minimally impaired MS patients are measurable by tests commonly used in clinical practice and identify specifically which parameters of gait cycle change in patients with EDSS 0-1.5.
Methods: We enrolled 65 MS patients with EDSS 0-1.5 (median age 35 years, range 25-51, 76% female), 29 MS patients with EDSS 2-2.5 (median age 37 years, range 22-57, 69% female) and 47 normal controls (median age 37 years, range 22-52, 77% female).

Tests performed include 25 foot walk test (25FWT), two minute walk test (2MWT), temporal and spatial gait analysis on GAITRite (velocity, cadence, step length, step time and percentage of double support).
Results: There is a significant difference in 25FWT between normal controls and patients with EDSS 0-1.5 as well in 2MWT. Gait assessment at fast speed of walking on GAITRite revealed significantly decreased cadence, prolonged step time, shorter step length and increase in percentage of double support time between patients with EDSS 0-1.5 and normal controls. These measures did not reach significance when measured at self selected speed of walking. The difference between fast speed of walking and self selected speed of walking is significantly lower in patients with EDSS 0-1.5 in comparison with normal controls.
Conclusions: Both tests of walking (25FWT, which is faster, as well as longer 2MWT) identified gait abnormalities in group of minimally impaired patients in comparison with normal controls.

It is clear that even minimally impaired MS patients have decreased ability to reach higher speed of walking (the difference between self selected and fast speed of walking) in comparison with normal controls.

P811

Reliability and validity of the narrow path walking test for people with multiple sclerosis

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Background: Decline in gait and balance control has been associated with increased risk of fall in people with Multiple Sclerosis (PwMS). More than 50% of PwMS fall at least once a year and more than 90% suffer from instability during gait. Reliable and clinically feasible methods of gait instability assessment are needed. Clinical measures of balance and gait function should demonstrate sensitivity to pathology and remain invariable when there is no change in function. In order to address these two problems we present a novel testing procedure "the Narrow Path Walking Test (NPWT)" to evaluate balance function during gait.

Objectives: Investigating the repeatability (test-retest reliability) of NPWT parameters under single and dual task conditions for PwMS; Validating the NPWT with gold standard clinical examination of PwMS as well as exploring which of the NPWT parameters is associated with activity levels in PwMS.

Methods: Fifteen PwMS performed 3 trials of the NPWT under single task (ST) and 3 trials of dual task (DT). Participants were patients from the Sheba MS Center, at different activity levels ranging from 2 to 5.5 on the Expanded Disability Status Scale (EDSS). Participants were instructed to walk within a 6-m narrow path normalized to pelvic width, without stepping out (i.e., step error). Trial time, number of steps, trial velocity, number of step errors, and number of cognitive task errors were determined. Concurrent validity was assessed using EDSS, Four Squares Step Test (FSST) and Multiple Sclerosis Walking Scale (MSWS-12) using partial correlation controlled for age.

Results: Test-retest agreement (ICC1,2) varied from 0.48 to 0.85 for ST and from 0.46 to 0.88 for DT, with no significant difference between the 2 trials except for trial velocity ($t=2.364$, $p=0.033$) were the 2nd trial was performed faster. Significant correlations were observed for trial time, velocity, and number of steps in ST and DT conditions with EDSS and MSWS-12 ($0.60< r< 0.73$). In addition number of step errors in the 2nd DT trial and average number of losing balance and falls in DT were significantly correlated with FSST ($r=0.65$, and $r=0.76$, respectively). Linear regression modeling sujest average trial velocity in DT predicts EDSS level ($F=12.99$,$p=0.004$); It is found in high correlation to EDSS ($r=0.72$) and the higher the EDSS level the slower the walking ($b=-0.16$, $\beta=-0.72$,$t=-3.60$,$p=0.004$).

Conclusions: The present results indicate that the NPWT testing procedure is reliable, reproducible and associated with the EDSS scores.

P812

Visual delay adaptation to reduce intention tremor in multiple sclerosis

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Background: Intention tremor is a disabling symptom affecting ~15% of those with multiple sclerosis (MS). Our previous work has shown that tremor may be due to an inability to adapt expected visual delays to match an increase in visual processing delay caused by demyelination of visual pathways.

Objectives: To examine whether external feedback delays can be used to reduce tremor by inducing gradual adaptation of expected visual delays, and determine whether these effects can be predicted through simulations of individual neuromotor control performances.

Methods: Subjects' closed-loop neuromotor control systems were characterized using a series of tracking tasks performed with a 1-D robotic manipulandum. The resulting control model was simulated to determine the effects of varying actual and expected feedback delays, and results were used to choose experimental feedback delays. Subjects used the manipulandum to place a cursor on a target during a series of random step displacements. Cursor position was delayed from hand position by 100 (sets 1-2), 200 (set 3), or 300ms (set 4). Tremor was examined before and after the adaptation task using digital spiral tracing, handwriting, the Nine Hole Peg Test (9HPT), and a step-tracking task.

Results: For a subject with severe tremor (TAS=3), simulations of the best-fit neuromotor control model indicated that manipulation of an external visual delay could be used to alter the subject's expected delay. Average submovement interval, used to characterize expected delay, was 317±109ms pre-training and increased significantly to 460±143ms post-training ($t(10) = 2.51$, $p = 0.01$). This corresponded with a decrease in 9HPT times by 28% (RH) and 11% (LH). During spiral tracing, tremor frequency increased (3.2 to 4.3 Hz) while tremor power declined by 80%. For a subject with mild tremor (TAS=1), simulations indicated external feedback delays would be ineffective at shifting expected delay. Consistent with model predictions, submovement intervals did not increase following training (pre: 329±174ms, post: 340±180ms). Pre- versus post-training comparisons showed no change in 9HPT for either hand (< 5% decrease), while post-training tremor power increased by 183%.

Conclusions: Experimental characterization of subjects' neuromotor control models predicted which subjects benefit from delay adaptation. For a subject with severe intention tremor, visual delay adaptation increased expected visual delay and decreased tremor amplitude, suggesting a new potential avenue for rehabilitation.

P813

Understanding patient comprehension in natalizumab administration/discontinuation and JC virus serology - a pilot study

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Background: Currently, there are few studies looking at how patients feel about their JC virus risk, the risk of possible PML, disease progression and decision making around continuing/discontinuing Natalizumab treatment. There are many resources, brochures, blogs and reviews available regarding Natalizumab treatment and the risks, but it is not known if patients have a good understanding of these, or if they meet their individual or group needs regarding this subject. This study aims to find out what patients understand, their comprehension of the situation around treatment and risk, and what they want and need to know.
Objectives: To assist the researchers to develop a tool to be used by MS clinics and centres to improve patient care, safety and outcomes for patients receiving treatment with Natalizumab.
Methods: This is a pilot study involving 40 patients- 10 at each of 4 different sites around Australia. Patients were interviewed by the MS Nurse and asked questions about their understanding of JC virus serology, the risks of PML and other possible side effects of Natalizumab and their general experience with infusions. The pooled study data was examined by the researchers to identify trends and areas of need.
Results: Patients responses were grouped according to themes. Results show that current education methods may not be adequate to give a clear and accurate understanding of JC serology and PML risk in patients receiving Natalizumab therapy, often despite targeted and lengthy education sessions prior to commencing treatment.
Conclusions: There are many variables around patient comprehension and understanding of risk and treatment options, particularly in Multiple Sclerosis where cognition issues also play a major role. In this time of rapid drug development and risk/benefit analysis, we often provide detailed information to patients, who may not truly comprehend all aspects of this information. This study aims to provide health care providers with an understanding of the patient perspective in Natalizumab therapy in order to guide practice towards new tools to assist patients with understanding risk.

P814

A novel characterization of gait pattern alterations in individuals with multiple sclerosis based on quantitative movement analysis

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Background: The assessment of gait abnormalities in individuals with Multiple Sclerosis (MS) represents a key factor in monitoring the progression of the disease and in evaluating the effectiveness of rehabilitation treatments. Even though nowadays sophisticated techniques are available to objectively evaluate the kinematic aspects of gait, the data they produced are scarcely suitable for clinical uses.
Objectives: We propose a novel characterization of gait kinematics in individuals with MS, based on a single measure (Gait Profile Score, GPS Baker et al., 2009) which is calculated on the basis of nine variables referred to pelvis, hip, knee and foot kinematics (Gait Variable Score, GVS) and obtained from a quantitative three-dimensional analysis of gait.
Methods: Forty-five patients suffering from relapsing-remitting MS (20 female, 25 male, mean age 46.7 years) with an Expanded Disability Status Scale (EDSS) score in the range 1.5-7 participated to the study; they were classified in three groups: mild (EDSS 1.5-3), moderate (3.5-5-5) and severe (>6). All participants underwent a gait analysis from which the GPS index was calculated, and their results were compared with those of a control group (CG) of healthy age and gender-matched subjects. Differences in the GPS scores induced by the pathology were assessed using one-way MANOVA setting the status (healthy, mild, moderate and severe) as independent variable and the GPS and GVS as dependent variables.
Results: GPS value of healthy subjects was 5.99°, while individuals with MS reported 7.22°, 9.93° and 13.65° respectively for mild, moderate and severe status. MANOVA revealed a significant influence of status on gait kinematics [$F(30,490.85) = 14.23$, $p < 0.001$, Wilks $\lambda = 0.16$, $\eta^2 = 0.46$]. Given the significance of the overall test, the univariate main effects were examined. Significant univariate main effects for status were obtained for GPS ($p < 0.001$) and eight out of nine GVS kinematic variables ($p < 0.001$). Significant pairwise differences were also obtained between all the status classes.
Conclusions: The differences in GPS between controls and individuals with MS at different stages suggest that such a measure is suitable for representing gait deviations from physiological patterns in MS. This index is thus potentially useful in monitoring the progression of the disease as well as providing ease in assessing the outcomes related to either pharmacologic or physical therapies.

P815

Factors affecting physical activity in minimally impaired people with multiple sclerosis

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Background: Despite the commonly known benefits of physical activity, evidence shows that people with multiple sclerosis (pwMS) are relatively inactive. There are several studies about factors affecting physical activity in pwMS. However, these factors have not investigated in minimally impaired pwMS [Expanded Disability Status Scale (EDSS) scores 0 - 3.5] who do not have remarkable symptoms and walking disturbance.

Objectives: The objective was to determine factors affecting physical activity in minimally impaired pwMS.
Methods: Fifty two minimally impaired pwMS (EDSS scores between 0 and 3.5) participated in this cross-sectional study. Physical activity was measured with Godin Leisure-Time Exercise Questionnaire (GLTEQ) and an accelerometer which was used for the 7-day period. Demographic data such as age, gender, body mass index (BMI), and employment status were recorded. Disability level, walking, fatigue, depression, cognitive function and quality of life were measured.
Results: Accelerometer counts were correlated with Six-Minute Walk Test (6MWT) ($r=0.29$, $p< 0.05$) employment status ($r=0.28$, $p< 0.05$), BMI ($r=0.34$, $p< 0.05$), and gender ($r=0.40$, $p< .005$). GLTEQ was correlated with 6MWT ($r=0.46$, $p< 0.05$), Timed 25-Foot Walk (T25FW) ($r=-0.50$, $p< 0.05$), International Questionnaire Investigating Quality of Life in MS ($r=0.36$, $p< 0.05$), EDSS ($r=-0.37$, $p< 0.05$), Fatigue Impact Scale ($r=-0.31$, $p< 0.05$), and Beck Depression Inventory ($r=-0.32$, $p< 0.05$). Cognitive function (Paced Auditory Serial Addition Test and MS Neuropsychological Questionnaire) was not correlated with physical activity (accelerometer and GLTEQ) ($p>0.05$). Multiple regression analysis showed that the predictors of physical activity were T25FW ($\beta=-0.422$), gender ($\beta=0.371$), and BMI ($\beta=0.268$).
Conclusions: Better walking (speed and endurance), low fatigue and depression levels, male gender and being an employee were related with higher physical activity in minimally impaired pwMS.

P816

The effects of video-game training on broad cognitive transfer in multiple sclerosis: a pilot, randomized controlled trial

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Background: Multiple sclerosis (MS) is a neurodegenerative disease of the central nervous system that results in diffuse nerve damage and associated physical and cognitive impairments. Of the few comprehensive rehabilitation options that exist in the MS literature, those that have been successful at eliciting broad cognitive improvements have focused on a multi-modal training approach, emphasizing complex cognitive processing which utilize multiple domains simultaneously.
Objectives: The current study sought to determine the feasibility of an eight-week, hybrid-variable priority (HVT) training program, with a secondary aim to assess the efficacy of the intervention at eliciting broad cognitive transfer effects.
Methods: Employing the videogame Space Fortress, we compared the HVT strategy-based intervention with a waitlist control group, to primarily assess skill acquisition and secondarily determine presence of cognitive transfer. Twenty-eight participants met inclusionary criteria for the study and were randomized to either a training or a waitlist control group. To assess broad transfer effects, a battery of neuropsychological tests was administered to all participants pre- and post-intervention.
Results: The results indicated an overall improvement in skill acquisition, and evidence for the feasibility of the intervention, but a lack of broad transfer to tasks of cognitive functioning. Participants in the training group, however, did show improvements on measures of spatial short-term memory.
Conclusions: Improvements to the current study would include a larger sample size based on post-hoc power analysis, a longer course of training to elicit greater game score improvement, the inclusion of only cognitively impaired individuals, and integration of subjective measures of improvement in addition to objective tests of cognitive performance.

P817

Effects of biofeedback-assisted stress management on symptoms and neurologic performance in patients with MS

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Background: Biofeedback-assisted stress management (BFSM) trains individuals to change physiologic parameters reflective of autonomic nervous system activation, through relaxation, deep breathing, and modification of stressful thought patterns. Although associations were observed between stress and neurologic symptoms from MS, evidence regarding the effects of BFSM in individuals with MS is lacking.
Objectives: To assess the effects of BFSM on self-reported symptom severity, neurologic performance tests, and blood markers of stress and inflammation, in patients with MS.
Methods: This is a randomized controlled parallel group proof-of-concept trial of BFSM versus usual care (UC). Subjects in the BFSM group received 8 weekly sessions of relaxation training with heart rate variability biofeedback. Subjects in the UC group continued with routine MS care. Assessments were conducted at baseline and at end of training, including: self-report measures (MOS Short-Form-36 (SF-36), Patient Health Questionnaire-8 (PHQ-8), General Anxiety Disorder-7 (GAD-7), Modified Fatigue Impact Scale (MFIS)); a psychophysiological stress assessment (PPSA); MS-specific performance tests (MS Functional Composite (MSFC)); and blood testing for makers of stress and inflammation. BFSM subjects were further divided into "responders" (BFSM-R, n=5) or "non-responders" (BFSM-NR, n=5) based on their ability to regulate physiologic parameters. Between-group differences were tested using the Mann-Whitney U test, and Cohen's d was computed. Significance level was set for $p< 0.1$.
Results: Nineteen subjects completed the study. At baseline, BFSM subjects (n=10) had a significantly shorter disease duration (8.5 (1-27) vs. 16 (8-31)years, $p=0.028$) and were less likely to take medications influencing cardiac function (20% vs. 78%, $p=0.023$) compared to UC subjects. There were statistically significant differences in change scores between BFSM-R and UC, favoring BFSM, on the PPSA ($p< .01$, $d=-2.15$), GAD-7 ($p=0.06$, $d=-0.93$), and MFIS ($p=0.04$, $d=-1.46$). No significant differences were found between UC and BFSM-NR. Statistically significant

differences were also noted between BFSM-R and BFSM-NR for CRP, GAD-7, and PHQ-8.
Conclusions: These results suggest that BFSM is effective in promoting self-management of stress in individuals with MS, and in improving symptom severity in those who were successful in learning how to regulate physiologic parameters. Studies with larger samples are needed to further assess the benefits of BFSM in MS.

P818

Concern about falling in people with MS: association with definite gait parameters measured by an instrumented treadmill

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Background: People with multiple sclerosis (MS) identify mobility limitations as one of the greatest challenges of this disease. Continued loss of mobility and falls are among their greatest concerns for the future.
Objectives: To determine if fear of falling is associated with spatial and temporal gait parameters in persons with MS, when measured by an instrumented treadmill.
Methods: This study was an observational case control study. Sixty-eight relapsing-remitting patients diagnosed with MS, 38 women and 30 men, aged 40.9 (S.D=11.9), were recruited from the Multiple Sclerosis Center, Sheba Medical Center, Tel-Hashomer, Israel and participated in this investigation. Twenty-five apparently healthy subjects, 14 women and 11 men, aged 39.5 (S.D= 9.4) served as controls. Gait spatiotemporal parameters were obtained using the Zebris FDM-T Treadmill (Zebris® Medical GmbH, Germany). The Falls Efficacy Scale International (FES-I) was used to assess the level of concern relating to falls during 16 activities of daily living, ranging from basic to more demanding activities including social activities that may contribute to quality of life.

MS subjects were divided into two groups: highly fearful and slightly concerned about falling. Allocation was determined according to the FES-I scores. The cut-off point for distribution was set at a score of 20. People with MS with scores >20 were defined as highly fearful and those ≤ 20 were defined as slightly concerned about falling.
Results: Forty-one people with MS were classified as patients highly fearful of falling (mean FES-I=35.3, S.D=9.5). Twenty-seven patients were classified as slightly concerned about falling (mean FES-I=16.7, S.D=1.2). Highly fearful of falling patients walked slower had a shorter step length, a wider base of support and prolonged double support phase compared to slightly concerned patients. Fearful patients also demonstrated elevated variability of the center of pressure (CoP) trajectory compared to slightly concerned MS patients. The FES-I was significantly correlated with 17 (out of 17) gait spatiotemporal parameters. The strongest correlation was observed in terms of CoP lateral variability and double support period; 0.70, 0.65 ($P< 0.001$), respectively.
Conclusions: Fear of falling and spatiotemporal gait alterations in people with MS are linked. Additionally, variability of the CoP during walking appears to be connected with the level of concern.

P819

Brain activity during motor imagery in multiple sclerosis

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Background: Mental imagery (MI) is defined as mental execution without any actual movement and involves neural networks overlapped with those activated during actual actions. Although widely investigated in stroke, very little is known about MI in Multiple Sclerosis (MS). In a recent study we showed a MI impairment in relapsing-remitting (RR) course compared to healthy controls (CTRL).
Objectives: We propose to investigate the neural correlates of MI impairment in MS patients using fMRI and behavioral tasks and to assess whether MI deficit is associated with disease severity.
Methods: We recruited 24 right-handed subjects, 8 RR (32.6±6.4 yrs; EDSS:1.5, 1-3.5), 8 clinically isolated syndrome (CIS) patients (31.88±6.10 yrs; EDSS:1, 0-1.5), 8 CTRL (29.6±3.4 yrs) undergoing MRI (1.5T GE scanner) with the following sequences: a) T2-W spin-echo; b) 3D-T1-W FSPGR; c) EPI for fMRI. Two tasks were performed during fMRI acquisition: (1) motor execution (ME) with subjects squeezing a ball with the dominant/non-dominant hand; (2) MI with subjects imagining to squeeze the ball with the dominant/non-dominant hand. The same tasks were performed before the MRI scan to record ball squeezes executed/imagined ratio (R) evaluating anisochrony in dominant and non-dominant hand.
Results: RR and CIS showed an increased R compared to CTRL: RR=1.76±0.69; CIS=1.41±0.17; CTRL=1.17±0.12. Regarding fMRI, ME showed a gradient of increased activity in MS (RR>CIS) than CTRL; intra-group comparison between MI and ME showed decreased activation in MI compared to ME in all the groups. Compared to CTRL, CIS patients were not significantly different in brain activation during MI, whereas RR patients showed increased activation with the non-dominant hand in the Thalamus bilaterally, R Precentral, R Fusiform gyrus, R Cingulum and L Inferior Parietal Lobule. Compared to CIS, RR patients showed increased activation with dominant hand in the L Postcentral, R Thalamus and L SMA and with non-dominant hand in R Pre- and Postcentral, R and L IPL, L Cingulum. No significant association was found between areas of brain activations and T2-LV in patients.
Conclusions: Brain activity during MI is higher in RR than in CTRL and CIS and that is associated with increasing asynchrony on the behavioral task. Interestingly, the strongest association is found with increased activity of L Cingulum connecting sites implicated in cognitive control. This pilot study is aimed to better clarify MI in MS to possibly identify new rehabilitation strategies towards a better QoL.

P820

Neuropsychological and neurophysiological assessment of a cognitive rehabilitation program for multiple sclerosis patients

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Background: Cognitive impairment is common in multiple sclerosis patients. One of the main challenges nowadays is to rehabilitate cognitive deficits in these patients.
Objectives: The main aim of the present study was to assess the possible benefits of applying a cognitive rehabilitation program to a sample of multiple sclerosis patients.
Methods: Thirty-three subjects were assigned to three groups (Healthy, Pathological Control and Treatment groups (N=11 for each group)). All groups were matched for socio-demographic (age, genre, education level and handedness) and clinical (EDSS and duration of disease) variables. A cognitive rehabilitation program was applied to the treatment group in 60 sessions over three months. The rehabilitation program was a modified version of Attention Program Training (APT). Behavioral responses, latency and topography of the ERP component (P3) were calculated before and after the rehabilitation program was delivered. Similarly, a neuropsychological assessment was conducted to evaluate changes in attention capacity (SDMT and PASAT) and level of depression (Beck Inventory) between the beginning and end of the therapy.
Results: There was a statistically significant improvement in reaction time in the Treatment group (45 milliseconds, t=3.05, p=0.012). No changes were found in the healthy and pathological control groups. No change in P3 latency was found in any group. Regarding the topography, there was a high correlation (r=0.91, p< 0.05) in the treatment group between the beginning and the end of the program; when the healthy and pathological groups were compared (Pathological Control and Treatment), lower correlations for the P3 maps were obtained (r=0.41 and r=0.71, respectively, p< 0.05).There were no statistical differences in the neuropsychological scores.
Conclusions: There was a better reaction time performance without a neuropsychological improvement. P3 latency was also blinded to the benefit, but the topography of this component reflected different patterns of brain activity between the pathological and healthy groups. All these results indicate the need for different levels of analysis for cognitive impairment and its potential rehabilitation.

P821

Gait variability, asymmetry, and bilateral coordination of gait during a long distance walk in persons with multiple sclerosis

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Background: Persons with multiple sclerosis (pwMS) have difficulties walking long distances yet the specific gait impairments underlying reduced walking endurance are poorly understood.
Objectives: To document gait variability, asymmetry and bilateral coordination of gait during a long distance walk, and determine if these gait parameters worsen as disability increases.
Methods: 80 persons with MS (age: 46.2 ± 10.7, 66 female, EDSS: 2.3 (0 - 6.5)) completed a six minute walk test (6MWT) wearing Opal body-worn sensors. Gait variability (Stride time variability (Stride CV)), gait asymmetry (GA, left vs. right leg swing times), and bilateral coordination of gait (phase coordination index (PCI)), were calculated based on heel strike and toe off times for the straight line walking segments. One-way between groups ANOVAs and Tukey's post-hoc tests compared gait parameters between mild (EDSS 0=2.5, n = 52) vs. moderate (EDSS 3-4, n = 23) vs. severe (EDSS =4.5- 6.5, n = 5) disability subgroups.
Results: Across subjects, the mean values (±SD) of Stride CV, GA and PCI were (3.1 ± 1.8%, 6.3 ± 10.1%, and 5.0 ± 4.3%, respectively. The severe disability group had greater variability and asymmetry (Stride CV = 5.8 ± 3.8%, GA = 24.5 ± 30.4%) than the mild (Stride CV = 2.7 ± 1.2%, GA = 4.4 ± 4.2%) or moderate disability group (Stride CV = 3.4 ± 1.7%, GA = 6.4 ± 8.4%; all, p $\leq$ 0.009), and more impaired coordination than the mild disability group (PCI = 10.6 ± 11.2% vs. 3.9 ± 2.0%, respectively, p = 0.002). There was a trend for bilateral coordination of gait to differ between the moderate and severe groups (PCI: 6.3 ± 4.8 vs. 10.6 ± 11.2, p = 0.08). None of the gait parameters differed significantly between the mild and moderate disability groups (all p > 0.05). Increased Stride CV, GA, and PCI were associated with shorter distance walked during the 6MWT (6MWT vs. Stride CV r = -0.57, vs. GA r = -0.47, vs. PCI r = -0.55, p < 0.01).
Conclusions: Gait variability, asymmetry and incoordination are associated with reduced walking endurance and most pronounced in pwMS with severe disability. Further studies should determine whether variability, symmetry and coordination of gait change over time, and whether a rehabilitation approach targeting these specific gait impairments could result in improved walking endurance.

P822

Characteristics of MS patients who follow-through with cognitive training

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Background: Up to 65% of multiple sclerosis (MS) patients experience cognitive deficits associated in the disease, and as a result, researchers have implemented cognitive training as a tool to address this problem. However, much like longitudinal interventions with other disease groups, these studies frequently report

high rates of attrition. Further investigation is needed in order to identify the characteristics of individuals who will follow-through with a cognitive training intervention.

Objectives: To identify the characteristics of MS patients who will complete a 6-week cognitive training study.

Methods: 73 participants were recruited for a study involving computer-based training in their homes for 30-minutes per day, 6 days of the week, for a 6-week period. The study also involved baseline and follow-up neuropsychological testing sessions. However, only 42 participants completed the study. Baseline data from completers and non-completers were compared on a number of variables, including demographics, MS Quality of Life (MSQOL), and Neuropsychological Assessment Battery Digits Backward.

Results: Completers scored higher on Social Functioning (t=-2.01, p=.048) and Overall Quality of Life (t=-2.128, p=.039) on the MSQOL and had higher scores on working memory (digits backward; t=-2.307, p=.024). Completers were equivalent with non-completers in terms of age, education, personality characteristics, length of time with MS, fatigue, anxiety, and depression. Additionally, there were no differences between completers and non-completers on measures of processing speed or single-trial verbal learning. week, for a 6-week period.

Conclusions: MS patients who reported a higher quality of life and performed better on a measure of working memory were more likely to complete a longitudinal study of cognitive training involving two in-person study appointments and 6-weeks of at-home training. Factors such as depression, anxiety, fatigue, and education did not differentiate those more likely to complete or drop-out of the study. In the future, researchers and clinicians should consider utilizing a brief working memory task and a quality of life self-report measure as screening tools to help identify ideal candidates for a cognitive training intervention.

P823

The relationship between core stability and balance in patients with multiple sclerosis

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Background: People with Multiple Sclerosis (MS) experience disabling balance and mobility impairments. Although core stability is an important component of balance, evidence on the relationship between core stability and balance in patients with MS is insufficient.

Objectives: To investigate the relationship between core stability, balance and functional mobility in patients with MS.

Methods: 24 ambulatory patients with MS (3 men, 21 women, age: 40.33±11.23 years EDSS: 1.31±1.37) participated in the study. Core stability was assessed in two parts; core endurance and core power. Core endurance was assessed using the McGill protocol (trunk flexion test, a modified Biering-Sorensen trunk extension test, right-left flexion tests and prone breach test). The tests were scored by the duration the individuals could maintain these isometric postures (recorded in seconds). Core power was assessed by maximum number of 'sit-ups' and maximum number of modified 'push-ups'. The number of successful repetitions in 30 second was recorded. Balance and functional mobility was assessed with the duration of single-limb stance and Timed "up and go" test (TUG) respectively. Spearman correlations were used to determine relationships between core stability, balance and functional mobility.

Results: Core endurance was significantly correlated with duration of single-limb stance (r= 0.490-0.615, p< 0.05) and TUG (r= -0.405_-0.643. p< 0.05) in patients with MS. Core power was significantly correlated with duration of single-limb stance (r= 0.440-0.726, p< 0.05) and TUG (r=-0.371_-0.649, p< 0.05) in patients with MS.

Conclusions: Core stability was related to balance and functional mobility. These results indicate that balance training should include core stability exercises in patients with MS.

P824

The state of MS: current insight into patient-neurologist relationships, barriers to communication and treatment satisfaction

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Background: The chronic, progressive, and variable nature of multiple sclerosis (MS) underscores the importance of establishing strong relationships between patients and their health care providers (HCPs), to promote effective communication and ensure high treatment satisfaction. Identifying existing gaps between the MS care paradigm and patient needs may promote dialogue around key issues related to disease management within the MS community.

Objectives: Gain insight into the current state of MS care from the perspective of patients and neurologists, in order to identify potential areas of collaboration between patients, family members, caregivers, patient advocacy organizations, physicians, and MS pharmaceutical manufacturers that may achieve better patient health-related outcomes and quality of life (QoL).

Methods: Online surveys were conducted in five countries (Germany, Italy, Spain, United Kingdom [UK], and United States [US]) in adult patients diagnosed with MS (n=982) and in neurologists who see at least one MS patient per year (n=844).

Results: Preliminary results indicate that the majority of patient respondents was from the US (52%), followed by Germany (15%), UK (13%), and Italy and Spain (10% each); the majority of neurologists responded from Germany and US (23% each), followed by Italy (22%), UK (17%), and Spain (16%). The majority of patients had relapsing-remitting MS (RRMS; 53%), followed by secondary progressive MS (SPMS; 25%), primary progressive MS (PPMS; 10%), and progressive-relapsing MS (PRMS; 5%). Of patient respondents, 71% were female, mean time since diagnosis was 13 years, and 65% had ever taken disease-modifying therapies (DMTs) for the treatment of MS. Patient respondents

provided information on HCP interactions (eg, quality and comfort of relationship), barriers to communication (eg, factors that interfere with communication), and treatment satisfaction (eg, goals, expectations, challenges). Neurologist respondents provided complementary information on patient interactions, barriers to communication, and patient treatment satisfaction.

Conclusions: Insights gained from survey data into the quality of patient-neurologist relationships, similarities and differences in perspectives, barriers that interfere with effective communication, and factors that drive treatment satisfaction will promote discussion and potential initiatives to improve disease management, treatment optimization, and QoL in patients living with MS.

P825

Value based medicine: enabling evidence-based and individualized treatment decisions for patients with multiple sclerosis

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Background: Advances in drug development have led to a rapid expansion in available treatments for patients with multiple sclerosis (MS) in recent years. However, benefit-risk profiles are not well understood beyond the population level. This, coupled with the absence of reliable predictors of outcomes, has created an unmet need for individualized, evidence-based treatment decisions in MS.

Objectives: Value Based Medicine (VBM) is an initiative through Biogen Idec aimed at transforming MS care by empowering better decision-making through scientific, clinical, and marketplace innovation.

Methods: The VBM initiative aims to develop a robust suite of tools to address key issues in MS treatment categorized into three aspects of care: monitor, evaluate, and treat. Tools related to monitor will enhance the ability of patients and clinicians to monitor MS through the collection of data at the site of care and remotely using novel technology. Tools related to evaluate will assist clinicians and patients with individualized evaluation of current disease state and disease prognosis, through integration and visualization of all collected data. Tools related to treat will support the development of individualized treatment plans for patients through the improved understanding of disease course and treatment response.

Results: With the development of these tools, patients may be able to recognize clinical changes earlier, have access to relevant data collected and visualized in a standardized manner, enabling better communication with their clinicians.

Clinicians may have better standardized outcome measures, reliable predictors of disease progression and treatment response, integrated longitudinal summaries of available measures, and better tools for individualized assessment of benefit-risk.

Payers will better understand the relative value of different treatment options.

Conclusions: The increased complexity of treatment decision making in MS requires the community to develop innovative tools and approaches to better serve patients, physicians and payers. Various approaches to advancing treatment decision making will be presented.

P826

Do patients with multiple sclerosis understand quantitative health information? A comparison of patients with a probabilistic national sample

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Background: Natalizumab (NAT) is a highly effective therapy in relapsing multiple sclerosis (MS), yet bears the risk of a progressive multifocal leukoencephalopathy as a rare but severe side effect. To make informed decisions about this treatment, patients need to understand quantitative information about its benefits and risks, which requires statistical numeracy. It is unknown, however, whether MS affects numeracy and how MS patients score in comparison to the general population.

Objectives: To compare numeracy between NAT treated MS patients and a probabilistic national sample; to identify variables that predict patients' numeracy.

Methods: Since 12/2011 the prospective, observational, multicenter study PERCEPT investigates NAT patients' and neurologists' benefit/risk perception and knowledge in routine practice in Germany. Numeracy of NAT patients was assessed as the proportion of correct answers on a standard test (1). So far, complete data from 211 patients were available (data collection ongoing) and were compared to published data on numeracy in a German probabilistic sample with N = 1,001 (1) with an analysis of variance controlling for age, sex, and education. Within patients, a multiple regression assessed which demographic variables (age, sex, education) and which MS variables (years with MS, disability, annual relapse rate) allowed predicting numeracy.

Results: Preliminary analyses showed that MS patients had slightly higher numeracy than the probabilistic national sample (72.8% vs. 69.0% correct answers, $p = .048$). Within patients, numeracy was predicted by education (beta = .26, $p = .0002$), years with MS (beta = .17, $p = .033$) and sex (beta = .15, $p = .026$), but not by age, disability, and annual relapse rate. The overall predictability of numeracy was low (R square = .112, $p = .0005$).

Conclusions: In these preliminary analyses, MS patients had slightly higher numeracy than a national probabilistic sample, controlled for age, sex, and education. One should therefore not assume that MS patients understand quantitative health information worse than the general population. Other variables typically known about patients (education, years with MS, sex) predicted their numeracy, but predictability was so low that using those variables in practice to judge who will understand quantitative health information cannot be recommended.

P827

Analysis of data from an adult day program for people with multiple sclerosis

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Background: B. Fit!, an outpatient wellness program serving individuals with MS, was launched in 2005 in a collaborative effort between the National Multiple Sclerosis Society (NMSS) Greater New England Chapter and The Boston Home (TBH). B. Fit!'s 35 active participants attend this model and well structured program of exercise; stress management; cognitive, health and wellness education; nutritional support; socialization; and recreation.
Objectives: The goal of this study is to conduct a systematic evaluation of B. Fit! to determine whether participation in the program results in improvements in mental functioning, quality of life, and perceptions of social support, and to determine which program components are most effective.
Methods: We surveyed participants at four points throughout the year to analyze changes in quality of life and mental and social functioning. We also investigated whether the length of time in the program and frequency of attendance contributed to better outcomes. In combination with satisfaction surveys, we interviewed participants to determine which activities they preferred and found most beneficial.
Results: Preliminary data show that B. Fit! participants´ mean Mental Health Inventory (MHI-5) score is comparable to that of another sample of MS patients in the US, and have a higher, (less distressed) mean MHI-5 score than patients with subthreshold depressive symptoms, major depression, and dysthymia, and people over the age of 65 with a disability. Modified Social Support Survey (MSSS) scores, which measure perceived social support, indicate that B. Fit! participants, when compared to patients with other chronic illnesses, felt that they had greater support in terms of material aid and assistance, but not in terms of the availability of people with which to share fun activities, provide affection, or emotional information. Mean MCS (Mental Composite Scores) of the SF-12 indicate that participants have a higher level of mental quality of life than the general MS and depressed population.
Conclusions: Results from the MHI-5 scores suggest that the psychological distress among B. Fit! participants is similar to that among the MS population in general, and that it is greater than among people with other chronic illnesses, although not as severe as among people with a mental illness or older populations with a physical disability. Future analyses will examine the relationship between psychological distress and perceived lack of social support and the impact of attending B.Fit! on both.

P828

A prospective study comparing the impact of three levels of support services on interferon β adherence in patients with relapsing MS: interim results

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Background: Suboptimal adherence to disease-modifying treatments (DMTs) is common in MS. MS LifeLines® (MSLL) is a support service provided to patients with relapsing MS (RMS) who are prescribed interferon beta-1a subcutaneously three times a week (IFN β-1a SC).
Objectives: To compare the impact of 3 levels of support services on patient-reported adherence and other patient-reported outcomes (PROs) in RMS patients prescribed IFN β-1a SC as their first or first-switch DMT.
Methods: In this ongoing, 12-month study, patients are allocated to Group A or B based on geographic location at initiation of MSLL support (baseline). Group A is randomized to a standard MSLL services group (who receive phone calls and nurse visits at set intervals) or a custom services group (who have the option of selecting which 'standard' services they receive, and who also receive educational materials, planning tools and reminders). Patients in more remote areas are allocated to Group B and receive phone calls and a visit for injection training. The primary endpoint is the difference in medication adherence, derived from the cumulative number of missed injections (as reported by patients every 4 weeks), between the standard and custom service groups. Patients complete various PRO questionnaires periodically, including Morisky Medication Adherence Scale-4 (MMAS-4) and MS International Quality of Life (MusiQoL).
Results: PRO responses at baseline are currently available from the first 163 enrolled patients (>50% of the total [N=306] to be enrolled). Of these 163 patients (mean [SD] age, 38.9 [9.9] years; 74% women), 77% were DMT-naïve and 62% reported depression since their MS diagnosis (45% were both). The MMAS-4 identified the following barriers to medication-taking in 35% of patients: 29% forget to take their medication, 9% are careless, 8% stop if they feel worse when taking it, and 6% stop when they feel better. On the MusiQoL, 32% reported having felt depressed or gloomy, and 38% reported problematic memory loss. Of the 52 patients allocated to the custom group, 92% chose to receive all available services. For the first 50% of patients, 6-month data on adherence and on the other PROs will also be presented.
Conclusions: In this ongoing study, cross-sectional data at baseline showed that 35% of DMT-naïve or first-switch RMS patients reported depression or reported sometimes forgetting to take their medication. The 6-month data will provide information on potential correlations between support services and adherence and persistence.

P829

Aerobic fitness and hippocampal volume in multiple sclerosis

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Background: The hippocampus is important for memory functions in multiple sclerosis (MS). Importantly, there is significant atrophy of this structure among persons with MS, and the volume of the hippocampus has been inversely associated with performance on tests of memory encoding. There is substantial evidence that aerobic fitness and exercise training yield improvements in hippocampal volume among older adults without MS, and one recent case study suggested similar benefits in MS. There is a

need for additional evidence of an association between aerobic fitness and hippocampal volume before investing considerable time, effort, and resources into clinical trials of aerobic exercise training in MS.
Objectives: This study examined the association between aerobic fitness and hippocampal volume in persons with MS.
Methods: We enrolled 25 persons with MS (EDSS = 1.0-6.5) who underwent an incremental exercise test on an electrically-braked, Lode cycle ergometer for objectively quantifying aerobic fitness as peak power output (W_p). Participants further underwent MRI using a 3T whole body MRI Siemens Trio scanner. Using 3D T1-weighted MR images, volumes of the right and left hippocampi were automatically calculated by the FMRIB's Integrated Registration and Segmentation Tool (FIRST)-algorithm FMRIB's Software Library (FSL). We examined the association using Spearman rho bivariate (r_s) and partial (pr_s) correlations controlling for age and EDSS scores.
Results: W_p was significantly associated with the average hippocampal volume (r_s=.41) and EDSS scores (r_s=-.77), but not age (r_s=-.31). The correlations were not statistically significant between hippocampal volume with age (r_s=-.15) or EDSS scores (r_s=-.12). The correlation between W_p and average hippocampal volume was independent of age and EDSS scores (pr_s=.50).
Conclusions: We provide novel evidence of an association between aerobic fitness and hippocampal volume, independent of age and disability status, in MS. This provides a stronger basis for examining aerobic exercise training as an approach for delaying or reversing atrophy of the hippocampus in MS.

P830

Physiological response to exercise in people with MS: peak or slope?

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Background: Research from our group indicates that people with Multiple Sclerosis (MS), diagnosed in the era of imaging and availability of disease modifying drugs (post 1995), show near normal function on submaximal tests but dramatically reduced function on maximal tests. There are a number of reasons for this:

(i) heart and lungs have low capacity to deliver oxygen to muscles;
(ii) negative physical and psychological sensations limit tolerance to maximal exercise; and
(iii) MS impairments (muscle weakness, spasticity, poor balance, lack of core strength) make strenuous muscular exertion costly.

The gold standard exercise test is maximal oxygen consumption (VO_{2max}) and is the outcome against which exercise interventions are tested. Several valuable parameters can be obtained from exercise testing: VO_{2max}; peak value at exercise tolerance (VO_{2peak}); and oxygen consumption over time. The latter indicates capacity of the body to economically respond to exercise demand.
Objectives: The purpose of this study was to identify variability in response to exercise in people with MS.
Methods: By pooling three studies assessing exercise capacity in people with MS, the largest data set on oxygen consumption during exercise has been compiled. A total of 169 people (mean age 44; 72% women; majority RRMS) were tested using maximal graded cycling protocol. Group based trajectory analysis was used to identify patterns of oxygen uptake over time.
Results: Ten distinct trajectories of exercise response were uncovered. The most striking source of variation was time to exercise termination which was < 8 minutes for 30 people (18%), between 8 and 15 minutes for 79 people (47%), and up to 27 minutes for the remaining 60 (35%). Peak oxygen consumption averaged 24 ml/kg/min (SD:9). Using normative data, 114 people (67%) had values < 15%ile and only 14 people (8%) had values >50%ile. The slope for change of oxygen uptake for the least energy economical group was 2.45 ml/kg/min/min, and their time to termination was short, compared with 0.47 for the most energy economical group, with a longer time to termination. These two extreme groups showed a ratio of slopes of 5.2.
Conclusions: The poor exercise tolerance in this relatively young and able population confirms previous findings. As exercise programs for MS must target all of the impairments and disabilities as well as aerobic capacity, evaluation of effectiveness might consider supplementing change in VO_{2peak} with change in slope.

P831

Falls among non-ambulatory individuals with multiple sclerosis: an international expert panel consensus statement

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Background: Falls are a significant health concern for individuals with multiple sclerosis (IwMS) and have been associated with physical injury, fear of falling and decreased quality of life. Over the last decade substantial research efforts have examined predictors, consequences and strategies to prevent falls in IwMS. A major limitation of this research is that it has almost exclusively focused on individuals capable of walking.
Objectives: To reach consensus on an operational definition of non-ambulatory IwMS and determine the prevalence of falls in this segment of the MS population.
Methods: An international expert panel was convened to determine a consensus- based operational definition of non-ambulatory IwMS. The definition was subsequently applied to data from a population-based study of falls in IwMS living in Stockholm (Ytterberg et al, 2013, J Rehabil Med 45:452-7) to determine the proportion of non-ambulatory IwMS and fall prevalence rate. In that study, performance and self-reported mobility data and self-reported data on falls in the last 3 months was collected from 164 IwMS ranging in age from 19-79 years old.

Results: The expert panel determined that "non-ambulatory" was best operationalized with performance-based measures as opposed to self-report measures. Inability to perform a timed walk test was identified as the best outcome to operationalize "non-ambulatory". Using these criteria, 39 of the 164 participants (24%) were identified as non-ambulatory. Of these 39 individuals, 11 reported falling at least once in the past 3 months (28%). Preliminary results showed that fall risk factors in non-ambulatory and ambulatory IwMS differed.
Conclusions: This novel research project yielded a useful operational definition of non-ambulatory IwMS. Nearly a quarter of the population-based sample was found to be non-ambulatory. The high prevalence of falls in this population highlights the need to better understand risk factors and consequences of falls among non-ambulatory IwMS. The International Classification of Functioning, Disability and Health could be used as a comprehensive framework to guide this research agenda. This work represents the initial phase of the development of a complex intervention specifically designed to reduce falls among non-ambulatory IwMS.

P832

Low-cost portable posturography for patients with multiple sclerosis using Wii balance board

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Background: Computer-based measurements of postural sway by force platform have been recently incorporated in research setting as reliable, objective and specific outcome measures for balance of patients with multiple sclerosis (MS). Despite this, the lack of standardisation, heterogeneity of equipments and their expensiveness make the application of these measurements not easily feasible in daily clinical setting.
Objectives: To verify whether the commercial Wii Balance Board System (WBBS) (Nintendo, Kyoto, Japan) has technical characteristics equivalent to laboratory-grade force platform in estimating postural sway of patients with MS.
Methods: Eighty-five patients (55 F, 30 M), with a mean (SD) age of 39.2 (10.2) years, mean (SD) MS duration of 12.4 (8.6) years and median [range] Expanded Disability Status of 3.0 [1.0-6.0] were recruited. The displacement (mm) of body's center of pressure (COP) under eyes opened and closed was estimated using a "gold-standard" laboratory-grade force platform (ProKin PK254P, Tecnobody) and the commercial WBBS. Data from the WBBS device were collected via Bluetooth using a freeware custom-written software running on a laptop computer [http://www.dia.uniroma3.it/~patrigna/portable_post/]. Inter-device reliability and validity of the WBBS in detecting patients who reported the occurrence of at least one accidental fall in the past year were investigated.
Results: Mean (SD) COP displacement were 311 (153) mm (eyes opened) and 414 (117) mm (eyes closed), and 560 (380) mm (eyes opened) and 648 (352) mm (eyes closed), as measured with ProKin and WBBS, respectively. Visual evaluation of the Bland-Altman plots did not show any relationship between the variances of measures with the size of the mean; however, the WBSS tends to overestimate postural sway when compared with the gold standard.

Inter-device reliability was good under eyes opened and excellent under eyes closed (ICC=0.677 and 0.857, respectively). Comparison of ROC curves showed no significant between-device difference in detecting the 31 (36.5%) fallers, with AUC (ProKin)=0.707 vs. AUC (WBBS)=0.721 under eyes opened condition (p=0.738) and AUC (ProKin)=0.749 vs. AUC (WBBS)=0.733 under eyes closed condition (p=0.538).
Conclusions: The low-expensive, portable, and user-friendly WBBS exhibited good-excellent reliability and fair validity. Therefore, it may overcome, at least partially, the main drawbacks of laboratory-grade force platforms, potentially providing an useful tool for multi-centre studies.

P833

Investigating of the effect of fascial mobilization on dynamic walking parameters in patients with multiple sclerosis

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Background: Plantar flexor spasticity decreases the normal hindfoot loading whereas increases midfoot and forefoot loading by preventing the normal initial contact and dynamic loading. Plantar flexor spasticity also prevents controlled plantigrad contact and push-off during walking in patients with Multiple Sclerosis (MS).
Objectives: To investigate that the effects of fascial mobilization technique on dynamic walking parameters in patients with MS.
Methods: 9 (5 male, 4 female) patients (EDSS scores between 2-4) and 14 healthy adults (7 male, 7 female) have participated in the study. Fascial mobilization technique (FM) was applied to plantar flexor muscles spasticity of plantar flexor muscles according to Modified Ashworth Scale. Dynamic loading parameters while walking (maximum loading pressure -N/cm2 and maximum loading time- ms for 1st and 5th metatarsal head, medial and lateral heel, midfoot) have been recorded by dynamic pedobarography, initially and immediately after the applications and 24 hours after application. Dynamic loading parameters of healthy adults (maximum loading pressure-N/cm2 and maximum loading time-ms for 1st and 5th metatarsal head, medial and lateral heel, midfoot) also recorded by dynamic pedobarography.
Results: No differences in the muscle tone have been recorded between initial and post treatment values (p>.05). After application of FM, medial heel maximal loading increased (p< .05) both feet and midfoot loading decreased in left feet (p< .05). There was a carryover effect 24 hours after the application. Comparison of MS and healthy adults showed that there were difference at heel medial loading (p< .05) in favor of healthy adults (p< .05). Immediately and 24 hours after application of FM, the difference between the MS and healthy adults was eliminated (p>.05).
Conclusions: Although there was no change in the muscle tone according to Modified Ashworth Scale, a significant improvement on dynamic loading parameters has been found by fascial mobilization technique. We believe that the MAS could not be a sensitive marker of all changes of muscle tonus.

P834

Vitamin D serum levels and balance are not related in multiple sclerosis

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Background: Balance impairment is a common and disabling MS symptom that can lead to falls and accidents. Postural sway has been validated as a predisposing factor to falls in MS. Serum Vitamin D (VitD) levels has been shown as a variable associated with postural sway in elderly. VitD supplementation reduces sway and falls in elderly fallers but effects in Multiple Sclerosis (MS) are not established

Objectives: To evaluate the relationship between sway and VitD serum levels in a group of MS patients in a cross-sectional designed study.

Methods: A group of MS patients attending the University MS Center of Cagliari during the winter of 2012 were included. Balance was assessed by postural sway analysis using a force platform. The patients were asked to stand barefoot as motionless as possible for 30 seconds on the plate keeping a stable and relaxed position with and without visual control. During the same period the patients were tested for VitD serum levels by RIA (Diasorin). Center of Pressure (COP) time series were post-processed to calculate the sway area, COP path length, COP displacements and velocity in the medio-lateral and antero-posterior directions. Correlation between all these sway parameters and VitD were tested by means of the Pearson product moment correlation analysis first without adjusting for confounding variables and then adjusting for age and EDSS.

Results: 49 MS patients 13 male and 36 female were enrolled. Mean age was 40.4 (DS±14.5) and mean EDSS was 2.8(DS±1.9) while median values of VitD serum levels were 14.4 (DS±16.9). None of the sway parameters considered for the eyes open and eyes closed test showed significant relationship with VitD serum levels also after adjusting for confounding factors.

Conclusions: The most common explanation to VitD effect on postural response in elderly is the direct action on muscle strength. In fact VitD receptors in muscles lead to protein synthesis and cell growth while supplementation in deficient individual improve muscle strength. Nevertheless the mechanisms of postural impairment in MS is more complex and involves a wide number of structures in the CNS where the effects of VitD, although questioned, basing to our data are limited.

P835

An exploratory study of the acceptability and efficacy of working memory training for individuals with pediatric-onset multiple sclerosis

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Background: Cognitive impairment occurs in 30-50% of children and adolescents with Multiple Sclerosis (MS), with prominent deficits in working memory (WM). Studies conducted in pediatric populations show improvement in WM capacity after intensive training using computerized activities that tax WM.

Objectives:

(1) To describe the feasibility of implementing a computer training program (Cogmed) in pediatric-onset MS patients by evaluating retention and tolerance of the program; and

(2) To explore how Cogmed training may predict changes on standardized neuropsychological measures.

Methods: Nine pediatric-onset MS patients (ages 14-24), identified as having cognitive dysfunction, were recruited from SickKids Hospital and underwent a 5-week (5 days/week, 45 minutes/day) computer training program that adapts task difficulty based on individual performance. Cognitive dysfunction was determined by performance falling ≥1SD below normative data on ≥1 neuropsychological measure, and/or subjective cognitive complaints. A Training Coach phoned all participants weekly to:

(i) inquire about attitude towards training and onset of health/other changes; and

(ii) provide motivational support. Change scores on standardized neuropsychological tests pre- and post-treatment served as preliminary efficacy data.

Semi-structured exit interviews were conducted with all participants to investigate their training experiences.

Results: Of 17 invited patients, 9 agreed to undergo training. Six patients who required no parental supervision (mean age 19.8 ± 4.2 years), completed the program within the recommended time frame; three did not complete (mean age 16.9 ± 2.8 years). On average, patients completed 44.6 ± 5.2 minutes of active training per session. Repeated measures ANOVA revealed improved performance from baseline to follow-up on a non-trained test of visual WM ($p < .01$) and a trend towards significance on a verbal WM task ($p < .10$). There was no improvement on control tests (i.e. tests that do not tax WM). No adverse symptoms were reported throughout the training period.

Conclusions: Select pediatric-onset MS patients can tolerate and complete an intensive cognitive rehabilitation program. Completion was influenced by whether individuals required parental motivation to do training. Further studies are needed to explore methods to increase adherence with intensive training programs. Replication of near transfer effects is required using a larger sample, controlled design, and a wider variety of tasks.

P836

Gait and cognition in MS: a complex interplay. Relationship between kinematic data and cognitive processing speed, verbal and visual memory

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Background: During the last years several studies suggested a relationship between cognitive and motor performance in persons with multiple sclerosis (MS), generally using as experimental setting the dual task that combines motor and cognitive fields during a single test. Nevertheless relationship between gait and cognition has been studied only using gait spatial-temporal (ST) parameters but not Kinematic analysis, which could give more insight on ambulation impairment.
Objectives: The aim of this study was to investigate the relationship between kinematic gait alterations and cognitive processing speed, verbal and visual memory (evaluated by means of the Symbol digit modality test (SDMT), the Selective reminding test (SRT, Amato et al.2006) and the Brief visuospatial memory test revised (BVMT-R Benedict R et al 1997)).
Methods: A group of patients attending the MS Center of Cagliari were enrolled. The inclusion criteria were a diagnosis of MS, the ability to walk independently without any assisting device for at least 20 meters and the absence of other pathologies that could affect gait.

An MS expert neurologist performed a Neurostatus evaluation and the cognitive tests. Kinematic and ST parameters were acquired using an optoelectronic system. The Gait Profile Score (GPS, Baker et al., 2009) was used as a synthetic index for the kinetics data. The relationship between gait and SDMT, SRT and BVMT-R (corrected for age and education) results was assessed by means of the Pearson product moment correlation analysis.
Results: 27 subjects were enrolled, 18 male and 9 female. Mean age at examination was 47.0 (SD=10.0), mean EDSS was 3.7 (SD=0.9) and mean GPS score was 9.51° (SD=2.4), for left side and 9.67° (SD=2.3) for the right side. None of the ST data correlated with any cognitive task. A significant positive correlation between GPS and SDMT corrected scores (r=0.304 p=0.026) and BVMT-R corrected scores (r=0.365, p=0.007) was found. No correlation was found between SRT and GPS.
Conclusions: In our analysis executive function and visual memory impairment are mildly related to walking alteration measured by GPS. No correlation were found between any ST and cognitive data suggesting that GPS could be more sensitive in defining interaction between walking and cognition. Further studies are needed with an adequate sample size to obtain definitive results. Moreover the evaluation of kinetic data in a dual task setting will be very useful.

P837
Postural adaptation during a Nintendo WII balance training

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Background: In the last years several works suggested the use of Nintendo Wii plus Balance board as effective tool in balance rehabilitation in MS. Nevertheless there is a scarce insight about postural adaptation induced by this device.
Objectives: To characterize the postural adaptation mechanisms induced by Nintendo Wii + Balance Board during a WII session and to define if they differs from patients (PT) versus controls (HC).
Methods: 20 MS PT and 16 age and gender matched HC were enrolled in the study. All participant were familiar with Nintendo Wii + Balance Board system. They played three games of the Wii-Fit suite namely Penguin Slide (PS), Table Tilt (TT) and Balance Bubble (BB). During the session, Center of Pressure (COP) time series were collected using a force platform placed under the Balance Board. Raw data were post-processed to calculate the COP displacements and velocity in mediolateral (ML) and anteroposterior (AP) directions. The statistical analysis was performed by means of a three-way MANOVA setting as independent variables the subject's status (MS or HC) direction (AP and ML) and played game (PS, BB and TT), while the dependent variables were COP displacements and velocities. Where necessary, univariate ANOVAs were performed for each dependent variable by setting the level of significance at $p < 0.0125$ (0.05/4) as per Bonferroni correction.
Results: MANOVA revealed a significant main effect of status (PTvsHC), game and direction ($p< 0.001$) on COP features. In particular, ANOVA revealed that COP displacements and velocities were found larger in ML direction with respect to AP ($p< 0.001$) for both groups, while no significant differences between HC and MS were found for any of the COP parameters. Different games induced different amplitude of both ML and AP displacements, while velocities in AP direction appear no influenced by the played game. On the contrary, PS was the game associated with the largest COP velocities in ML ($p< 0.001$ when compared to TT and BB)
Conclusions: The Wii-Fit games in MS induced an unbalanced activity in sagittal and frontal planes. The prevalence of ML movements is not related to MS features but is rather an intrinsic feature of the games. Our data gave more insight on the postural mechanisms during a Wii games session, nevertheless further studies are needed to understand whether a long-term unbalanced activity might influence the postural sway and thus a more accurate choose of Wii games must be considered.

Reproductive aspects and pregnancy

P838
Glatiramer acetate and pregnancy in women with multiple sclerosis - results from the German multiple sclerosis and pregnancy registry

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Background: MS is the most common autoimmune disease of the central nervous system, which mainly affects young women of reproductive age.

Only limited data are available on whether GLAT exposure during pregnancy has an effect on perinatal outcome.
Objectives: To determine safety of glatiramer acetate (GLAT) exposure during pregnancy of patients with multiple sclerosis (MS).
Methods: We compared the outcome of pregnancies of patients with MS exposed to GLAT with pregnancies of disease-matched unexposed controls and healthy women.

Women with MS were enrolled into the German MS and pregnancy registry at any point during pregnancy. Healthy Controls (HC) were women without teratogenic exposures in pregnancy. These women had contacted the Motherisk General (Toronto) or nausea and vomiting of pregnancy (NVP) lines to inquire about safety of non-teratogenic drugs.

MS controls were matched 1:1 with healthy controls according to age (+/- 1 year) and body mass index (+/- 1).
Results: We collected data on 274 pregnancies: 90 with exposure to GLAT, 97 MS controls unexposed to disease-modifying therapies (DMTs) during pregnancy and 87 pregnancies of healthy controls.

While 30 of the exposed pregnancies are still ongoing we did not document any malformation in the GLAT exposed pregnancies (0/0%) so far. The rate of spontaneous abortions did not differ compared to MS diseased non-exposed controls. 4 (4,12 %) infants of the DMT unexposed women were born with malformations of non-genetic origin: 2 with an atrial septal defect, 1 with dysmelia of tibia and fibula of the right leg. 1 newborn of the DMT unexposed women with MS was diagnosed with Wolf Hirschhorn syndrome (aberration of chromosome 4).
Conclusions: Glatiramer acetate exposure does not constitute a major human teratogen. Final data including the outcome of ongoing pregnancies will be presented at the time of the meeting. As the sample size of our cohort is still small, further studies are needed to prove the safety of glatiramer acetate exposure during pregnancy in MS.

P839

Delayed-release dimethyl fumarate and pregnancy: preclinical studies and pregnancy outcomes from clinical trials and postmarketing experience

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Background: As women of childbearing age make up a large proportion of the multiple sclerosis population, it is important to evaluate effects of delayed-release dimethyl fumarate (DMF) exposure on pregnancy.
Objectives: To present preclinical data from animal reproductive toxicology studies and the outcomes of pregnancies occurring during the delayed-release DMF clinical development program, as well as postmarketing experience.
Methods: Reproductive and developmental toxicology was evaluated in rats and rabbits. Clinical studies of delayed-release DMF included 2,665 MS patients, 320 psoriasis patients, 101 rheumatoid arthritis patients, and 338 healthy volunteers; subjects were required to use reliable contraception and immediately discontinue drug in the event of pregnancy. Outcomes as of January 31, 2014 are reported.
Results: No evidence of impaired fertility in rats or teratogenicity in rats and rabbits given delayed-release DMF was apparent at doses attenuating maternal weight gain. Pregnancy outcomes are known for 38 of 44 pregnancies in subjects who received delayed-release DMF in clinical trials, for all pregnancies (13) in subjects who received placebo, and for 3 of 4 pregnancies in subjects who received glatiramer acetate (GA). Information is pending for 5 patients who received delayed-release DMF in clinical trials. In patients exposed to delayed-release DMF, 25 live births (57%), 3 spontaneous abortions (7%), and 10 elective terminations (23%) were reported. In placebo recipients, 9 live births (69%), 2 spontaneous abortions (15%), and 2 elective terminations (15%) were reported. In GA recipients, 1 live birth (25%) and 2 elective terminations (50%) were reported. No fetal abnormalities were reported. The incidence of spontaneous abortion was consistent with the expected rate of early pregnancy loss in the general population (12-22%). There were 45 pregnancies reported from postmarketing experience; however, only 7 outcomes were known at the time of this analysis (information pending for 37 patients and 1 patient lost to follow-up): 0 live births, 6 spontaneous abortions, and 1 elective termination. As with the pregnancy outcomes from clinical trials, no fetal abnormalities were reported.
Conclusions: Based on available clinical evidence, gestational exposure to delayed-release DMF during the first trimester was not associated with increased risk of fetal abnormalities or adverse pregnancy outcomes. Outcomes continue to be monitored through a pregnancy registry and updated data will be presented.

P840

Multiple sclerosis is more severe after menopause in a longitudinally followed clinical cohort

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Background: Almost half of women living with multiple sclerosis (MS) are post-menopausal. While puberty and pregnancy represent key modulatory periods for MS, little is known about menopause. We have previously described worsening of MS severity after menopause in an online research platform, but now focused on a well-phenotyped clinical cohort.
Objectives: To determine the effect of menopause on MS course in a well detailed clinical cohort.
Methods: We linked reproductive survey data with prospectively collected disease severity measures for women with MS in CLIMB, a deeply phenotyped large Northeastern US MS clinic cohort. Disease severity was measured by the Expanded Disability

Status Scale (EDSS). Menopausal status was the primary exposure.

Results: Over half the 455 respondents were postmenopausal. Median age at natural menopause was 51.5 years. Women with surgically induced menopause reported earlier menopausal age ($p< 0.001$) and more hormone replacement therapy (HRT, p=0.02) use than natural menopause.

In our primary analysis of 87 women followed longitudinally through their menopausal transition, we detected an inflection point in EDSS changes at menopause. [AM1] The estimated increase in the average yearly change was 0.14 units per year (95% CI: 0.047, 0.23, p=0.003 in this linear spline mixed effects model. Similar results were noted in both natural and surgical menopause.

In a secondary model examining 275 women whose entire follow up was either during their pre- or post-menopausal period, there was a significant worsening of EDSS after menopause, adjusting for age and disease duration; the difference between the groups was 0.088 EDSS points per year (95% CI: 0.021, 0.16; p=0.011).

No differences in relapse rate and no effects of HRT use were noted, but numbers for these exploratory analyses were small.

Conclusions: We observed a worsening of MS severity after menopause, consistent with our prior reports from an online research platform. Larger studies are required to explore a potential role for HRT.

P841

Risk of neuromyelitis optica spectrum disorder relapse associated with pregnancy on Japanese patients

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Background: There are a few reports describing the influence of pregnancy on the clinical course of neuromyelitis optica spectrum disorder (NMOSD). Previously reports reviewed the annualized relapse rate (ARR) was calculated for each trimester during pregnancy and the postpartum period. The highest ARR was during the first trimester Postpartum.

Objectives: To identify factors related to the risk of relapse during pregnancy of Japanese NMOSD patients.

Methods: In pregnant NMOSD patients who developed the disease before pregnancy, those with and without relapsing during the year before pregnancy were compared. We analyzed the ARR for disease onset and/or relapse associated with pregnancy and investigated the year before pregnancy (BP), each trimester during pregnancy (1st, DP1; 2nd, DP2; 3rd, DP3) and each trimester postpartum (1st, PP1; 2nd, PP2; 3rd, PP3; 4th PP4).

Results: All 139 Japanese women with NMOSD tested positive for anti-AQP4 antibody. Total fifty-six pregnancies in 47 women were included in the study. Twenty patients relapsed with developed pregnancy-associated NMOSD. The highest ARR was 1.80, during the PP1. Eleven of 47 pregnant patients who developed the disease before pregnancy, those with recurrence during the year before pregnancy were significantly more likely have pregnancy-associated relapse than those of 2 patients without recurrence during the year before pregnancy ($P < 0.05$). These cases suggest that treatment with a suitable amount of corticosteroids and immunosuppressants might be useful for stabilizing patients with NMOSD during pregnancy and the postpartum period. We measured anti-AQP4 antibody titers in two babies, and found that the titers became negative within a few months after birth. These babies developed normally without neurological deficits. and the maternal anti-AQP4 antibody might not directly damage the fetal central nervous system.

Conclusions: In pregnant NMOSD patients, disease activity should be stabilized to prevent pregnancy-associated relapse, and immunosuppressant treatment should be considered. The two babies with anti-AQP4 antibody positive, developed normally. The maternal anti-AQP4 antibody might not directly damage the fetal central nervous system, indicating that it is unnecessary to provide special treatment for babies born of mothers with positive anti-AQP4 antibody titers. And this study show that the ARR of NMOSD increased significantly during PP1 in Japanese women, as previously reported.

P842

Pregnancy outcomes in the alemtuzumab MS clinical development program

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Background: Alemtuzumab, approved in over 30 countries for treatment of relapsing-remitting multiple sclerosis (RRMS), showed superior efficacy over subcutaneous interferon beta-1a (SC IFNB-1a) and manageable safety in 3 randomized studies. Alemtuzumab is usually eliminated from the body by 1 month after administration; however, animal data suggest potential transfer to fetus during gestation and postpartum in breast milk. Label guidelines recommend contraception for women of childbearing potential for ≥4 months after treatment.

Objectives: To report pregnancy outcomes in alemtuzumab-treated patients in the clinical development program and serious adverse events (SAEs) in their fetuses/infants.

Methods: In 36-month, phase 2 CAMMS223 (NCT00050778) and 24-month, phase 3 CARE-MS I (NCT00530348) and CARE-MS II (NCT00548405) core studies, RRMS patients received alemtuzumab intravenously 12 or 24 mg/day (only 12 mg in CARE-MS I) on 5 consecutive days at baseline and on 3 consecutive days 12 months (and 24 months in CAMMS223 subgroup) later, or SC IFNB-1a 44 μg 3 times weekly. Follow-up continued in an extension (NCT00930553) with as-needed alemtuzumab re-treatment (first-time treatment for former SC IFNB-1a patients). Contraception was required, but pregnancies did occur.

Patients becoming pregnant could remain on study with alemtuzumab suspended. Information on pregnancy outcomes and SAEs in fetuses/infants was collected.
Results: As of October 17, 2013 (N=1486), 139 pregnancies occurred in 104 patients, resulting in 67 live births, 14 elective abortions, 24 spontaneous abortions, 1 stillbirth, 4 unknown outcomes, and 29 ongoing. Most pregnancies (133/139) occurred >4 months after last alemtuzumab dose. Spontaneous abortion rate was age-related (overall: 17% of pregnancies; < 35 y: 12%; ≥35 y: 32%). SAEs occurred in 12 fetuses/infants; each SAE was a unique event type. Grade 4 thyrotoxic crisis occurred in a 21-day-old, full-term infant whose mother developed Basedow's disease during pregnancy; infant recovered after treatment with iodine, methylprednisolone, and propylthiouracil. Two elective abortions followed fetal defect SAEs (hypoplastic left heart syndrome/cystic hygroma and anembryonic gestation).
Conclusions: Spontaneous abortion risk in alemtuzumab-treated patients was comparable to that in the general population. No trends emerged in types of fetus/infant SAEs. Women of childbearing potential should continue contraception for 4 months after alemtuzumab. A new pregnancy registry will collect prospective data.

P843

Pregnancy outcomes and disease activity after exposure to natalizumab in patients with multiple sclerosis

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Background: Little is known on pregnancy outcomes and disease activity after in utero exposure to natalizuamb in multiple sclerosis (MS) patients.
Objectives: To assess the impact of natalizumab on pregnancy outcomes in an Italian cohort of MS patients.
Methods: We recruited natalizumab exposed (NE) pregnancies in MS patients prospectively followed-up in Italian MS Centres, in the period 2010-2013. Exposure to natalizumab was defined as suspension of the drug < three months prior to conception. Pregnancy outcomes and clinical relapses during pregnancy were compared with data from the Italian pregnancy dataset (Amato et al. 2008). Data on pregnancy outcomes for the Italian population were also available. All the patients were administered a structured interview which gathered detailed information on pregnancy course and outcomes, as well as on possible confounders. Group comparisons were assessed through the chi^2 test or the analysis of variance with Bonferroni correction for multiple comparisons, when appropriate.
Results: So far 28 pregnancies were recruited. Pregnancies resulted in 20 live births, six spontaneous abortions and two voluntary abortions (one due to Down Syndrome). Proportion of spontaneous abortion in NE pregnancies (21.4%) was higher than that previously observed in interferon-beta exposed (IFNBE) pregnancies (8%; p=0.015), although it fell within the upper limit of that expected in the general population. Proportion of pre-term deliveries (29.4%), mean birth-weight (2904gr) and birth-length (48.9cm) were comparable to those of IFNBE pregnancies. The occurrence of relapses during the NE pregnancies (25%) was higher than that observed in the IFNBE patients (13.3%; p=0.01).
Conclusions: Our findings do not show any major safety issues due to pregnancy exposure to natalizumab in terms of spontaneous abortion and other outcomes. Detailed analyses of relapse-rate before, during and after pregnancy are ongoing.

P844

Pregnancy issues in multiple sclerosis patients: preliminary results from a multicenter retrospective cohort study

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Background: The frequency of childlessness in Multiple Sclerosis (MS) women may be higher than in the general population. This is not necessarily explained by infertility; rather, it could be caused by a reduced propensity to maternity due to a number of reasons including concerns about disability and postponement of maternity while on immunomodulatory drugs. MS may also affect the delivery procedure, with a higher number of planned caesarean deliveries (CD) in MS women, and breastfeeding, since women may choose not to breastfeed so they can resume their preventive therapies.
Objectives: To assess whether: 1) there is a higher frequency of childlessness in MS women and to examine its possible causes, 2) there is a higher proportion of CD and 3) the frequency of breastfeeding is reduced in MS women.
Methods: Female MS patients aged >43 years attending seven MS Centers in Emilia-Romagna, Italy, and control subjects (CS) aged >45 years enrolled at the Breast Cancer Screening Center of Modena, were asked to fill-out a self-administered anonymous questionnaire exploring pregnancy issues.
Results: Of 219 patients and 201 CS, fifty MS women (23%) were childless, compared to 21 controls (10%) (p=0.0007). The lack of a stable relationship was more frequent in the MS cohort (43; 19% vs 25; 12%) (p=0.048), though after correction for relationship status and thyroid dysfunction, MS diagnosis still determined two-fold higher odds of childlessness (OR: 2.4; p=0.005). We found similar rates of miscarriage in MS (46; 26%) compared

to CS (39; 23%) but a higher rate of elective abortions (EA) (36; 21% vs 19; 11%) (p=0.01). There were no significant differences in the proportion of pregnancies achieved by assisted reproductive technology (4; 2.5% vs 2: 1,1%). The main reported reasons for childlessness were similar in MS patients and CS: "no stable relationship" (37% vs 38%), followed by "no desire to have children (27% vs 28%) and "infertility" (16% vs 14%). CDs were more frequent in MS (46; 29% vs 34; 17%) (p=0.0229) while there were no differences in the percentage of women who did not breast-feed (41; 24% vs 33; 18%).

Conclusions: MS is associated with higher frequencies of childlessness, elective abortions and CD while it does not affect the frequency of breast-feeding. Our results indicate that childlessness in MS may in part be due to the lack of a stable relationship and to higher rates of elective abortion rather than to infertility issues.

P845

Sex life of women with multiple sclerosis: qualitative study

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Background: Spasticity, tremor, muscle weakness, sensorial disorders, depression, cognitive problems, bladder and bowel dysfunction and sexual dysfunction are commonly seen in multiple sclerosis (MS) and these conditions significantly affect the quality of life of patients. Sexual functioning of a woman is a complex process affected by psychological, physiological and personal factors. Sexual function of a woman is affected by diseases and disabilities, reduces the quality of life and may affect the family relations as well.

Objectives: To define the thoughts, emotions, experiences and perceptions of women with MS and to determine how women are affected physically and emotionally by MS, the study was performed using qualitative research methods.

Methods: In the study performed via in-depth interviews, a semi-structured questionnaire was used. 24 women aged ≥ 18 years were invited to the study, and 3 of them were excluded as they stated that they don´t want to talk about their sex life. An approval was obtained from the Ethical Board of the Faculty of Health Sciences, Selcuk University. All participants were informed about the fact that the interview would be taped, transcribed, and their names would be kept confidential before collecting data. Transcribed interviews were qualitatively and thematically analysed using content data analysis to code and categorize emerging themes.

Results: Three main topics were established. The first one was "uncertainty". Women had one of the following complaints: numbness in hand and feet, loss of sensation, balance or visual problems. Women were worried about the effects of MS on physical health and degree of disease progression. Their psychological state was negatively affected by this condition. The second topic was "changes in sex life". Particularly, lack of sexual desire, lack of sexual stimulation due to insensitivity, unable to reach orgasm and decreased sexual satisfaction were noted. In addition, two women stated that they experience urinary incontinence during the intercourse. The third topic was "feeling of inadequacy". Feeling of uselessness was dominant amongst women due to problems with married life and their role as a woman.

Conclusions: Women may experience physical and emotional challenges affecting sexual life negatively after MS, but cannot discuss their problems frankly with healthcare providers. So, healthcare providers should evaluate MS patients meticulously as to sexual life and direct such patients to appropriate health centres.

P846

Pregnancy outcomes for female patients and partners of male patients in the teriflunomide clinical development program

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Background: Teriflunomide is a once-daily oral immunomodulator approved for relapsing-remitting multiple sclerosis (MS). Evidence from animal studies suggests a potential risk of teratogenicity associated with teriflunomide. During the teriflunomide clinical studies, pregnancy was therefore excluded before starting treatment, and use of reliable contraception was required for women of childbearing potential and men with partners of childbearing potential.

Objectives: To summarize pregnancy outcomes for female patients and partners of male patients enrolled in the phase 2 and 3 studies in the teriflunomide clinical development program.

Methods: Patients received teriflunomide 14 mg or 7 mg, interferon beta, or placebo. Despite the requirement for use of reliable contraception, pregnancies were reported. Upon learning of pregnancy, female patients were instructed to discontinue treatment and undergo an accelerated elimination procedure. Pregnancy outcome information was collected using a Drug Exposure Via Parent form (data cut-off Oct 2013).

Results: A total of 83 pregnancies in female patients were reported in the teriflunomide clinical trial program; of these, 70 occurred in women treated with teriflunomide. There were 26 live births in female teriflunomide-treated patients, and all newborns were healthy, with no structural or functional abnormalities. The known rate of spontaneous abortion in teriflunomide-treated female patients was within the range reported for women without MS; median birth weight and gestational age at birth for newborns of teriflunomide-treated female patients were within the ranges reported for newborns born to mothers without MS.

An additional 22 pregnancies were reported in the partners of male patients, 19 of which occurred in partners of male patients treated with teriflunomide. Sixteen live births were reported for partners of teriflunomide-treated men; all newborns were healthy and free of structural or functional abnormalities.

Conclusions: Data from the teriflunomide clinical development program have shown no evidence of human teratogenicity. This is consistent with findings from the pregnancy registry and extensive post-marketing studies of patients treated with leflunomide. Pregnancy outcomes among female patients who received teriflunomide and partners of teriflunomide-treated male patients were consistent with those in the non-MS population. Global teriflunomide pregnancy registries are presently collecting prospective data from pregnancies in the post-marketing setting.

P847

Exclusive breastfeeding and postpartum multiple sclerosis relapses

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Background: Women with multiple sclerosis (MS) experience an elevated relapse risk after birth. The effect of exclusive breastfeeding on the postpartum relapse risk is unclear.
Objectives: To determine the effect of exclusive breastfeeding on the postpartum relapse risk and to investigate the effect of introducing supplemental feedings on the postpartum relapse risk.
Methods: Pregnant women with MS were followed prospectively (2008-2012) one year postpartum in a nationwide German MS and pregnancy registry.

Cox regression was used to analyze if breastfeeding exclusively for at least 2 months influenced the time to first relapse in the first 6 or 12 months postpartum after adjusting for confounders of disease activity.

Results: Of 201 women, 120 intended to breastfed exclusively for at least 2 months (60%) and 81 (40%) breastfed only some (n=42), or did not breastfed at all (n=39). 29 (24%) women who intended to breastfed exclusively for at least 2 month had a relapse in the first 6 months postpartum compared to 31 (38%) who did not breastfed exclusively (crude HR 1.8 (ITT population), 95% CI 1.09-2.99; p=0.02). After adjustment for age and disease activity before and during pregnancy the adjusted HR changed to 1.7 (95% CI: 1.02- 2.85; p=0.04). The time to first postpartum relapse did after the introduction of supplemental feedings not differ between women who previously breastfed exclusively and those who did not (p=0.36).
Conclusions: Our study provides further evidence that the women with MS who choose to breastfeed exclusively should be supported as this does not increase the risk of postpartum relapses.

P848

Ovarian reserve and sex hormone levels in multiple sclerosis patients

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Background: Hormonal alterations such as higher levels of prolactin and hyperandrogenism have been suggested as one of the possible causes associated with impairment of the fertility in women with multiple sclerosis (MS). However, the ovarian reserve, a measure of the fertility potential, has not been systematically studied in MS patients followed in clinical practice conditions.
Objectives: The aim of our cross-sectional study was to evaluate the ovarian reserve and the integrity of the hypothalamic-pituitary-gonadal (HPG) axis in a Spanish cohort of fertile women with MS under usual disease modifying treatment (DMT) and to compare with a group of healthy volunteers.
Methods: 25 female MS patients and 25 age-matched healthy controls were included in the study. In their early follicular phase of menstruation (2^{nd}-5^{th}day) each participant underwent to a transvaginal ultrasound to evaluate ovarian volume and antral follicle count (AFC) and the same day, a determination of sex hormones and antimüllerian hormone (AMH) levels. Patients were excluded if they had a history of being treated with gonadotoxic treatments and/or had a history of any known endocrine disease or infertility (e.g., polycystic ovary syndrome). MS patients were free from steroid treatment at least for 3 months.
Results: Oral contraception consumption was similar between patients and volunteers (28% vs 24%, p=0.747). MS patients showed significantly higher concentrations of prolactin, total and free testosterone and a significantly lower serum concentration of 17-β-estradiol. MS patients exhibited higher levels of inhibine A and lower levels of inhibine B. Mean ovarian volume, total AFC and AMH did not show significant differences between both groups. In patients, an annualized relapse rate (ARR) > 0.5 was significantly associated with lower levels of AMH, mean ovarian volume and total AFC. Patients under treatment with high dose of interferon beta (IFNβ) had a lower mean ovarian volume and reduced levels of estrogens than those treated with low dose or other DMT, but with similar levels of AMH.
Conclusions: MS patients in their childbearing age do not have compromised ovarian reserve but they exhibit hormonal alterations usually associated with impaired fertility: higher levels of prolactin and androgens with reduced levels of estrogens. If the clinical activity of the disease and/or the use of high-dose IFNβ influence the ovarian reserve deserves to be studied in a large cohort.

P849

Sexual dysfunction and incidence of depression in MS patients

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Background: Multiple sclerosis (MS) is one of the most frequent diseases of the central nervous system and usually occurs at the age when people would be expected to be in the prime of their sexual lives.
Objectives: In everyday practice sexual dysfunction is underestimated because clinicians mostly concentrate on the classical neurological deficits and often overlook symptoms that can seriously affect the quality of life.
Methods: We included in our study 97 patients with relapse remitting multiple sclerosis from our MS register, 22 men and 75 women with established diagnosis of relapse remitting multiple sclerosis according to McDonald criteria. Patients completed the questionnaires (Sexual satisfaction scale-SSS, Beck Depression Scale-BDS), and underwent neurological assessment (Expanded Disability Status Scale- EDSS).

Results: All patients were in the group with EDSS 2 to 4 points (mobile patients). There was no statistically significant difference in BDS and SSS values according to EDSS score. Correlation coefficients were calculated (BDS and SSS) for men (p=0, 42) and for women (p=0, 44), positive correlation was found. There was no statistically significant difference in BDS and SSS values according to gender, disease duration or immunomodulatory therapy.
Conclusions: In our group of patients despite low EDSS score (fully ambulatory without aid, self sufficient patients) we found positive correlation between sexual dysfunction and depression showing that even in such patients quality of life can be decreased. Sexual dysfunction and depression are mostly underrecognized from neurologists because they are not part of routine testing; therefore some additional questionnaires should be used in evaluation in MS patients, even with low EDSS score in order to improve their quality of life.

P850

Prevalence of sexual dysfunction in multiple sclerosis

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Background: Multiple sclerosis (MS) is a chronic inflammatory disease of central nervous system in which demyelination and axonal loss occurs in brain and spinal cord and affects 2.5 million people worldwide. These patients suffer from various comorbidities even with treatments that change the natural evolution of the disease, it is important an approach integrated. Currently sexuality is recognized as one of the major determinants of quality of life in patients with chronic diseases and sexual satisfaction is considered a relevant variable in social, family and emotional relationships.

It was observed that the sexual dysfunction (SD) affects 82 to 91% of men and 59 to 72% of women with MS, even on light and moderate cases compared to 13% in healthy controls. Also factors such as depression, problems with self-esteem and overall impact on quality of life interfere with sexual health of these patients.
Objectives: Assess sexual dysfunction by The Multiple Sclerosis Intimicy and Sexuality Questionnaire-19 (MSISQ-19), translated to Portuguese in patients with MS.
Methods: Were evaluated by MSISQ-19 translated to Portuguese and validity (MSISQ-19-BR) 204 individuals, 70 of the control group and 134 patients with MS.
Results: The incidence of sexual dysfunction in our study was 72% in women and 54% men.

The MSISQ-19-BR scale is reproducible, reliable and valid for the Brazilian population and may be used as a tool for assessing the impact of sexual dysfunction in patients with MS.Most research suggests that MS has a negative impact on relationships and the satisfaction with romantic relationship is lower in MS patients than in general population, with many partners showing significant stress due illness. Previous studies suggested that some interventions can minimize the impact of DS. It is important to consider, however, that despite the high prevalence of sexual dysfunction in MS, we should not neglect other conditions that may also be causing difficulties concerning sexuality such as hypertension, diabetes, continence problems, psychiatric disorders and effect of drugs.
Conclusions: This study confirms the need to approach the sexuality theme in MS patients for treatment. The use of a standardized tool may facilitate the initial approach and introduce it naturally in clinical practice. The MSISQ-19-BR is a valuable tool to help physicians and patients in this matter so delicate and allow for a global treatment, to reduce the impact of MS on quality of life.

P851

Evolving trends in reproductive practices among women with multiple sclerosis: insights from an online patient powered research network

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Background: Reproductive hormones impact the onset and course of multiple sclerosis (MS). Recent interest in reproductive health in MS, as well as broader changes in women's health, such as the results of the Women's Health Initiative, may have led to altered reproductive practices in MS patients.
Objectives: To explore trends in reported reproductive characteristics in women from a large online research platform (PatientsLikeMe.com, PLM).
Methods: All adult female PLM members with MS, with at least 3 logins in the prior 90 days, received an email invitation to complete an online reproductive survey. Cohorts were divided by decade of birth, and by age at MS diagnosis.
Results: The 513 subjects returning the survey within 2 weeks were: 91% white, 80% with some college education, with mean age of 48.6 years. Mean age at symptom onset was 34 and 74% had relapsing remitting MS. Respondents were similar in all characteristics ($p>0.2$) to all survey recipients (n=1301), except for age (48.6 vs 46.3, $p< 0.001$).

From the women born in the 1940s through women born in the 1980s, age at menarche (12.7), ever-use of hormonal contraceptives (HC, 86%), mean age of first birth (24) and total number of births (2.1) remained stable over time. The rates of women limiting their pregnancy due to infertility and lack of desire to have children remained stable, but a trend of increasing concerns regarding teratogenicity of disease modifying therapy (DMT) was noted in later decades. Declining use of hormone replacement therapy (HRT) was also noted over time, with less use of HRT in postmenopausal women born in the 1960s compared with those born in the 1940s (38% vs 59%, p=0.06); only 3.1% vs. 31.2% reported HRT use within 3 years of menopause, p=0.028.

Women diagnosed with MS earlier in life (ages 15-25) on average breastfed fewer months per birth compared to those diagnosed at a later age (e.g. ages 45 - 55, 1.8 ± 0.6 months vs 5.6 ± 0.8 months respectively, p=0.045).
Conclusions: In this large survey we identified some reproductive practices in women with MS that appeared stable over the past few decades, such as parity and HC use. However, we also noted emerging patient-reported concerns regarding teratogenic effects of DMTs, as well as declining rates of HRT and breastfeeding

duration, that may reflect wider social changes as well as evolving clinical practices in MS and women´s health. Further study is required to confirm these findings in clinical cohorts and assess interactions between reproductive practices and disease course.

P852

The role of breastfeeding in multiple sclerosis patients

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Background: Multiple sclerosis (MS) is more common in women of child-bearing age. Pregnancy and breastfeeding (BF) must be planned carefully because although disease activity decreases during the pregnancy, there is a higher risk of relapse after delivery. Traditionally, breastfeeding was not recommended, but this lately become a controversial point.
Objectives: Our aim is to know the course of the disease in MS patients depending on the type of feeding.
Methods: We observed all pregnancies in relapsing-remitting multiple sclerosis (RRMS) women at Virgen Macarena University Hospital from 2004 to 2014, and interviewed them by phone call about pregnancy and feeding after delivery. We divided them in two groups to compare: breastfeeding (BG) vs non breastfeeding (NBG). The collected data included age at pregnancy, EDSS, duration of the disease, previous treatment and time to restart treatment after the delivery, relapse before, during the pregnancy and feeding and after the feeding. We also collect time to progresión of the disease (more than 1 point in the EDSS).
Results: We identified 130 pregnancies in that period but only 79 completed the interview. Mean age at pregnancy was 31.52 years-old. 67.1% of our patients chose breastfeeding instead of artificial feeding. Results also showed that patients who chose breastfeeding were those with a lower relapse rate. Patients who chose artificial feeding restarted their treatment following the delivery with a mean wait period of 4.96 days. The mean length of follow up was 4.89 years. Baselines, pregnancy and feeding characteristics showed no statistically significant differences between the two groups ($p>0.05$), except for the time to restart treatment ($p=0.03$). We also found no differences between groups regarding EDSS, duration of disease, the annualized rate of relapses before or during pregnancy and feeding and time to progression ($p>0.05$).
Conclusions: The role of breastfeeding in RRMS patients is controversial because of the higher risk of relapse after delivery. Our study shows that the patients with lower rates of relapse were those who choose natural breastfeeding. The patients with higher relapse rates were those who chose artificial feeding and restarted the treatment sooner. Despite this, however, all patients presented the same course of disease regardless of the type of feeding. For this reason, we think that breastfeeding could play a protective role, at least, in patients with low relapse rates.

P853

Examining the effectiveness of pregnancy counseling in women with multiple sclerosis of childbearing potential

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Background: MS is twice as prevalent in females as males and typically has an onset between the ages of 18-45 which is commonly known to be of child bearing age. Unplanned pregnancies account for half of all pregnancies in the United States and can pose potential risks. Women typically are advised to discontinue interferon-beta therapy before trying to conceive; however, if a neurologist does not counsel or discuss such issues with a MS patient of childbearing potential, unplanned pregnancy can occur and risks related to exposure, although remain unclear, still can occur for the women and her child. By proactively counseling and discussing issues with such patients from the start of their diagnosis, can not only prevent unplanned pregnancies but will also lessen risks. Through proper counseling, physician/patient relations can be enhanced as well as increase the MS patient's knowledge of pregnancy risks and options.
Objectives: To demonstrate the effectiveness of counseling and discussion of pregnancy risks during clinic visits through planned vs unplanned pregnancy outcomes.
Methods: Over thirty female MS patients of child bearing potential (18-45) who have been a patient of record at Phoenix Neurological Associates over the last two years were identified and a retrospective chart review was performed to determine which patients received counseling, how often and what the incidence of planned, unplanned or no pregnancy outcomes were.
Results: Retrospectively reported planned pregnancy rates and no pregnancy rates were higher than unplanned pregnancy in patients who were counseled continuously throughout the course of DMT treatment.
Conclusions: Early data suggests that proper, consistent pregnancy counseling can lower the risk of unplanned pregnancy. Exact numbers and incidence rates will be available and provided by the time of the meeting in September.

P854

Sexual dysfunctions in female patients with relapsing-remitting multiple sclerosis

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Background: Sexual dysfunction is a common symptom in Multiple Sclerosis (MS), even among young patients. But still, sexuality is a taboo for patients as well as doctors. Especially regarding women there is a lack of data on sexual functioning.
Objectives: The aim of the study was to evaluate the frequency and characteristics of sexual dysfunctions in female MS patients with relapsing-remitting MS compared to otherwise healthy female headache patients.
Methods: Consecutive female patients of the outpatient MS clinic and of the outpatient Headache clinic were invited to participate in the study. Sexual functioning was evaluated by the Multiple Sclerosis Intimacy and Sexuality Questionnaire (MSISQ-19; adapted for headache patients), a self-rating questionnaire. It divides sexual dysfunctions in primary, secondary, and tertiary sexual dysfunctions, i.e. affection of genitals and sexual sensations by MS, impairments caused by MS affecting sexuality indirectly, and impact of MS on psychosocial and emotional aspects of sexuality, respectively. We evaluated depression (Beck

Depression Inventory) and quality of life by the Short-form-36 questionnaire (SF-36).
Results: We screened 82 patients, 42 MS patients (34.7 ± 6.7 years, median EDSS 1.8) and 31 patients with primary headaches (32 ± 8.7 years) were included. Of the 42 MS patients 35.7 % had any form of sexual dysfunction, with primary sexual dysfunction being the most common form (40 %), and 33.3 % having tertiary sexual dysfunction. Of the 31 headache patients 22.6 % had any form of sexual dysfunction, with primary and tertiary sexual dysfunctions being the most common forms. The frequency of sexual dysfunctions did not differ significantly between MS and headache patients, but headache patients more often had a combination of primary, secondary and tertiary sexual dysfunction. Depression and the psychological and physical scale of the SF-36 correlated significantly with sexual dysfunction in both groups.
Conclusions: A considerable number of females with MS suffers from sexual dysfunctions. This is associated with depressive symptoms and reduced quality of life. Doctors should pay attention to this and integrate it into the therapeutic approach.

P855

Fecundity in women with multiple sclerosis: an observational retrospective mono-centric study

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Background: Multiple sclerosis (MS) is a frequent neurological disease mostly affecting women of childbearing age. Many questions arise when counseling MS patients on the effects of MS on pregnancy and vice-versa. Reassurance can often be given regarding contraception, pregnancy management, pregnancy outcome and the risk of the child developing MS. However, evidence lacks concerning impact of MS and disease modifying treatment (DMT) on the time to pregnancy. A better knowledge of these could drive advice and therapeutic strategy during time to conception.
Objectives: The aim of this study is to assess time to pregnancy, in a French cohort of MS women.
Methods: This observational retrospective study, included consecutively 115 women with Multiple Sclerosis (Polman et al 2011) admitted in the Pitié-Salpêtrière MS clinic between December 2013 and January 2014. Information was given before inclusion and self-questionnaires were completed during the daily-hospitalization.
Results: Among the 115 female patients included : mean age was 45.39 years [21 to 78], 56.5% had a relapsing remitting form, 34.8% a secondary progressive form, and 8.7% a primary progressive form. Mean disease duration was 11.97 years [1 to 37]. Mean EDSS score at inclusion was 4.18 [0 to 7] and average MSS score was 5.05 [0.24 to 9.08]. Self-questionnaires showed that 95 women had a parental project whereas 20 women (17.4% of the group) have chosen to stay childless, by choice or by recommendation of their neurologist. Among the 95 women with a parental project, 11 have never been spontaneously pregnant. The average time to spontaneous pregnancy reported for 124 out of 216 pregnancies (92 time to pregnancies reported "not known") was 8.57 months (SD = 18.22) when pregnancy occurred before MS onset, and 7.53 months (SD = 10.45) after MS onset (p=0.69).
Conclusions: This study reveals that time to pregnancy is similar before and after MS onset, and not different from the French general population. According to the time to spontaneous pregnancy, it seems reasonable to maintain IFN and GA until conception (and even during pregnancy). Safety of others DMT remains partially unknown and need to be further evaluated.

P856

Progestin content of oral contraceptives and the risk of multiple sclerosis

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Background: The types of progestins used in oral contraceptives have different biological effects on androgen, gluco-and mineral corticoid receptors and vary in their anti-and pro-estrogenic effects. In addition, higher doses of estrogen may have differential effects.
Objectives: To determine whether the risk of MS varies by the type of progestin or amount of ethinyl estradiol contained in combined oral contraceptives (COC).
Methods: We conducted a population-based nested case-control study for the membership of Kaiser Permanente Southern California (KPSC). We identified females ages 14-48 years with incident MS or clinically isolated syndrome (CIS) between 2008 and 2011. Ten controls per case were matched on age, race/ethnicity and membership characteristics. Oral contraceptive use up to ten years prior to symptom onset/index date was obtained from the complete electronic health record. Data were analyzed using conditional logistic regression adjusted for age, smoking, obesity, live births and abortions.
Results: We identified 400 women with incident MS/CIS and 3992 matched controls. The most common form of hormonal contraceptive was COCs used by 40% of cases and 31.9% of controls. The majority (70.6%) of women who took COCs, used more than one type. The most common progestins in COCs were norethindrone and levonorgestrel . Most COCs used contained between 0.03 and 0.049mg of ethinyl estradiol. Ever having used any COC in the 10 years prior to symptom onset was associated with an increased risk of MS/CIS (OR 1.44, 95%CI 1.14-1.82, p=0.002). This increased risk was found regardless of the type of progestin or estrogen dose used most recently prior to symptom onset/index date.
Conclusions: We did not detect a difference in the association between OC use and MS/CIS based on the progestin content or estrogen dose in this contemporary cohort. This may be due to the relatively large number of multiple progestin-type users and the restricted range of estrogen content (i.e. few high or low dose users). Larger studies and meta-analyses with more variation in types of COCs used are needed to confirm these findings.

Risk management for disease therapies

P857

Maraviroc and JC - virus associated immune reconstitution inflammatory syndrome

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Background: In patients with Progressive Multifocal Leukoencephalopathy secondary to Natalizumab (PML-NZB) immune reconstitution inflammatory syndrome (IRIS) has been regarded as nearly universal. Currently there are no evidence-based treatments for IRIS. Glucocorticoids are commonly used, but this may inhibit JC virus clearance and worsen underlying PML. Chemokine receptor 5-positive (CCR5+) T cells have been indirectly implicated in IRIS pathophysiology. Maraviroc, a CCR5 antagonist approved for the treatment of human immunodeficiency virus (HIV) infection, may blunt IRIS in PML-NTZ. To our knowledge, there is only a single published case report of effective use of Maraviroc in this clinical setting.
Objectives: To report on two cases of Maraviroc-treated disseminated PML-NTZ from NYU MS Center in which no clinically or radiologically significant IRIS was observed over 3-15 months of follow up.
Methods: Case series.
Results: The first patient is 49 year old woman with relapsing remitting multiple sclerosis (RRMS) on NZB for 3 years and 6months who presented for a second opinion with 3 months of worsening numbness, ataxia, and dysarthria. Brain MRI suggested wide -spread PML and spinal fluid analysis confirmed the diagnosis, with 390 JCV copies/mL detected (Focus Diagnostics). Maraviroc 300mg twice a day was initiated after plasmapheresis (PLEX). Patient remained free of clinically significantl IRIS. Patient's clinical status and MRI stabilized over 6 month and Maraviroc was tapered. Patient was discharged home in stable condition; her EDSS is 6.5. Second patient is 47 year old woman with RRMS on NZB for 6 years and 9 months who developed progressive right lower extremity weakness. Brain MRI revealed a new T2 lesion in the left motor cortex and spinal fluid analysis confirmed the diagnosis, with 18 JCV copies/mL detected (NIH laboratory). Maraviroc was initiated after PLEX. No significant radiological or clinical IRIS has yet to be seen.
Conclusions: Development of IRIS is considered near universal in PML-NZB, yet we did not observe it in our two Maraviroc treated patients. Furthermore, the drug was well tolerated by our patients and has been shown to have good safety profile. We advocate for a clinical trial of this drug in NZB - associated PML. However, until a clinical trial can be organized, it is important to increase the body of literature that documents clinicians' experience with the use of Maraviroc to treat the JC virus - associated IRIS.

P858

Fifteen non-fatal outcomes in natalizumab-associated PML/IRIS: the effects of early diagnosis and evolving novel therapeutic approaches

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Background: An often disabling or fatal opportunistic brain infection caused by the John Cunningham virus (JCV), progressive multifocal leukoencephalopathy (PML) occurs in immune-compromised states, including treatment of multiple sclerosis (MS) with natalizumab.

We treated fifteen patients with MS, five from Rush and ten community-referred, with PML occurring after 14-70 months of natalizumab treatments. Two had prior immunosuppressants.
Objectives: To present an all-surviving cohort of 15 patients with natalizumab-associated progressive multifocal leukoencephalopathy (PML) in whom management strategies evolved to a core of early detection by MRI, immunostimulation with filgrastim, and cautious administration of corticoids.
Methods: To minimize injury, we strive for preclinical diagnosis of PML by obtaining tri-annual cranial MRIs and accelerated JCV elimination via immunostimulation with filgrastim, which amplifies lymphocyte production and endothelial adhesion. The initial variably added plasma exchange, oral mefloquin, mirtazapine, and maraviroc evolved as inessential. Anticonvulsants and unaggressive corticoids are administered at the onset of PML and IRIS, respectively.
Results: There were no fatalities. MRI discovered asymptomatic PML in 4 patients. Three patients had initially negative CSF JCV qPCR. Six of 15 recovered to baseline and 2 with residual deficits to high functioning. The remaining 7 patients' outcomes were significantly worse than pre-PML.
Conclusions: Our multipronged protocol consisting of early PML detection followed by empirical management probably prevented fatal outcomes, even in patients with delayed diagnosis. Early and frequent MRIs and administration of filgrastim likely contributed to favorable resolution of PML/IRIS in 8 of 15 patients. This approach warrants further exploration.

P859

Managing the switch from natalizumab: the washout is hazardous and should be avoided

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Background: Natalizumab-treated relapsing remitting multiple sclerosis (RRMS) patients may face a high risk of progressive multifocal leukoencephalopathy (PML). Actually, in RRMS patients treated for more then 24 months and having a JCV-antibody index >1.5, the risk of PML is about 1:125. Thus, natalizumab discontinuation is highly suggested in these patients and a washout period of three months is generally adopted before starting a new second line therapy. However, MS reactivation has been observed, especially after 3-4 months after the last dose of natalizumab, raising concern that the washout may expose patients to severe relapses.
Objectives: To evaluate, in a large cohort of MS patients, the risk and timing of disease reactivation after natalizumab discontinuation.
Methods: In the period March 2011-September 2013, natalizumab was stopped in 66 RRMS patients. Reasons for

discontinuation were: pregnancy in 6 patients, high PML risk in 60. Fifty-eight patients were switched to other therapies after a three month-washout period: 50 switched to fingolimod, 6 to interferon beta (IFNb), 2 to cyclophosphamide. Two patients remained therapy free. All the patients were followed for at least six months with monthly clinical examination and brain MRI performed before starting the new therapy and in case of relapse.

Results: Between month 4 and 6 after natalizumab break, clinical and/or neuroimaging evidence of disease reactivation was observed in 100% IFNb-treated, 44% (22/50) fingolimod-treated and 100% cyclophosphamide-treated patients. Of the 6 females that planned pregnancy, 3 had a mild disease reactivation between months 4 and 5, while three remained disease free. Both untreated patients relapsed. All together, 35/66 (53%) patients had disease reactivation.

Conclusions: Our observations strongly confirm that, independently from the subsequent therapeutic options, a three month-washout after natalizumab is hazardous since it exposes the patients to a >50% risk of disease reactivation between month 4 and 6. Thus, any washout, especially in patients having had very active disease prior to natalizumab, should be kept to a minimum or even avoided. Worth of interest are the six patients that decided to plan the pregnancy: three patients remained disease-free and three had mild relapses, than all remained asymptomatic throughout the pregnancy, confirming the protective effect of pregnancy even in patients with particularly active MS.

P860

MS FIRST - a longitudinal, prospective, comparative drug safety module for use in MS clinical practice

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Background: The comparative long term safety profile of disease-modifying therapy (DMT) in MS treatment is unknown. Although adverse events such as infection risk and liver function test (LFT) abnormalities are known possible side effects, incidence and trends over time in serious adverse events (SAEs) are less well characterised in real world clinical practice. MS FIRST, a sub-study of the MSBase registry, is an Australian multi-centre study to implement a user-friendly safety module to track safety outcomes in MS patients over the long term.

Objectives: The primary objective of this study is to track and compare the incidence of safety outcomes in MS patients who either receive DMT (including fingolimod according to approved label) or who are on no treatment. The secondary objective is to characterize the longitudinal distribution of lymphocyte count and LFT abnormalities in fingolimod treated patients.

Methods: Data from the module was extracted on 10 April 2014. Patients contributing a baseline and at least 1 post-baseline follow-up visit were included in the descriptive analyses (years of follow-up; frequency of event counts).

Results: As at the date of data compilation, MS FIRST had enrolled 1704 patients (3462 clinic visits, cumulative 802.7 person-years of follow-up) since enrolment on 1 Jan 2012. Mean (SD) follow-up was 5.7 (6.7) months. Of the 904 patients who contributed at least 1 post-baseline visit, 703 (77.8%) has been exposed to DMT during follow-up. Of these, 462 (65.7%) had exposure to fingolimod. Across the observation period 14 infective SAEs, 14 Herpes Zoster events, 2 malignancies (excluding non melanoma skin cancer, NMSC) and 5 NMSC were recorded on fingolimod, compared with 9 infection SAE's, 8 Herpes Zoster, 4 malignancies and 7 NMSC on any other DMT. 575 patients on fingolimod contributed 2300 lymphocyte counts and LFT´s. Of these, 21 (3.7%) recorded at least one lymphocyte count < 0.2 whilst no patients recorded a count < 0.1. 74 (12.9%) patients recorded a gamma glutamyl transpeptidase (GGT) level > x5 the upper limit of normal (ULN) at least once on fingolimod.

7 (1.2%) recorded an alanine transaminase (ALT) level >5x ULN.

Conclusions: The establishment of a large, prospective multi-drug safety module for use in routine practice has been successful to date in Australia. It could provide important insights into the incidence and timing of treatment-associated SAE´s. Modelling trends and detecting signals in associated pathology could assist clinicians in identifying patients at risk.

P861

Longitudinal serum JCV indexes in MS patients with natalizumab-associated progressive multifocal leukoencephalopathy

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Background: The presence of John Cunningham Virus (JCV) serum antibodies is a risk factor for the development of progressive multifocal leukoencephalopathy (PML) in multiple sclerosis (MS) patients treated with natalizumab. We hypothesize that higher anti-JCV antibody indexes result in a higher risk of developing PML.

Objectives: To evaluate pre-PML longitudinal serum JCV indexes in four MS patients who developed natalizumab-associated PML.

Methods: We measured 3-monthly longitudinal serum samples in four MS patients who developed PML during natalizumab treatment in the MS Center Amsterdam, The Netherlands. Sampling took place from before natalizumab initiation until the development of PML. Indexes were compared to those from all other MS patients treated with natalizumab in the MS Center Amsterdam (n=140). Anti- JVC antibodies were measured using the STRATIFY test.

Results: All four patients show rather stable high serum JCV indexes before developing PML. Compared to all patients using natalizumab, patients who developed PML had higher anti-JCV mean antibody indexes. No increase of the JCV index was seen before the diagnosis PML.

Conclusions: Higher anti-JCV antibody indexes might increase the risk of PML within the JCV positive patients on natalizumab. Whether the height of the anti-JCV antibody index is a specific risk factor that may be safely applied in daily practice, needs to be confirmed.

P862

Varicella-zoster virus (VZV) experience in fingolimod clinical studies and the post-marketing setting

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Background: Fingolimod, a S1P receptor modulator, selectively redistributes CD4+CCR7+ naïve and central memory T cells to the lymphoid organs, without reducing the circulating effector memory T cells. In controlled clinical studies, the overall infection rate with fingolimod 0.5 mg was similar to that with placebo. In the post-marketing setting (PMS) in 2013, a fatal case of a varicella-zoster virus (VZV) infection was reported with fingolimod 0.5 mg.
Objectives: To report the rate and severity of VZV infections with fingolimod 0.5 mg in clinical studies and in PMS.
Methods: In the analyzed clinical trials, rates of adverse events (per 1000 patient-years, PY) are based on pooled data from the completed controlled phase 2 and three phase 3 studies (FREEDOMS, FREEDOMS II and TRANSFORMS) and their long-term extensions. In PMS, we evaluated the cumulative reporting rate every six months since 2010. We also report data on VZV reactivation (herpes zoster, HZ) by severity and clinical complication.
Results: As of 28 Feb 2014, more than 91,500 patients received fingolimod in clinical trials and the PMS with at least 135,800 PY of exposure. Data from controlled clinical trials showed that the rates of VZV infections were 11 with fingolimod 0.5 mg vs. 6/1000 PY with placebo, and were unchanged in the extension (up to 7 years) phases. In the controlled phases of clinical trials, uncomplicated cutaneous HZ constituted 95% of all cases (10.4/1000 PY with fingolimod 0.5 mg); no cases of disseminated HZ were reported with 0.5 mg. In PMS, the cumulative reporting rate of VZV infections was 7.2/1000 PY and the rates have remained consistent. The overall reporting rate of complicated HZ is estimated at about 0.3/1000 PY. Based on analysis from the FDA adverse event reporting system database, the proportion of serious HZ infections on fingolimod is comparable to other MS DMTs. Non-cutaneous manifestations were uncommon. Only a few cases of recurrent, non-serious dermatomal HZ cases have been reported to date.
Conclusions: Rates of VZV infections in fingolimod clinical trials were low with fingolimod 0.5 mg, but higher than in placebo recipients. VZV infection rates remained stable over time in extension studies and PMS. These cases were mostly uncomplicated cutaneous HZ. The risk of VZV infections is well manageable with appropriate vigilance for signs/symptoms of infections and with clear recommendations on immunization against varicella.

P863

Treatment of multiple sclerosis patients after 24 Natalizumab doses: a prospective observational study: the TY-STOP

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Background: Natalizumab is the most effective drug for relapsing-remitting multiple sclerosis (RR-MS) but could rarely be associated with progressive multifocal leukoencephalopathy (PML), whose risk increases after 24 natalizumab administrations.
Objectives: To evaluate the efficacy of different available therapies, on mean annualized relapse rate (ARR) and magnetic resonance imaging (MRI) activity after 24 natalizumab doses, in patients with RR-MS.
Methods: Spontaneous, prospective, multicenter, observational, 1-year study to evaluate disease course 24 doses of natalizumab. Primary outcome: ARR. Secondary outcomes: annual MRI activity, mean confirmed EDSS at 1 year.
Results: One-hundred twenty four patients, out of 130 recruited, completed the follow-up and were objective of statistical analyses conducted according to: *1*) the decision to continue/discontinue natalizumab after 24 doses [intention-to-treat (ITT) population]; *2*) the treatment received during the observation period ["as treated" (AT) population]. In the "AT" population the distribution of patients was: *1*)patients continuing natalizumab during the year of follow-up (CONTINUERS);*2*)patients discontinuing natalizumab during the year of follow up (QUITTERS);*3*)patients switching among different therapies, including natalizumab (SWITCHERS). No significant differences in demographic and baseline clinical characteristics were found comparing the different groups of treatment. The ITT population analysis showed that clinical (p=0.004) and radiological (p=0.018) activity was significantly lower in patients continuing natalizumab (N=43) than in those stopping it (N=81) This meant a protective effect of natalizumab continuation on clinical activity (odds ratio (OR),0.33; 95% confidence interval (CI),0.15-0.70) and radiological activity (OR,0.35;95%CI,0.15-0.79). The "AT" population analysis showed that clinical (p=0.003) and radiological activity (p=0.002) were significantly lower in CONTINUERS than in QUITTERS or in SWITCHERS. This confirmed a protective effect on the risk of relapse in CONTINUERS compared to QUITTERS (OR,4.40;95%CI,1.72-11.23) or SWITCHERS (OR,3.28;95%CI,0.99-10.79). One PML was observed, with full recovery of neurological signs and symptoms.
Conclusions: Clinical and MRI reactivation occurs more frequently in patients stopping natalizumab than in those continuing it. Therapy discontinuation should be considered only if the risk of PML is high and outweighs the benefits of continuing the drug.

P864

Risk of relapse after natalizumab discontinuation: which is the best treatment option?

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Background: To date, no direct comparisons among disease modifying drugs (DMDs) have been performed to evaluate the best treatment option for reducing the relapse risk occurring after natalizumab (NTZ) discontinuation in Multiple Sclerosis (MS) patients.
Objectives: To evaluate predictors of risk of relapses after NTZ stop and to compare the effect of different treatment regimens in reducing this risk.
Methods: An unselected cohort of 613 MS patients who discontinued NTZ therapy were extracted from the Italian iMedWeb registry. The relapse risk was estimated through a Poisson regression model adjusted for the following covariates: sex, age, first drug after NTZ, reason for discontinuation, comorbidity, previous DMDs/immunosuppressant (IS) exposure, disease duration, EDSS, number of infusions, relapses (yes/no) during NTZ and in the year before NTZ. Patients who started, after NTZ, a treatment with fingolimod (FIN) or with other DMDs were propensity score (PS)-matched on a 1-to-1 basis at the switching date, and the relapse risk was estimated by a Poisson model.
Results: At least one clinical relapse after stopping NTZ was experienced by 264/613 (43.07%) patients. During the follow-up (17±14.09 months), 441 (71.9%) patients received at least 1 DMDs prescription after a mean wash-out time of 4.66±6.26 months, whereas 172 (28.1%) were not treated at all. The Poisson regression analysis demonstrated that a higher number of relapses in the year before and during NTZ treatment was correlated to the risk of relapses (IRR=1.59, p=0.0001 and IRR=1.39, p=0.002, respectively) after NTZ discontinuation. During the follow-up, patients not treated at all resulted at higher relapse risk in comparison to patients receiving FIN (IRR=4.38, p< 0.0001) or other DMDs (IRR=2.43, p< 0.0001). A significant lower risk of relapses was found in patients treated with FIN in comparison to those treated with other DMDs (IRR=0.55, p=0.0014) and this result was confirmed to be unbiased by the Poisson model performed in 88 patients receiving FIN and 88 PS-matched patients receiving other DMDs (IRR=0.65, p=0.024).
Conclusions: Our results confirm the risk of clinical disease reactivation after NTZ suspension, and indicate that an alternative treatment should be prompt resumed mainly in patients with a previous very active course. Moreover, our results demonstrate a superiority of FIN in comparison to other DMDs in preventing relapses after NTZ discontinuation.

P865
Anti-JC virus antibodies in a Portuguese multiple sclerosis cohort

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Background: Patients with Multiple Sclerosis (MS) treated with Natalizumab (NTZ) have increased risk of developing Progressive Multifocal Leucoencephalopathy (PML) when positive for anti-JC virus antibodies (JCV+). The stratification of PML risk during NTZ treatment is crucial in determining patient safety and follow-up. The estimated prevalence of JCV+ in MS cohorts is 57.6% with an annual risk of seroconversion < 3%.
Objectives: Determine the prevalence of JCV+ in a Portuguese MS cohort. Estimate the annual seroconversion rate in MS patients treated with NTZ.
Methods: Chart review of consecutive MS patients with criteria for 2nd line therapy. All performed the JCV antibody test (JCVt). Seroconversion rate was estimated in patients on NTZ treatment with initial negative JCVt who repeated the test during treatment.
Results: 222 patients performed JCVt with 63.1% being JCV+ (63.5% in first-generation ELISA test vs. 62.8% in second-generation). The prevalence of JCV+ was higher in males (69.9% vs. 60.5%, p=0.2), and increased with age (< 20 years: 50%, 20-39 years: 61.2%, 40-49 years: 64.3%, 50-59 years: 67.4%, ≥60 years: 77.8%; r=0.970, p=0.006). 41 of the patients treated with NTZ who were initially seronegative repeated the test, 75.6% female, with median age at NTZ treatment onset of 38.5±10.6 years and median treatment duration of 34.6±20.3 months. 22% (9/41) of these patients presented seroconversion and there was a positive correlation between seroconversion and treatment duration (r=0.971, p=0.006), 1st year 2.4% (1/41), 2nd year 3% (1/33), 3rd year 8% (2/25), 4th year 15.8% (3/19) and 5th year 20% (2/10). The median treatment duration until seroconversion was 34.6±20.1 months. No correlation was found between seroconversion and gender, previous disease modification therapy, initial or final disability score, relapse rate before or during treatment.
Conclusions: The prevalence of anti-JCV antibody in our sample is in agreement with the values described in the literature, however we found a seroconversion rate higher than expected and with a progressive increase with treatment duration. No other predictors of seroconversion were identified. To the best of our knowledge this is the first study reporting an increase in the seroconversion rate with treatment duration, however our cohort is very small to draw definite conclusions. Studies with larger samples should be performed in order to confirm these results.

P866

Persistence of JCV in CSF of MS patients with PML

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Background: Multiple sclerosis (MS) patients treated with natalizumab are at risk for developing Progressive Multifocal Leukoencephalopathy (PML), a demyelinating disease due to infection with JC virus (JCV). Presence of JCV in the cerebrospinal fluid (CSF) is an important finding in the diagnosis of PML and is not seen in MS patients without PML. Longitudinal data investigating the clearance of JCV from the CSF in PML patients is being collected with the increasing cases of PML.
Objectives: Assess CSF of PML patients longitudinally for the presence of JCV.
Methods: CSF from 65 MS patients diagnosed with PML was tested at least once after initial diagnosis. Repeat CSF sampling occurred at a mean time of 55 days after initial testing. In 36 patients, CSF was analyzed at a third time point. JCV DNA copy number was detected by quantitative real-time polymerase chain reaction (qPCR) with a detection limit of 10 copies/mL (c/mL).
Results: In 39/65 (60%) of the patients JCV DNA copy numbers decreased after initial diagnosis. In twelve of these individuals (18%) JCV DNA was undetectable at the time of the second CSF sampling. In 26/65 patients (40%), however, JCV DNA was increased in the repeat CSF. In most of these cases, JCV copy numbers at least doubled and increased to as much as 4000% in some cases. Demographic and clinical characteristics did not differ significantly between patients with increase and decrease in their repeat CSF even with evidence of immune reconstitution syndrome (IRIS).
Conclusions: Persistence of JCV in the CSF is seen in a large percentage of MS patients with PML. Which factors affect clearance of JCV from CSF needs further investigation.

P867

Long-term safety of rituximab in MS and other autoimmune disorders

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Background: Rituximab is an anti-CD20 monoclonal antibody, used off-label in various autoimmune disorders including MS, NMO, and myasthenia gravis. Early stage clinical trials and several case series have shown remarkable efficacy. However, the long-term safety of sustained peripheral B-cell depletion is unknown.
Objectives: We examined the incidence of infections and malignancies in patients who received continuous rituximab therapy for at least 3 years.
Methods: This was a retrospective study examining the incidence of infections including serious infections, opportunistic infections, and malignancies in patients who received 6 monthly intravenous cycles of rituximab infusions continuously for at least 3 years.
Results: 23 patients (mean age 40 years) received continuous 6-monthly rituximab infusions for a period ranging from 36 to 72 months (mean: 51.1 months). CD19 counts were 0 at month 12 and remained 0 at last observation. Rituximab treated patients included NMO (n=15), MS (n=5), and Anti-MuSK antibody positive MG (n=3). Forty-eight MS patients (mean age: 41 years) receiving interferon beta or glatiramer acetate therapy observed for 3 years, served as control group. There was no significant difference in the incidence of infections between the two groups. Four serious infections (requiring hospitalization) in the rituximab and 7 in the control group were observed (p=ns). No opportunistic infections or malignancy were observed in either group during the observation period ranging up to 72 months in the rituximab group and 36 months in the control group. Pre and post rituximab showed remarkable reduction in disease activity. Efficacy data will also be presented.
Conclusions: Although preliminary, our observations do not indicate an increased risk of infections with prolonged peripheral B-cell depletion in patients who received rituximab infusions for up to 6 years continuously. While encouraging, these observations must be viewed with caution due to the small number of patients. Larger studies are warranted to confirm these findings.

P868

Analysis of data from RRMS alemtuzumab-treated patients in the clinical program to evaluate incidence rates of malignancy

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Background: Alemtuzumab is approved in >30 countries for relapsing-remitting multiple sclerosis (RRMS). In 3 clinical trials, alemtuzumab demonstrated superior efficacy vs subcutaneous interferon-beta-1a (SC IFNB-1a) with a consistent and manageable safety profile. Annual malignancy rates during controlled studies were the same for alemtuzumab and SC IFNB-1a (0.003 events/year),including rates for basal cell carcinomas which were seen with both treatments.
Objectives: To report incidence and types of malignancies in alemtuzumab-treated patients in the MS clinical trial program.
Methods: The alemtuzumab MS clinical trial program included the CAMMS223 phase 2 study (NCT00050778), Comparison of Alemtuzumab and Rebif Efficacy in Multiple Sclerosis (CARE-MS) I (NCT00530348) and II (NCT00530348) phase 3 studies, and an ongoing extension (NCT00930553). Patients were randomized to alemtuzumab 12 mg (and 24 mg in CAMMS223 and CARE-MS II) or SC IFNB-1a 44 mcg 3 times weekly. Standardized incidence ratios (SIRs) were calculated to quantify cancer risk in alemtuzumab-treated patients compared with a

retrospective MS cohort (US Clinformatics Data Mart; N=32,348). Age- and sex-adjusted incidence rates were used to determine the expected number of cases in the background cohort; confidence intervals (CIs) that include 1 suggest no additional risk of malignancy with alemtuzumab.
Results: Through the first year of the CARE-MS extension, 1486 patients received alemtuzumab in the MS clinical trial program (median follow-up 53.4 months, 6483 person-years); of these, 23 (1.5%) had a diagnosed malignancy. No meaningful incidence trends were seen by number of treatment courses, years of follow-up, or cumulative alemtuzumab dose (overall SIR 1.27; 95% CI 0.85-1.92). Thyroid papillary carcinoma (SIR 1.57; 95% CI 0.70-3.49) was the most common malignancy; all cases were incidental findings during evaluation or treatment of autoimmune thyroid disorders. The other most common malignancies were breast cancer (SIR 0.85; 95% CI 0.35-2.04), malignant melanoma (SIR 0.88; 95% CI 0.33-2.35), and cervical carcinoma (SIR 1.93; 95% CI 0.48-7.72).
Conclusions: The risk of malignancies with alemtuzumab in the MS clinical trial program was not greater than that in the large MS cohort. Rate of thyroid malignancies was likely inflated by ascertainment bias. Risk of malignancies will continue to be assessed in long-term studies.

P869

Longitudinal analysis of anti JCV-serostatus and antibody levels for PML-risk assessment in natalizumab-treated MS-patients

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Background: Serostatus of anti-JCV-antibodies serves as an established risk predictor for natalizumab-associated progressive multifocal leukoencephalopathy (PML). More recently, the level of antibodies (index) as determined using a second generation anti-JCV-ELISA has also been proposed to further refine the risk to develop PML.
Objectives:

a) To examine the association between anti-JCV antibody index and PML risk in an independent cohort of natalizumab-treated patients;
b) to assess proportion of patients with serostatus changes and longitudinal stability of anti-JCV-index.

Methods: 1,009 blood samples from 471 longitudinally observed patients were collected nationwide for routine testing for anti-natalizumab antibodies during open label treatment between 2007 and 2010. From 3 of 6 patients who later developed PML two samples each were available 24.5-35.3 months prior to diagnosis (pre-PML samples, no prior immunosuppression). Testing for anti-JCV antibodies was performed in a blinded fashion using the second generation two-step ELISA (STRATIFY JCV DxSelect™).
Results: All 6 pre-PML samples were seropositive. Pre-PML samples showed higher antibody index (3.6 (3.5-3.6), median (25-75 percentile)) than seropositive patients who did not develop PML (n=269; 2.4 (1.0-3.1); p= 0.0492), first of longitudinal samples analyzed). Also longitudinally high anti JCV-index in pre-PML samples remained stable (3.6 (3.2-3.8), 3.7 (3.6-9.1) months interval).

17 of 150 patients (11.3%; 4% of the total cohort) who were initially seronegative converted to seropositivity over an interval of 7.4 (4.3-11.9) months. 10 of these 17 patients (58.8%) remained below the anti-JCV index threshold of 0.9 over an interval of 6.9 (4.5-10.8) months. 10 of 272 patients (3.6%; 2.4% of the total cohort) seropositive at first testing reverted from anti-JCV antibody-positive to -negative. Antibody levels indicate fluctuations around the lower cut point of the assay. Seropositive patients showed a slight decrease in index (-0.1 (-0.3 to -0.1) over a duration of 5.5 (3.3-8.7) months (p=0.0007).
Conclusions: Our data from an independent German cohort followed up in the post marketing setting supports the potential of anti JCV-index to further refine the risk to develop PML. Use of anti JCV-index may also assist in risk stratification for patients with changing serostatus.

P870

Late onset PML in a natalizumab-treated MS patient

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Background: Progressive multifocal leucoencephalopathy (PML) is a well-documented side effect of natalizumab, a treatment for Multiple Sclerosis (MS). PML risk is expected to be low when the drug is eliminated from the body (washout period approximately 3 months).
Objectives: We present a case of PML in a natalizumab-treated MS patient three and a half (3,5) months after natalizumab discontinuation.
Methods: A 37-year-old female, diagnosed with MS in 2002 received interferon-b1a for eight years. Due to a high number of relapses natalizumab was initiated and the patient's condition stabilized for 2,5 years (EDSS: 5) but then the drug had to be discontinued temporarily because of recurrent urinary infections. The patient's condition was stable for three months after natalizumab discontinuation and only complained of extreme fatigue and weight loss. Brain MRI at this point only showed lesions compatible with MS. Three and a half months after natalizumab discontinuation the patient exhibited marked deterioration with vomiting, vertigo, horizontal nystagmus and ataxia. A repeat brain MRI showed a non-enhancing lesion in both cerebellar penduncles compatible with PML and CSF analysis showed 150 JCV copies.
Results: The patient received high dose of corticosteroids and IVIG with initial improvement but deterioration later on, both clinically and on the MRI, (spreading of the lesion in the brainstem). The patient received further corticosteroid treatment (both IV and per os) as well as immunomodulatory treatment with glatiramer acetate and approximately 8 months later her condition has stabilized with moderate improvement (EDSS: 7). Plasma exchange was not employed as the drug was considered to have been eliminated from the patient's body. Repeat MRIs showed gradual improvement of the lesion and at no point was lesion enhancement detected which would be indicative of immune reconstitution inflammatory syndrome (IRIS).

Conclusions: As this late-onset PML case shows, clinicians should remain vigilant for PML or IRIS manifestations even after the natalizumab washout period.

P871

Evidence of cortical lesions in PML patients and differences from cortical lesions observed in multiple sclerosis: an imaging study

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Background: Progressive multifocal leukoencephalopathy (PML) is a rare disease with high morbidity and mortality rates, that primarily affects immunocompromised patients. It has become in the limelight as serious adverse event of some new multiple sclerosis (MS) drugs such as Natalizumab, Fingolimod, and Dimethyl Fumarate. PML does not appear exclusively a white matter (WM) disease, since several "cortical" signs as seizures, behavioural disturbances, aphasia and cortical blindness have been described. The involvement of GM has been confirmed by pathological studies but its identification in vivo is challenging with conventional MRI techniques. Double inversion recovery (DIR) is a relative new unconventional MRI sequence having a higher specificity for GM compared to other conventional sequences.
Objectives: Aim of our study is to assess the potential application of DIR sequence to confirm and describe in vivo the GM involvement in PML patients. Furthermore, we evaluate the ability of DIR in distinguishing different diseases that affect primarily the WM and particularly in the diagnosis of PML in MS patients.
Methods: Two patients with HIV-PML, 1 with HIV-related leukoencephalopathy, 3 MS subjects, 2 patients with metachromatic leukodystrophy and 5 healthy controls were recruited in the study. An experienced neurologist and a neuroradiologist interpreted the 3D DIR images by consensus.
Results: The analysis of DIR images revealed that typical PML lesion is represented by a diffuse hemispheric WM hyperintensity that often involves cortical GM and does not respect the WM/GM junction involving also the cortical GM but not extending to the pial surface. Some focal pure intracortical lesions, again not extending to the pial surface, were also observed. On the contrary, in MS patients, several pure intracortical lesions some of which extending to the pial surface, were observed. In HIV-related leukoencephalopathy and metachromatic leukodystrophy lesions were limited to the WM and never entered the GM.
Conclusions: Based on our preliminary observations, we can confirm in vivo pathological data revealing that PML is also a GM disease although not extending up to pial surface differently from MS. Moreover, 3D DIR sequence may be helpful in distinguishing PML from other WM pathologies as HIV-leukoencephalopathy in immunocompromised patients.. Further studies on larger populations are required to confirm such observation that could be helpful to distinguish PML from MS cortical lesions.

P872

Application of serum natalizumab levels during plasma exchange in multiple sclerosis patients with progressive multifocal leukoencephalopathy

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Background: Progressive multifocal leukoencephalopathy (PML) is a serious adverse event of natalizumab treatment. Restoring immune function by plasmapheresis (PLEX) is important for the outcome of PML. It is unknown whether the current standard (five times PLEX) is the optimal treatment strategy.
Objectives: To evaluate if measuring serum natalizumab levels before and during PLEX may optimize the current treatment strategy.
Methods: In four multiple sclerosis (MS) patients who developed natalizumab-associated PML we measured serum natalizumab concentrations at the time of the diagnosis of PML and before and after every PLEX treatment.
Results: The required number of PLEX treatments to reach subtherapeutic serum natalizumab concentrations (below 1 μg/mL) depended on the serum natalizumab concentration at the time of PML diagnosis. The number of PLEX treatments needed varied between 4 and 7 in these patients.
Conclusions: Measuring serum natalizumab concentrations before and during PLEX might be helpful to determine the optimum number of PLEX treatments in individual natalizumab-associated PML patients. This can avoid redundant PLEX treatments or prolonged exposure to potentially harmful, i.e. bioactive levels of natalizumab.

P873

Rituximab in high activity MS patients after natalizumab withdrawal

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Background: Natalizumab provides a good control of relapsing activity in MS patients with suboptimal response to immunomodulators. When natalizumab is withdrawn, it is customary to associate fingolimod, with good clinical results. However, some patients may continue suffering relapses and their therapeutic management may be problematic.
Objectives: To analyze over 6 months the clinical and MRI variables of a group of patients previously treated with natalizumab and most of them with fingolimod, who had received rituximab due to persistent relapse activity.
Methods: Ten patients were included in the study, 80% females, with a mean age of 38 years and a disease duration of 8 years. The mean EDSS was 3.1. All but 2 had been treated with fingolimod after natalizumab withdrawal (in one case due to lack of drug availability and in other case due to comorbidities). EDSS, number of relapses, adverse events were recorded every 3 months. MRI variables were analysed at months 0 and 6. Informed consent was obtained from each patient.
Results: No adverse events were observed. EDSS was stable or improved in 62.5% of patients. One patient showed confirmed

progression at 6 months. A single patient, with a high annualized relapse rate before natalizumab had one relapse. No new T2 lesions or contrast enhancing lesions were found at 6 months, and the mean percentage of brain volumen change was 0.4%.
Conclusions: Rituximab has shown in phase II trials a significant therapeutic potential for MS. At present, new humanized and humane anti CD-20 monoclonal antibodies have replaced rituximab in ongoing clinical trials. Rituximab has been available in medicine for many years and has a well known safety profile. Although it is not an approved drug for MS, in selected cases such those of this study, it offers the possibility of being used as a rescue medication with a reasonable risk/benefit balance.

P874
Life threatening asthmatic crisis after prolonged fingolimod treatment
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Background: Fingolimod (FTY) is the first oral therapy approved for the treatment of relapsing remitting multiple sclerosis (RRMS). Its therapeutic effect in MS is exerted through the interaction with sphingosine - 1 phosphate receptors (S1P1-R), expressed on lymph cells and on a wide range of other cell types, including airway endothelial and smooth muscle cells where they mediate bronchoconstriction.
Objectives: We report the case of a woman with RRMS who experienced a life threatening episode of asthmatic bronchitis after six month of FTY treatment.
Methods: Description of a clinical case.
Results: A 49-yrs woman with history of mild, non-treatment requiring asthma during adolescence was diagnosed with RRMS in 1999. She switched through several disease modifying treatments due to either disease progression (interferon-beta 1-B, glatiramer acetate), or high risk of severe adverse events (mitoxantrone, natalizumab). In September 2012, 6 months after the last therapy was stopped, she was started with FTY 0.5 mg daily. A pneumologic evaluation with spirometry performed before treatment start was normal. After 6 months of treatment, the patient experienced a life-threating asthmatic crisis. The episode required patient's hospitalization and treatment with high doses of steroids and beta-2 agonists. FTY treatment was promptly stopped. She slowly recovered, and the respiratory parameters returned to normality on a follow-up spirometry three weeks later.
Conclusions: The patient with asthmatic hyper-reactivity in her youth, developed a serious respiratory adverse event after 6 months of FTY therapy. Published data show rather transitory worsening of the pulmonary function occurring within one month after FTY start both in MS and non-MS moderately asthmatic patients (1-3). Based on its mechanism of action, FTY might have precipitated bronchoconstriction in this case even in the long term.

P875
High dose glucocorticoid treatment does not contribute to reduced bone mineral density in patients with multiple sclerosis
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Background: Patients with Multiple Sclerosis (MS) are at increased risk of reduced bone mineral density (BMD). A contributing factor might be treatment with high-dose glucocorticoids (GC).
Objectives: The aims of this cross-sectional study were to assess bone mass in a large cohort of patients with MS and to evaluate the importance of high-dose short-term GC treatment and other risk factors that affect BMD in patients with MS.
Methods: 260 patients (202 women, 58 males) from the Danish Multiple Sclerosis Center had in the period 2012 to 2013 received high-dose short-term GC treatment and therefore had their lumbar (L2-L4) and proximal femoral bone BMD measured by dual x-ray absorptiometry. BMD measurements were compared to a healthy age-matched reference population (Z-scores). Osteoporosis was defined as a T score ≤ -2.5, and osteopenia as a T score of < -1 to > -2.5 according to WHO. Cumulative GC dose during the time period with MS, age, body mass index, serum 25(OH) vitamin D levels, disease duration and severity were obtained from medical records and the Danish Multiple Sclerosis Registry. Disease severity was assessed by the Expanded Disability Status Scale. All factors were included in a multivariate regression analysis to evaluate the independent association of each risk factor and reduced BMD in MS patients.
Results: Osteopenia was present in 38% and osteoporosis in 7% of the study population. The mean Z-score was significantly below zero, indicating a decreased BMD in our MS patients. By univariate regression analyses we found an association ($p=0.002$) between GC dose and BMD, but multiple linear regression analysis showed no significant association between GC dose and BMD in lumbar spine or in femoral bone. In contrast, age ($p=0.002$, $p=0.007$), BMI ($p=0.041$, $p< 0.0001$) and disease severity ($p=0.033$, $p< 0.0001$) were independently associated with both lumbar and femoral BMD, respectively.
Conclusions: Reduced BMD was prevalent in patients with MS. GC treatment appears not to be the primary underlying cause of secondary osteoporosis in MS patients. The principal factors associated with reduced BMD in our MS patients were increasing disease severity, increasing age and low BMI, which is in line with other studies investigating smaller cohorts.

P876
Two-years clinical and radiological activity during fingolimod post-natalizumab in relapsing-remitting multiple sclerosis. Single-center experience
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Background: Until 2011, natalizumab (NTZ) was the treatment of choice in patients with relapsing-remitting multiple sclerosis (RRMS) who had not responded after a course of immunomodulatory therapy (IMT), and in those with aggressive onset of disease. In some patients, the risk of progressive multifocal leukoencephalopathy (PML) causes NTZ withdrawal despite its efficacy. Fingolimod was approved in 2011 with the same indications as NTZ.
Objectives: We present here a series of patients treated with NTZ >2 years and with positive antibodies to JC virus (JCV), in whom NTZ was discontinued due to safety reasons and replaced by fingolimod. We analysed the clinical, radiological activity and adverse reactions during the 2 years with fingolimod.
Methods: We selected all JCV-positive patients treated with NTZ >2 years who discontinued treatment between January 2012 and January 2014. Fingolimod 0.5 mg daily was initiated 3 months after NTZ withdrawal. Electrocardiogram (ECG) monitoring was performed during the first dose, and magnetic resonance imaging (MRI) of the brain was performed at baseline (3 months after NTZ) and at 3, 6, 12 and 24 months after fingolimod initiation; laboratory values were determined at baseline, 1 month and every 3 months.
Results: From January 2012 to January 2014, 20 patients (12 women) with a mean age of 39.9 years and a mean MS history of 8.9 years discontinued NTZ after a mean of 26 doses, due to risk of PML. All patients were seropositive for JCV; four had received prior immunosuppressant drugs. At NTZ discontinuation, they were clinically and radiologically stable (mean EDSS 3.5, relapse rate 0.5, annualized relapse rate (ARR) 0.27). After the first dose, ECG abnormalities were found at 24h in 21.1% of patients, 5% symptomatic. All of them resolved later on. 25% of patients had asymptomatic hypertransaminasemia, and 83% persistent lymphopenia. There were no treatment discontinuations. Mean time on fingolimod was 24.6 months. The ARR was 0.11, and EDSS at 2 years remained stable (3.5). 40% of patients had radiological activity at month 3. Later on, only 1 patient showed radiological activity at month 24. No relevant adverse reactions were observed.
Conclusions: The increased risk of PML is one of the most common reasons for NTZ discontinuation, which is associated with clinical and radiological relapse. Our experience suggests that fingolimod is an effective alternative option for controlling disease activity in these patients.

P877

A pilot study to assess disease state stability, efficacy, and tolerability in a natalizumab to dimethyl fumarate crossover design

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Background: While natalizumab therapy is highly efficacious for the treatment of multiple sclerosis (MS), it does carry significant risk for progressive multifocal leukoencephalopathy (PML) in patients who have an immunosuppressant history, >=24 natalizumab doses, and JCV index of >=1.5. A crossover to dimethyl fumarate (DMF) is an option for many of these patients. The data regarding a crossover of this nature does not yet exist.
Objectives: To determine disease stability for 24 weeks in patients who have crossed over from natalizumab to dimethyl fumarate therapy.
Methods: 30 subjects at high risk (>2 years on therapy, JCV index >= 1.5) for developing PML on natalizumab were enrolled in a study to monitor their transition to dimethyl fumarate. Patients were observed for 24 weeks, starting from their last dose of natalizumab therapy. Outcome measures included disease stability defined by EDSS, annualized relapse rate, modified fatigue impact scale (MFIS) and the symbol digit modalities test (SDMT), visual analog scale (VAS), and timed 25 foot walk test. MRI activity including spectroscopy was measured at Week 0, Week 4, Week 16, and Week 24.
Results: Of the 15 patients who have completed the study, mean MS disease duration was 16 years, mean number of natalizumab infusions was 67, and the mean length of the last 6 infusion cycles was 37 days. From the date of the last infusion, it took an average of 22 days to begin the 120 mg dose of DMF and another 6.8 days to titrate to the 240 mg dose. At baseline, the JCV index scores ranged from 1.24-3.95. EDSS score increased by 0.5 in 4 of the 15 subjects. Thus far, 2 of the 15 subjects have transitioned back to natalizumab.
Conclusions: The data lock completing the study will occur in August of 2014. All outcome measures will be presented.

P878

High seroconversion in a cohort MS patients treated with natalizumab in Amsterdam

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Background: John Cunningham virus (JCV) seropositivity is a risk factor for the development of progressive multifocal leukoencephalopathy (PML) during natalizumab treatment in multiple sclerosis (MS) patients. It has been suggested that patients who are initially tested seronegative can convert to seropositive during natalizumab treatment.
Objectives: The aim of the present study was to evaluate the rate of conversion in MS patients during natalizumab treatment.
Methods: Between March 2007 and July 2013, 168 MS patients started natalizumab in the MS Center of the VU Medical Center in Amsterdam. From all patients, pre-natalizumab and follow-up serum samples were obtained 3-monthly and stored at - 80ºC until analysis. A second-generation JCV antibody enzyme-linked immunosorbent assay (ELISA) (STRATIFY JCV) was used to determine the JCV antibody titers in serum by Unilabs, (Copenhagen, Denmark), masked for clinical data.
Results: At baseline, 64 patients were tested seronegative. Of these patients, 13 (20,3%) converted to seropositive. Conversions occurred between 18-72 months.
Conclusions: The seroconversion rates in our MS patients treated with natalizumab are higher than published until now. This incidence of seroconversion in natalizumab treated MS patients appears to be much higher than in the general population. This might indicate a higher vulnerability due to natalizumab treatment.

P879

Disability progression associated with transitioning treatment after 2-years of natalizumab therapy as reported by NARCOMS participants

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Background: The consequences of transitioning to another treatment after 2 years of natalizumab (NAT) have not been fully determined.

Objectives: To retrospectively compare disability progression, as measured by increase in Patient Determined Disease Steps (PDDS), in NARCOMS participants who remained on NAT or transitioned to fingolimod (FIN) or injectable therapies (INJ) after 2 years of NAT.

Methods: NARCOMS participants with PDDS reported at the start of and ≥6 months after 2 years of NAT were included (N=527); groups were based on treatments used after 2 years of NAT (NAT, N=406; FIN, N=50; INJ, N=71). Propensity scores (derived from age, sex, PDDS, and prior relapse activity) were used to form 50 and 45 participant pairs between NAT-FIN and NAT-INJ groups, respectively. For paired and unpaired datasets, participant proportions with PDDS increase and mean PDDS increase were compared using Chi Square tests and ANOVA, respectively.

Results: During 2 years on NAT there were no significant differences in disability progression between groups. Over the entire study, median months of follow-up (NAT=48; FIN=54; INJ=60; $P<0.0001$) varied significantly. In the unpaired analysis participant proportions with PDDS increase (NAT=31%; FIN=46%; INJ=42%; $P=0.02$) and mean PDDS increase (NAT=0.31; FIN=0.58; INJ=0.71; $P=0.004$) were significantly different. Age, gender, and starting PDDS were associated with change in PDDS (all $P<0.03$); total follow-up time was not ($P=0.11$). In the paired NAT-FIN analysis, 24% of NAT and 46% of FIN participants reported PDDS increase ($P=0.02$), and mean PDDS increase was 0.1 for NAT and 0.5 for FIN ($P=0.02$). In the paired NAT-INJ analysis, 33% of NAT and 47% of INJ participants reported PDDS increase ($P=0.20$), and mean PDDS increase was 0.2 for NAT and 0.7 for INJ ($P=0.06$). The differences in proportions with PDDS increase and mean PDDS increase were not statistically significant in either of the matched groups after correcting for multiple comparisons.

Conclusions: Transitioning to a different therapy after 2 years of NAT was associated with an increased likelihood of self-reported disability progression and increased mean disability. Similar findings were observed using propensity score paired analyses, although these differences were not statistically significant. Additional studies are needed to compare the clinical outcomes associated with transitioning treatment after 2 years of NAT.

P880

Management of infusion-associated reactions in alemtuzumab-treated relapsing-remitting multiple sclerosis patients

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Background: Alemtuzumab, a humanized monoclonal antibody directed against CD52, is approved in over 30 countries for treatment of relapsing-remitting multiple sclerosis (RRMS). Alemtuzumab demonstrated superior efficacy over subcutaneous interferon beta-1a and manageable safety in the 2-year, phase 3 CARE-MS I and II studies. Infusion-associated reactions (IARs) were the most common treatment-related adverse events (AEs) during clinical development.

Objectives: To report incidence and severity of alemtuzumab IARs during 3-year follow-up of the pooled CARE-MS studies, and to discuss IAR management practices.

Methods: Patients with RRMS (treatment-naive [CARE-MS I; NCT00530348] or who had relapsed on prior therapy [CARE-MS II; NCT00548405]) received intravenous infusions of alemtuzumab 12 mg/day on 5 consecutive days at baseline and on 3 consecutive days 12 months later, and as-needed alemtuzumab re-treatment in an ongoing extension study (NCT00930553). IARs were defined as any AE occurring between start and stop of any infusion or within 24 hours after the end of infusion. IAR incidence was based on the number of treated patients in each treatment course.

Results: 811 patients were treated with alemtuzumab in the core studies; 742 entered the extension. Over 80% of patients did not receive treatment in Year 3. Marked decreases in IARs from Course 1 (84.7%) to Course 2 (68.6%) persisted in Course 3 (62.9%). IAR frequency was highest with initial alemtuzumab exposure and generally decreased with each infusion day. IARs were predominantly mild to moderate; none led to study withdrawal or death. Serious IARs were rare (3.1% of patients). The most common IAR types were skin disorders (predominantly rash), headache, pyrexia, and nausea. One case of anaphylaxis occurred during Course 3 and resolved with treatment; alemtuzumab treatment was discontinued. Effective IAR management included practices before infusion (patient education and premedication [methylprednisolone]), during infusion (monitoring, symptomatic treatment, and infusion interruption/rate adjustment), and after infusion (continued monitoring for 2 hours and symptomatic treatment [antihistamines, antipyretics, and/or antiemetics]). Few patients required infusion interruption or adjustment.

Conclusions: IARs were common with alemtuzumab but were predominantly mild or moderate and decreased with treatment courses. IARs were minimized or managed during clinical trials with appropriate patient education, medication, monitoring, and infusion adjustment.

P881

JCV antibody index and adhesion molecule expression levels in natalizumab-treated multiple sclerosis patients - a cross-sectional study

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Background: The presence of anti-JC virus (JCV) antibodies is a risk factor for progressive multifocal leukoencephalopathy (PML) development in natalizumab (NTZ)-treated multiple sclerosis (MS) patients. According to recent studies JCV reactivation occurs during NTZ therapy and leads to an increase in the index levels of anti-JCV antibodies. Furthermore, an anti-JCV-Ab index level of above >1.5 (no prior immunosuppressive treatment of patients preexposed) has been suggested to correlate with an increased PML risk.
Objectives: To explore an association between the anti-JCV antibody index in NTZ-treated MS patients and adhesion molecule (AM) expression levels on lymphocytes.
Methods: We included 16 MS patients with a positive anti-JCV antibody (JCV-ab) serostatus (12 MS patients with anti-JCV-Ab index level of above >1.5) and 23 JCV-ab seronegative MS patients during NTZ treatment. Anti-JCV antibody status was determined with the STRATIFY JCVTM ELISA test. The expression levels of ICAM-1, -2, -3, CD11a, CD49d, CD29 were measured on CD3+ T, CD19+ B, natural killer (NK) and NKT cells by flow cytometry.
Results: We found a significant higher (p=0.015) surface expression of CD11a on B-cells in MS patients with anti-JCV antibodies index >1.5 compared to JCV-Ab seronegative MS patients. Furthermore there was a positive correlation between anti-JCV antibody index and expression levels of ICAM-1 on B-cells (p=0.007, r=0,654).
Conclusions: Our data indicate a potential link between the receptor/ligand pair LFA-1 (CD11a)/ICAM-1 on B-cells and JC V serostatus.

P882

Anti JC virus antibodies in pediatric multiple sclerosis

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Background: Natalizumab has been shown to be a very effective treatment in severe pediatric multiple sclerosis. So far no cases of progressive multifocal leukoencephalopathy have been reported in multiple sclerosis patients in this age group. Prevalence for anti JC virus antibodies in children below age 21 has been reported to be 21% in the United States.
Objectives: As the majority of patients needing escalation therapy in the German center for MS in childhood and adolescence was found to be seropositive for anti JC virus antibodies we analyzed the prevalence of anti JC virus antibodies, the conversion rate and the influence of the anti JC virus antibody status on the clinical course in a large pediatric multiple sclerosis cohort.
Methods: Anti-JC virus antibodies were analyzed in serum samples within 6 months of disease onset and during the course of the disease using the JCV_{TM} DxSelect™ test.
Results: 51.6% of 256 patients were found to be positive for anti-JC virus antibodies at onset of disease. No correlation of antibody status with age at onset, relapse rate, EDSS progression or MRI lesion load was found. Analyzing 693 follow-up serum samples in 154 patients we found high titer stability and an annual conversion rate of 4.37%.
Conclusions: In a large cohort of pediatric MS patients no evidence was found that seropositivity for anti-JC virus antibodies is associated with a severe clinical course. Surprisingly seroprevalence for anti-JC virus antibodies was more than twice as high as anticipated in this age group raising the question if the infection with JC virus increases the risk to develop MS.

P883

Impact of JCV seropositivity in natalizumab-relapsing-remitting-multiple sclerosis treated patients: an observational study

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Background: Natalizumab (NTZ) is a monoclonal antibody directed against α-4 integrines approved for highly active relapsing-remitting multiple scleroses (MS). Despite demonstrated efficiency on disease course, NTZ is unfortunately associated with a risk of developing progressive multifocal leukoencephalopathy (PML) that led to restriction use depending of PML risk stratification established from 3 factors: JC-virus seropositive status, prior immune therapy and treatment exposure duration (over two years). In a "real-life" clinical setting, the impact of this risk stratification on physician decision and particularly JCV serological status has been rarely evaluated.
Objectives: The objective was to study the impact of JCV seropositivity in NTZ-RR-MS treated patients. NTZ discontinuation decision-making was compared to individual risk of PML defined by its stratification.
Methods: MS patients from two French MS centers, whose JCV serological status was known and NTZ treatment duration was six months minimum were included between October 2011 and December 2013. According PML risk stratification, we evaluated a PML risk level for all patients.
Results: 121 MS-patients (86 females and 35 males) were included. Mean age was 41 years, mean disease duration was 13 years and median EDSS was 3. Mean duration of NTZ treatment was 38 months. Among the patients, the rate of JCV seropositive was 71,9% (87/121). 12% (15/121) had a higher PML risk because of the presence of three risk factors and 43 % (52/121) a intermediary risk because they had two factors PML risks including JCV seropositive and NTZ exposure more than 24 months. PML risks for others patients (55%: 54/121) was assessed lower. In the subgroup of patient with a higher PML risk, 93% (14/15) discontinuated NTZ even thought all of JCV seronegative patients carried on treatment. In intermediary sub-group, NTZ was stopped in 24/52 (46%) patients. The main reason of interrupting NTZ was the PML risk in 55% of this cohort.
Conclusions: This observational study found a correlation between the PML risk and the rate of NTZ discontinuation. JCV seropositivity had a real impact on decision to carry on with NTZ treatment. PML risk stratification is a tool using in "real-life" by physician to discuss NTZ continuation in each patient. The development of anti-JCV antibody index may allowed physicians to refine his decision.

P884

Disease reactivation in multiple sclerosis pregnant women who stopped natalizumab during the first gestational period

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Background: Multiple Sclerosis (MS) is an inflammatory disease leading to demyelination and axonal loss. A prompt treatment is mandatory to prevent disease activity. However, MS often affects women in their childbearing. Pregnancies in women with severe relapsing-remitting MS treated with natalizumab (NAT) currently constitute a major challenge for the risk of reactivation after drug discontinuation. In current literature, a few data of NAT efficacy and safety in pregnancy are available.
Objectives: We describe 3 women with MS, that used NAT only during the first gestational weeks (GW) and experienced a reactivation of MS during pregnancy.
Methods: Observational case reports.
Results: In our centre, 5 MS patients became pregnant during NAT treatment. Of these, 3 women experienced a relapse. A 34 year-old woman was treated with NAT from the age of 31. She was diagnosed to be pregnant 15 days after the last NAT infusion. Three months after NAT cessation (during 20th week gestation), she presented hypoesthesia/dysestesia at right limbs, treated with IV steroid therapy with optimal response. The other case is a 32 year-old woman treated with NAT from the age of 29, who was diagnosed to be pregnant 20 days after the last NAT infusion. Five months after NAT cessation (during 27th week gestation), she presented right limbs hyposthenia/hypoesthesia, treated with IV steroid therapy with good response. The 3rd case is a 29 year-old woman treated with NAT from the age of 28, who was diagnosed to be pregnant 15 days after the last NAT infusion. Seven months after NAT cessation (during 26th week gestation), she presented lower limb hypoesthesia, treated with IV steroid therapy with good response.
Conclusions: NAT withdrawal for pregnancy may cause severe relapses but continuation might have unknown effects on the infantile immune system, due to alpha4-integrin effects on mammalian development and hematopoiesis. For this reason NAT is advised to be stopped 3 months prior to a planned pregnancy. However accidental pregnancy during MS treatment occur, so it's important to collect cases with MS reactivation during pregnancy. To conclude, although the poor number of our sample, the known protective effects of pregnancy on MS course would not seem to be confirmed in women who stopped NAT during GW.

P885

Multiple sclerosis as immune related adverse event after ipilimumab treatment in metastatic melanoma

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Background: Ipilimumab (IPI) blocks the cytotoxic T-lymphocyte antigen-4 (CTLA4) and leads to a release of a CTLA4-mediated inhibition of T-cell immunoreactions. IPI has shown increased survival rates in metastatic melanoma patients. However, IPI causes a broad spectrum of immune-related adverse events (irAEs) in about two-thirds of patients. Neurological irAES have been reported as single cases of aseptic meningitis, Guillain-Barré syndrome, facial palsy, myasthenia gravis, and Tolosa-Hunt syndrome.
Objectives: This is the first case of multiple sclerosis as irAE after IPI treatment.
Methods: A 30-year-old patient presented with metastatic melanoma originating from a nodular melanoma at his right auricle. Brain MRI on staging showed four T2 hyperintense periventricular lesions, cerebrospinal fluid analysis (CSF) revealed lymphoycytic pleocytosis and positive oligoclonal bands. Following surgery and chemotherapy he had been put on IPI treatment at 3 mg/kg body weight. Shortly later cerebral MRI showed a new T2-lesion. Chemotherapy with paclitaxel and carboplatin followed, but due to ongoing tumor disease IPI was applied again and could finally achieve tumor remission. During IPI treatment thermhypaesthesia of both feet occurred and cerebral MRI displayed disease progression (5 Gadolinium-positive lesions).
Results: Brain biopsy ruled out cerebral metastases, but detected a multiple sclerosis lesion with histopathological pattern I according to Lucchinetti et al. One month later optic neuritis occurred as a first clinical manifestation and the diagnosis of multiple sclerois was established. High dose glucocorticosteroids achieved partial remission of visual deficits. Follow-up MRI showed further disease progression, immunophenotyping detected increased CD4/CD8-ratio in CSF and activation of T- and B-cells in blood. Immunmodulatory treatment with interferon-beta was started and the patient is continuously under follow-up.
Conclusions: The temporal association of IPI administration and progression of an radiologically isolated syndrome to highly active MS is demonstrated clearly in this case promoting a causal relationship. Since IPI can only augment a pre-exisiting antigen specific immune response, MS as severe irAE unlikely reflects IPI overtreatment but the price for tumor control in a susceptible patient. Neurologists need to be alert of irAE associated with IPI treatment since the use of these agents is increasing, prompt GC treatment is effective and side effects might be misdiagnosed as brain metastases.

P886

Prevalence of anti-JC-virus antibodies in an Austrian MS-cohort: impact of gender, age and pre-treatment and analysis of cut-off-index

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Background: John Cunningham virus (JCV) is known to cause rare progressive multifocal leukoencephalopathy (PML) in natalizumab-treated MS-patients. Measuring of Anti-JCV Antibodies by two-step JCV-Stratify enzyme linked immunosorbent assay (STRATIFY JCV$_{TM}$ DxSelect™) in patient's sera has become a routine procedure in PML-risk stratification before/under natalizumab (Tysabri®, Biogen Idec) treatment. Beside qualitative JCV-status, quantitative index of Anti-JCV-Antibodies is important for PML-risk stratification.

Objectives: To investigate JCV-prevalence, impact of gender and age, and to compare predictive value for different cut-off-indices.
Methods: n=143 patients (107 female, 36 male) at the outpatient clinic for multiple sclerosis in Innsbruck, Austria have been tested for Anti-JCV Antibodies. Laboratory test by JCV-Stratify ELISA was performed at Unilabs, Denmark. In a retrospective analysis, prevalence of Anti-JCV Antibodies, considering gender, age and prior natalizumab-treatment, as well as prevalence and conversion rate for different cut-off-indices were analyzed.
Results: With the actual cut-off-index of 0.2-0.4, 62.9% of MS-patients were tested positive for Anti-JCV Antibodies at first testing, showing a significant (p=0.014) correlation with age. There was found a trend for higher prevalence in male (61.7% in female vs. 66.7% in male), whereas natalizumab-treatment did not show any effect on Anti-JCV Antibody index. For cut-off-indices of 0.9 and 1.5, rates of JCV-negative patients at first testing increased to 52.5 and 62.2%, respectively. For these higher indices, negative predictive value in retesting increased as well, showing a lower rate of seroconverters (from 8.3% at index 0.4 to 2.8% at index 1.5).
Conclusions: Prevalence for Anti-JCV Antibodies in the Innsbruck area is approximately 63%, whereby age and gender appear to be influencing variables. By setting higher cut-off-indices, negative predictive value of the test could be increased.

P887

Is there an increased cancer risk in people with relapsing multiple sclerosis taking cladribine?

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Background: A large phase III trial (CLARITY) of oral cladribine (Movectro) in people with relapsing multiple sclerosis (pwRMS) reported significant efficacy. However, concerns over the safety of cladribine have been raised by American and European regulatory bodies. Specifically, a suspected increased cancer risk was key for the rejection of cladribine for pwRMS by the European Medicines Agency (EMA) in 2011. The small number of observed cancer during CLARITY may be insufficient to definitively assess cancer risk.
Objectives: We compared the cancer risk of cladribine in CLARITY with other disease-modifying therapies (DMTs) used in the treatment of pwRMS.
Methods: All phase III trials of approved DMTs for pwRMS were included. Fisher´s exact test was used to compare cancer rates.
Results: Twelve phase III trials reported cancer rates and were therefore included in this meta-analysis. Study heterogeneity was not significant. Investigated treatments in these trials were cladribine, dimethyl fumarate, fingolimod, teriflunomide, natalizumab, alemtuzumab and glatiramer acetate. The seven studies comparing treatment versus placebo (as opposed to interferon-beta 1a (IFN beta-1a)) were initially used for analysis. The cancer rate in the CLARITY treatment group (1.13%) was not increased compared to all other treatment groups, whether including placebo controlled trials only (0.6%, p= 0.1139) or all other trials, ie. including those with an IFN beta-1a comparator arm (1.05%, p=0.0951). No cancer was detected in pwRMS assigned to placebo in CLARITY whilst the combined cancer rate of all other placebo groups was 1.19% (p=0.0159).

The cancer rate of zero in the CLARITY placebo group is also lower than in the recently reported phase III trial of cladribine in people with clinically isolated syndrome suggestive of demyelination (ORACLE), p=0.0012. In contrast, no difference was detected between cancer rates in the treatment groups of CLARITY and ORACLE (p=1).
Conclusions: Our study suggests cladribine in doses used in CLARITY and ORACLE does not increase the risk of cancer in pwRMS. The impression that it would appears driven by an unusually low cancer rate in the placebo group of CLARITY. Long term follow-up will help determine the cancer risk of all DMTs. Given its efficacy, tolerability and convenience cladribine should be reconsidered for treatment of pwRMS.

P888

Fingolimod after natalizumab - Is there a rebound of MS activity?

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Background: Many patients with Multiple Sclerosis (MS) on Natalizumab (NTZ) are being switched to Fingolimod (FTY) because of safety issues related to Progressive Multifocal Leucoencephalopathy risk. The safety and efficacy of this switch is unknown and the possibility of disease activity rebound after the switch is a current concern.
Objectives: Evaluate the clinical response to FTY in patients previously treated with NTZ. Assess predictors of relapse or disability progression during FTY treatment.
Methods: Retrospective review of clinical charts of MS patients treated for 6 months with FTY after NTZ. Clinical response was assessed based on annualized relapse rate (ARR) and Expanded Disability Status Scale (EDSS) score. Statistical analysis was performed using SPSS software.
Results: 29 patients were switched from NTZ to FTY, 89.7% because of positive JC virus antibody, 3.4% after adverse events and 6.9% because of inefficacy. The mean age at MS beginning was 27.5±8.9 years and the mean duration of NTZ treatment was 28.7±14.9 months. At FTY onset the mean age was 38.2±8.6 years and the mean disease duration was 10.4±6.4. The mean time between the last NTZ infusion and the beginning of FTY was 2.3±1.8 months and the mean FTY treatment duration was 17.4±6.6 months.

After the switch to FTY, the ARR decreased in 20.7% of patients and increased in 31%, without significant difference

between ARR before and after FTY (0.5±0.9 on NTZ vs. 0.7±1.2 on FTY, p=0.4). 31% of patients had relapses with NTZ, 24.1% still had relapses with FTY and 20.7% only had relapses after starting FTY. The patients who presented relapses with FTY had a higher previous ARR than the patients without relapses (0.8±0.8 vs. 0.3±0.9, p=0.04). The EDSS score decreased in 31% and increased in 27.6%, also without significant difference between EDSS score before and after FTY (2.7±1.5 on NTZ vs. 2.8±1.8 on FTY, p=0.6). No significant difference in age, sex, disease duration or previous EDSS score was found in the patients with relapses or disability progression during FTY treatment.
Conclusions: No significant rebound of disease activity was seen after switching NTZ to FTY in our patients. A higher ARR previous to FTY was the only factor associated with relapse risk during treatment. No safety issues were raised but longer follow-up is needed before drawing conclusions. This study suggests that FTY may be an efficacious choice after NTZ in the majority of MS patients.

Stem cells and cell-based therapy

P889

MicroRNA and gene expression profiling related to stem cells immune regulatory function in experimental autoimmune encephalomyelitis (EAE)

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Background: The molecular mechanisms that regulate the immune function of bone marrow mesenchymal stem cells (BMSC) are not known. We have shown previously that freshly isolated BMSC (fBMSC) when induced to express neuronal stem cell markers (nBMSC) lost their immunoregulatory function in EAE model. Recently, microRNAs (miRNA) have been shown to be involved in regulation of several immune responses both in innate and acquired immunity of many cell type.
Objectives: We have performed global analysis of protein-coding genes and microRNAs expression in two stem cells populations with different immune regulatory potential assessed in animal model of multiple sclerosis, EAE.
Methods: MicroRNA expression profile from freshly isolated BMSC and neuronal differentiated BMSC was assessed using miRCURY LNA Array. Gene expression profile was assessed with Affymetrix GeneChip and differentially expressed genes were selected according to data clastering method. To examine the role of miRNAs in regulation of immunomodulation potential of BMSC we analyzed expression of miRNAs in relation to their target genes involved in immunity. Next potential miRNAs targets and differentially expressed genes were submitted to the functional annotation toll provided by GeneCodis database.
Results: When clustering samples and genes, 30 of 757 miRNA passed the filtering criteria on variation across samples; standard deviation > 1.0. Nineteen miRNAs were found to be up regulated and 11 down regulated during the neuronal differentiation process. Among up regulated miRNAs: mir-146a and mir-155 were correlated with targets annotated to processes related to immune and inflammatory response. Only one significantly down regulated miRNA, mir-451 was related to regulation of inflammatory process. According to Affymetrix GeneChip analysis we found 762 genes down regulated and 245 genes up regulated during stem cells neuronal differentiation. Among up regulated genes we found groups functionally annotated to inflammatory response, toll-like receptor signaling pathway, positive regulation of interferon-gamma biosynthetic process and positive regulation of interleukin-6 production.
Conclusions: Neuronal differentiation of bone marrow stem cells generated pattern of miRNAs with gene targets involved in immunomodulation. This study provides new insight into molecular mechanisms related to immunoregulatory potential of stem cells.

P890

Cytogenetic analysis of culture-expanded human mesenchymal stem cells used in a phase I clinical trial in multiple sclerosis

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Background: In a recently completed Phase I study of autologous mesenchymal stem cell (MSC) transplantation in multiple sclerosis (MS), human MSCs were culture-expanded ex-vivo and infused IV. During culture, cells may become genomically unstable, leading to chromosomal aberrations.
Objectives: To examine MSCs utilized in a Phase I clinical trial for cytogenetic changes due to the ex-vivo culture-expansion and examine potential differences in genomic stability between MS-derived MSCs and non-MS derived control MSCs.
Methods: Bone-marrow-derived MSCs were culture-expanded to a target yield of at least 1-2 x 10^6 per kg (approximately 2-3 passages) in low glucose DMEM containing 10% fetal bovine serum and 10 ng/ml human fibroblast growth factor-2 then cryopreserved. DNA was extracted from aliquots of the final passage of cultured MSCs and peripheral blood mononuclear cells (PBMCs) from 19 trial participants (mean age 46 years, 7 relapsing-remitting MS, 12 secondary progressive MS) and 5 normal donor controls matched on age, sex, and culture protocol. Experimental and reference DNA samples were analyzed by Ambry Genetics by comparative genomic hybridization on the 12x135K StemArray platform, a stem cell-focused array-based comparative genomic hybridization technique with probes covering the entire genome plus increased probe coverage of genes relevant to stem cell and cancer biology.
Results: Some deletions and amplifications were detected in both the MS and control MSC samples when compared to the reference DNA; however, the majority of chromosomal alterations detected in this study were present in both MSC and germline (corresponding PBMC) samples. A few MS MSC samples had areas with copy number changes in telomeric and centromeric regions when compared to PBMCs. There was no significant increase in copy number changes in MS MSCs samples compared to control MSCs.

Conclusions: Overall, chromosomal differences between MSC and corresponding PBMC samples were very minor. The only regions exhibiting chromosomal changes were centromeric and telomeric regions. These chromosomal regions are known to have a lower density of coded genes, so these changes are less likely to be of significance. These data demonstrate that the ex-vivo expansion of MSCs, according to our clinical trial protocol, does not result in cells exhibiting genomic instability, supporting the safety of the cell product used in the trial.

P891

The effects of activated immune and CNS-resident cells on human oligodendrocyte progenitor cell survival and differentiation

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Background: In the injured CNS, successful remyelination is largely dependent on the survival and differentiation of oligodendrocyte progenitor cells. Within MS lesions, oligodendrocytes and oligodendrocyte progenitor cells (OPCs) are exposed to secreted products derived from both infiltrating immune cells and CNS-resident cells. Such products may be considered either pro-inflammatory or anti-inflammatory, and have the potential to contribute to both injury and repair processes. During development and following inflammatory injury, astrocytes also significantly contribute to these mechanisms.
Objectives: The overall objective of the current study was to determine how functionally distinct pro-inflammatory and anti-inflammatory human immune cell subsets, which are implicated in MS, can directly and/or indirectly impact human oligodendrocyte progenitor cell (OPC) survival and differentiation.
Methods: Polarized T cell and myeloid cell supernatants were directly or indirectly (via astrocytes) applied to A2B5+ human OPCs. Survival, proliferation, and differentiation of OPCs was measured via TUNEL, Ki67, and immunohistochemistry, respectively.
Results: Using A2B5+ neural progenitors, pro-inflammatory T cell (T_h1/T_h17) and M1-polarized myeloid cell supernatants resulted in TNFα-dependent cell death and an overall decrease in the percentage of O4+ and GalC+ oligodendrocyte lineage cells. Similarly, astrocyte-conditioned media collected from astrocytes pre-exposed to the same pro-inflammatory supernatants also resulted in decreased OPC differentiation; however, this was not due to cell death and was mediated through astrocyte-derived CXCL10. The use of similar media did not exert a measurable biological effect on mature O1+/MBP+ oligodendrocytes. T_h2 and M2 macrophage or microglia supernatants had neither a direct nor indirect impact on OPC differentiation.
Conclusions: We conclude that pro-inflammatory immune cell responses can both directly and indirectly (via astrocytes) impact the fate of immature oligodendrocyte-lineage cells, with OPCs more vulnerable to injury compared to mature oligodendrocytes. Given that astrocytes secrete a variety of factors that regulate OPC biology, it may be possible to direct the myelin repair process through modulating astrocytes. Our work suggests that future therapeutics in MS, particularly those that aim to improve the remyelination process in progressive multiple sclerosis, could be complimentary in targeting astrocytes, microglia, and infiltrated immune cells.

P892

Immune function monitoring in a phase I trial of autologous culture-expanded mesenchymal stem cell transplantation for relapsing multiple sclerosis

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Background: Mesenchymal stem cells (MSCs) have immunomodulatory, tissue-protective, and repair-promoting properties *in vitro* and in animal models, but human MSCs paradoxically induce *in vitro* Th17 responses by human peripheral blood mononuclear cells (PBMCs) under some conditions. Published data on in vivo immune effects of MSC transplantation are limited.
Objectives: To assess in vivo immunological effects of a single dose of autologous MSCs administered intravenously in patients with relapsing forms of multiple sclerosis (MS).
Methods: In a single-arm open-label Phase I trial of a single IV infusion of autologous culture-expanded MSCs for relapsing forms of MS, PBMCs were isolated and cryopreserved, using strict SOPs, from 22 of 24 participants twice before and twice after (Months -1, 0, 1, and 3) MSC infusion. Entry criteria included Expanded Disability Status Scale (EDSS) 3.0-6.5, and clinical or radiographic disease activity in the prior 2 years. Mean MSC cell dosage was $1.9x10^6$ MSC/kg (range 1.3-2.0) requiring 1-3 passages; post-thaw viability ≥95%. The primary immunological outcomes were the treatment-associated changes in percent of Th1 and Th17 CD4+ T cell responses within activated PBMCs measured by flow cytometry and intracellular cytokine staining. Overall proliferation (tritiated thymidine incorporation) and cytokine secretion (ELISA) of activated PBMCs were also assessed.
Results: 15 women and 7 men, mean age 46.4, participated, 8 with relapsing-remitting and 14 with secondary progressive MS, 27.2% with baseline enhancing brain lesions, and mean EDSS 5.4. MSC infusion was tolerated well with no treatment-related severe or serious adverse events. Neither disease activation nor significant improvement in new disease activity was observed. PBMC recoveries (generally >80%) and viabilities (generally >90%) did not differ between visits or between pre- and post-infusion samples. Variability was high, but an estimated 16% increase in the percentage of CD4+IL17+ T cells from the aggregated two pre-infusion visits to the Month 1 post treatment visit (95% confidence interval: -2% to +36%; p=0.08) was followed by an estimated 19% drop (2% to 33%; p=0.03) to former levels. PBMC proliferation curves rose slightly at Month 1 ($3.3×10^3$ across doses, p=0.04).

Conclusions: Though not definitive, these data are consistent with possible augmentation of Th17 responses by MSC transplantation in some MS patients. This potentially adverse consequence should be monitored in future trials.

P893

Exosomes released by mesenchymal stem cell populations promote differentiation and maturation of oligodendrocytes

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Background: Novel cell therapy-based treatments for MS aim to promote central nervous system (CNS) repair and regeneration, in order to halt or reverse the progression of established disability. Early clinical trials utilizing mesenchymal stem cells (MSCs) and MSC-derived neural progenitors (MSC-NPs) are currently underway to investigate their safety and efficacy in MS patient populations. MSCs and MSC-NPs have been shown to promote neurological recovery in animal models of MS through both immunoregulatory and trophic mechanisms, which alter the lesion environment to promote endogenous repair. Specifically, MSCs and MSC-NPs can proximally influence oligodendroglial and neuronal differentiation from progenitor populations. However, the mechanism(s) by which MSCs and MSC-NPs exert their trophic effects on oligodendrocytes remains unknown.
Objectives: To determine whether the release of exosomes (microvesicles) by MSCs and MSC-NPs can mediate trophic effects on oligodendrocyte differentiation from neural stem cells.
Methods: MSCs and MSC-NPs were isolated and characterized from a panel of bone marrow samples from both control and MS patients. Exosomes were isolated from MSC and MSC-NP conditioned media using Exoquick precipitation reagent, and characterized by western blot. Purified exosomes were added to rat brain-derived neural stem cells (rNSCs), which differentiate into oligodendrocytes, neurons, and astrocytes upon growth factor withdrawal. Degree of oligodendrocyte differentiation in response to exosome treatment was assayed by immunocytochemistry and quantitative PCR.
Results: Purified exosomes from MSCs/MSC-NPs but not control cells dose-dependently promoted the differentiation of rNSCs into oligodendrocytes, as shown by increased RNA and protein expression of mature oligodendrocyte markers MBP, GalC, and PLP. Neuronal and astroglial differentiation was decreased or unchanged, respectively. Candidate exosome-associated proteins and microRNA species associated with effects on oligodendroglial differentiation were investigated.
Conclusions: These observations suggest that the regenerative effects of MSC/MSC-NPs on oligodendroglial cells may be mediated by exosome release. The optimization of exosome release by MSC/MSC-NPs may have therapeutic implications in influencing the rate of CNS repair and remyelination in MS.

P894

Localization and viability of neural stem cells after therapeutic intrathecal transplantation in experimental autoimmune encephalomyelitis

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Background: Intrathecal transplantation of adult neural stem/precursor cells (NPCs) ameliorates disease severity in experimental autoimmune encephalomyelitis (EAE), the animal model of multiple sclerosis. Understanding the kinetics of NPC behavior in vivo might advance clinical translation.
Objectives: Analyze the influence of neuroinflammation on the localization and viability of intrathecally transplanted NPCs.
Methods: NPCs were derived from the subventricular zone of 8-week old female C5Bl/6 mice. EAE was induced in syngeneic mice by subcutaneous immunization with myelin oligodendrocyte glycoprotein 35-55 peptide. One million GFP-labelled NPCs were transplanted intrathecally in the cisterna magna of EAE mice at peak of disease severity or healthy matched controls (HC). NPC survival and localization was assessed by immunofluorescence and immunohistochemistry at 1, 7 and 60 days post transplantation (dpt). Each group comprised at least 4 animals.
Results: At 1 dpt, NPCs distributed within few millimetres from the injection site (2,46 ±0,90 mm in EAE; 2,78 ± 0,57 in HC), no further migration was observed at 7 and 60 dpt. At 1 dpt, 8,1% of transplanted NPCs survived in HC and 7,5% in EAE. At 7 dpt the number of surviving NPCs further decreased in both groups (HC: 2,6%; EAE: 4,6%). Indeed, a fraction of transplanted NPCs expressed the apoptotic marker activated caspase 3, with EAE mice showing a trend of reduced apoptosis at 1 dpt (HC: 3,2%; EAE: 1,5%) and at 7 dpt (HC: 3,8%; EAE: 1,4%; $p< 0,05$). Consistently, at 60 dpt NPCs transplanted in EAE mice displayed increased survival (2,7%) when compared to HC (0,3%; $p< 0,05$). In both groups, transplanted NPCs localized mainly in the subarachnoid spaces of the fourth ventricle or surrounding meninges at 1 dpt (EAE: 94,8% of surviving NPCs; HC: 87,6%) and 7 dpt (EAE: 98,5%; HC: 89,4%), with a small quota of NPCs integrating in the parenchyma. At 60 dpt, 93,5 % of surviving NPCs retained their meningeal localization in the EAE group, while in the HC group 89,4% of the surviving NPCs were found in the parenchyma.
Conclusions: The inflammatory environment of EAE likely does not affect NPC survival in the immediate post-transplant phase. However, 7 and 60 dpt follow-up of transplanted NPCs showed increased survival of NPCs in the EAE group compared to HC as well as different NPC localizations in the two groups, suggesting that neuroinflammation might have long-lasting effects on the behaviour of transplanted NPCs.

P895

Clinical experience in autologous hematopoietic stem cell transplantation in refractory aggressive multiple sclerosis

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Background: Despite treatment with current first and second-line therapies, some MS patients suffer a breakthrough form, reaching significant disability in the early stages. Autologous bone marrow transplantation (BMT) is used as a compassionate treatment for aggressive and refractory MS.
Objectives: To describe the clinical experience of a series of refractory patients with aggressive multiple sclerosis subject in which a BMT was performed at some point in their evolution.
Methods: This is a descriptive study about the case mix of relapsing-remitting (RRMS) or secondary progressive MS with relapses (SPMS) in which a BMT was performed from 2 tertiary referral hospitals in Valencia (Spain) from February 1999 to March 2014. Presence of attacks, the Expanded Disability Status Scale (EDSS) and presence of adverse events were quarterly assessed.
Results: Twenty-seven patients underwent BMT (19 women, mean age at diagnosis of 27.1 [SD 7.7]), 16 RRMS (72%) and 11 SPMS (44%). Mean evolution time from first attack to BMT was 8.4 years (SD 4.0), and the median of previous treatments was 3 (range 1-6). The annualized relapse rate (ARR) the year before BMT was 1.2 (SD 0.82), and the median EDSS was 4.5 (range 2-7). Most patients (63%) have been followed for a period of time ≥4 years. No patients have required disease-modifying therapies during the first 4 years after BMT, only 7 patients started other therapies after a mean of 4.3 (SD 2.8) years. ARR decreased significantly at 2 (mean 0.13 [SD 0.22], p=0.001) and 4 (mean 0.20 [SD 0.36], p=0.002) years of follow-up. Most RRMS patients (83.3%) remained free from sustained-progression at the end of follow up period compared with 28.6% of SPMS patients (p=0.044). Sustained improvement was observed in 11 patients (44%), 10 RRMS patients. K-M curves showed differences in favour of patients with RRMS (median time 48 months [SE 11.5]) compared to SPMS (p = 0.039). Sixteen patients had immediate adverse reactions to BMT, 2 of them serious; 3 patients developed neoplasms (1 carcinoma in situ of the cervix, 2 breast cancers), with median time to diagnosis of 4.9 years (SD 0.6).
Conclusions: BMT is an effective alternative treatment for aggressive and refractory RRMS patients. Improvement of long-time disability has been observed in this subgroup of patients. There is no evidence of modification of clinical course in SPMS patients. Most patients have suffered short-term toxicity, but without long-term disability or mortality.

P896

Therapeutic potential of human mesenchymal cells in the animal model of MS

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Background: In MS, therapy with autologous mesenchymal stem cells has an attractive potential due to their immunomodulatory and neuroprotective properties. Ongoing clinical trials are evaluating safety and efficacy aspects. Among the various theoretical questions regarding this therapeutic approach, one refers to the suitability of the own cells from patients. A few papers have evaluated *in vitro* features of stem cells from patients compared to those of healthy controls but *in vivo* experiments have not been carried out.
Objectives: To evaluate the features of human bone marrow mesenchymal stem cells (hBM-MSC) from patients of MS and healthy controls both *in vitro* and in the animal model of MS, experimental autoimmune encephalomyelitis (EAE).
Methods: Eight MS patients were selected with no disease modifying drugs and no antecedent of immunosupresant therapy. hBM-MSC from healthy donors and MS patients were isolated by puncture of iliac crest and expanded *in vitro*. The differentiation potential, colony forming unit (CFU) capacity, antiproliferative and immunomodulatory properties and the production of neurotrophic factors were compared between two groups of hBMSC. The osteogenic, adipogenic and chondrogenic differentiation was analysed by confocal microscopy. CFU and antiproliferative capacity were determined by crystal violet count and MTT assays, respectively. Neurotrophic and cytokine production were determined by ELISA assays.

For *in vivo* experiments, EAE was induced using MOG35-55 in C57BL/6J mice. On days 0, 2 and 7 postinduction, 2x10^6 of hBM-MSC from controls and patients, were injected intravenously, in the different groups of animals. Next to induction, clinical signs were evaluated daily comparing between both groups of mice.
Results: No differences were found between patients and controls in any of the *in vitro* parameters. *In vivo* assays showed a significant reduction of the severity score of EAE both in animals treated with stem cells from patients and from controls.
Conclusions: One of the biological features of MS is the chronic and exaggerated passage of inflammatory cells across the blood-brain-barrier into the CNS. In MS, this continous inflammatory stimulus of the bone marrow might theoretically affect the therapeutic potential of the hBMSCs. However, our results, showing similar *in vivo* efficacy of those cells as compared to healthy controls, add evidence to the suitability of the autologous use of hBM-MSC in patients.

P897

Increased CXCL10 production in bone marrow MSCs and monocytes of severe MS patients before and after AHSCT treatment

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Background: The CXC motif chemokine 10 (CXCL10) is an inflammatory chemokine that may be produced by different cell types as monocytes, dendritic cells (DC), endothelial and epithelial cells and keratinocytes, in response to IFNg. CXCL10 is involved in inflammatory processes ongoing in RR and SP forms of MS and in a previous work we showed that bone marrow (BM) derived mesenchymal stem cells (MSCs) isolated from severe MS show increased production of CXCL10. We hypothesized that BM may be indicative of a more wide inflammatory status and may be involved in disease course and in the outcome of

autologous hematopoietic stem cell transplantation (AHSCT) treatment.
Objectives: To confirm CXCL10 over production and to identify molecular signature in bone marrow mesenchymal stem cells (MSCs) and circulating monocytes isolated from RR and SP MS patients that undergo autologous hematopoietic stem cell transplantation (AHSCT); to extend this analysis after AHSCT to test if therapy has modifying effects on MSCs and circulating monocytes.
Methods: MSCs and monocytes were isolated from 19 MS that undergone to AHSCT before and, 7 of them, 2 years or more after transplant. CXCL10 production was detected after LPS/IFN-g stimulation. TLR4 signalling pathways was investigated by mean of transcription factors phosphorilation/activation level. RT-PCR of activated transcription factors was performed to quantify their expression. All experiments were conducted in parallel with 24 matched healthy donors (HD).
Results: CXCL10 expression was significantly increased in both peripheral circulating monocytes and BM MSCs comparing to HD. We show that CXCL10 production is determined by an altered signalling pathway downstream TLR4, with the involvement of STAT1, NFkB, p38, JNK and CREB. All up regulated transcription factors are more phosphorilated in MS patient sample. These features are not modified after AHSCT.
Conclusions: We demonstrate that in severe MS cells residing in two anatomical compartments are characterized by significantly increased production of CXCL10, due to altered signalling pathways of innate immune reaction mediated by TLR4 probably associated with severe disease phenotype. This characteristic is not modified by AHSCT, suggesting that when T lymphocytes are reset other possible components of MS pathology, as CXCL10 over production, do not determine therapy outcome.

P898

Autologous hematopoietic stem cell transplant in patients with neuromyelitis optica

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Background: Neuromyelitis Optica (NMO) is an idiopathic inflammatory demyelinating disease of the CNS, specifically an autoimmune astrocytopathy, that preferentially affects the optic nerves and spinal cord. Frequent attacks result in significant disability with considerable morbidity and mortality. Treatments for this disease are highly toxic and only minimally effective.
Objectives: We have undertaken a trial of autologous non-ablative stem cell transplantation (AHSCT) in NMO to determine if such treatment leads to disease remission and freedom from immunosuppressive medication. We hypothesize a 50% reduction in the proportion of patients experiencing relapse events over a three-year period. Secondary outcomes include relapse rate, EDSS, vision measures and survival.
Methods: This open-label single centre trial was approved by the Calgary Conjoint Health and Research Ethics Board and Health Canada. Eligible patients have confirmed NMO, ages 18-65 and EDSS < =6.5 with =>1 relapse in 12 months or =>2 relapses in 24 months despite maintenance therapy. Using a cyclophosphamide/rituximab/ATG protocol, patients undergo mobilization/harvesting of stem cells followed by a conditioning/stem cell infusion 4-8 weeks later.
Results: Over the past three years, we have transplanted three patients. Patient 1 (28F) was transplanted in May 2011 with a pre-transplant annualized relapse rate (ARR) of 5 and baseline EDSS 4.0. Transplant complications included transient asymptomatic febrile neutropenia and volume overload. Her ARR 36 months post-transplant is 0 with EDSS 2.0. Patient 2 (36F) was transplanted without complications in April 2012 with a pre-transplant ARR of 4 and baseline EDSS 4.5. Her ARR 24 months post-transplant is 1 with EDSS 4.0. Patient 3 (37M) was transplanted in January 2014 with a pre-transplant ARR of 1.3 and a baseline EDSS of 3.0, with month-6 results to be presented.
Conclusions: This transplant regimen appears tolerable in relatively young and healthy patients with active NMO, allowing for a dramatic to complete reduction in relapses. If these results persist over a longer time period and in subsequent patients, AHSCT may prove to be an effective and tolerable option for NMO patients.

P899

Generation and characterization of neuralized mesenchymal stem cells from multiple sclerosis patients

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Background: In the last few years a lot of preclinical studies showed the theraputic potential of MSC in MS.Moreover, few clinical stuies showed positive results with the use of MSC in MS. The mechanisim of action of MSC in the disease model are mainly via immunomodulation and neuroprotection. The possibility of tissue repair with the use of MSC is very debatatble and seems to be not a major mechanisim in the action of disease amelioration. Therefore, the employment of neuroprecursors derived from MSC (MSC-NPs) instead of naïve MSC can be useful also in terms of neuro-regeneration and remyelination (while keeping the immunomodulatory properties) similar to potential of neural stem cells without ethical hurdles that neural stem cells have in clinical applications.
Objectives: To generate and characterize neuralized mesenchymal stem cells from multiple sclerosis patients.
Methods: MSC were isolated from the BM of five MS patients. The MSC were cultured in a medium contatining EGF, bFGF and B27 supplement to generate MSC-NPs. The MSC-NPs were characterized using FACS. The neural differentaion of MSC-NPs by culturing with neural differentiation commercial media (NeuroCult™ NS-A).The immunomdualtory effects of MSC-NPs were tested with lymphocytes suppression assay *in vitro*.
Results: We were able to generate MSC-NPs successfully from 5 donors. The spheres stained positively for the neurosphere markers Nestin and PS-Ncam (>90%) while loosing the mesenchymal markers CD90 and CD105 (< 5%). As well the MSC-NPs lost their ability to differentiate in mesodermal tissues (adipocytes and osteocytes). MSC-NPs differentiated successfully to neurons (MAP2 marker), astrocytes (GFAP marker) and oligodendrocytes (CNPase marker). Morevover, MSC-NPs induced dose-dependent suppression of lymphocytes.

Conclusions: The generation of MSC-NPs (which hold the neural differentiation potential of neural stem cell) from naïve MSC isolated while keeping there immunomodulatory properties open for us new possibilities for neuroregeneration and remyelination in future autologous cell-therapy treatments for multiple sclerosis.

P900

Anti-neural precursor cell (NPCs) - immune response in experimental autoimmune encephalomyelitis (EAE)

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Background: Autoantibodies may contribute to the pathogenesis of Multiple sclerosis (MS). EAE is a T-cell mediated experimental model that simulates MS, sharing many clinical, immunological and pathological aspects.

Objectives: We examined the potential humoral responses in EAE against central nervous system NPCs.

Methods: MOG_{35-55}-EAE was induced in C57bl/6 mice and blood-sampling was performed on day 17 (acute phase) along with naive group. The humoral immunity in EAE was examined by performing Western blotting. As substrates, total protein extract from NPCs and normal spinal cord (SC) stained with naive (Naïve-AS) - and MOG-EAE (EAE-AS) - antiserum, were used. Immunocytochemistry (ICC) was conducted on dissociated NPCs (day 7); immunohistochemistry (IHC) and double immunofluorescence (dIF) were also performed on BrdU labeled normal mouse brain sections from neonates, postnates and adults (P3, P17 and 3months, respectively) using the EAE- and Naïve-AS. A commercially available MOG antibody (anti-MOG) was used aiming to identify MOG- expressing cells. Neuropathological evaluation was performed using conventional and confocal microscopy.

Results: Western blot analysis using EAE-AS revealed anti-MOG autoantibodies (26-28KDa) in SC- though not in NPCs- substrate. However, two specific bands (60KDa and 40-46KDa) were identified when NPCs-substrate was used. Naïve-AS exhibited no similar results in either substrate. ICC data showed higher integrated density in dissociated NPCs stained with EAE- compared to Naive-AS ($p< 0.0046$). IHC, in all three ages, revealed a cell population, located in subventricular zone and periventricularly, that was positive for EAE- though negative for Naive-AS ($p< 0.0001$). Additionally, dIF revealed that EAE-AS had significantly higher affinity to BrdU(+) cells, when compared with anti-MOG: P3:45.43±8.112% versus 10.92±4.225%($p< 0.0027$); P17:50.64±9.207% versus 10.13±2.519% ($p< 0.0005$); 3months:88.54±7.542% versus 19.49±11.43% ($p< 0.0035$), respectively.

Conclusions: Activation of the immune system against MOG_{35-55} induces the production of antibodies apart from those related directly to the specific antigen. Furthermore, these antibodies may recognize yet unidentified epitopes other than MOG on NPCs, in SVZ. Our observations indicate a potential role of humoral response against NPCs in EAE and might provide a better understanding of MS autoimmunity.

P901

Identification of an alternative to fetal bovine serum for the culture expansion of human mesenchymal stem cells for use in clinical studies

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Background: Culture-expanded mesenchymal stem cells (MSCs) were evaluated for therapeutic safety in a recently completed Phase I trial in multiple sclerosis. As in our trial, standard ex-vivo culture techniques utilize a number of animal-derived components which may present a safety issue. Use of xenofree reagents is desirable to ensure the safety of human MSC cell products.

Objectives: To evaluate a xenofree culture expansion media that results in efficient expansion of human bone marrow derived MSCs for use in human clinical trials.

Methods: Bone marrow aspirates were obtained from normal donors after obtaining written informed consent. Mononuclear cells were isolated by Percoll density gradient centrifugation, and cells from each donor were divided equally and grown in parallel in low glucose DMEM containing either 10% fetal bovine serum (FBS) or 5% Good Manufacturing Practice (GMP) grade PLUS supplement (human platelet lysate). Cultures were passaged when cells were 70% confluent. Cells were expanded for 3 passages and theoretical yield was calculated. MSC purity was assessed by presence of the surface markers CD105 and CD73 and absence of the hematopoietic cell surface markers CD45 and CD14.

Results: Cultures containing PLUS supplement (n=5) had a 2-fold increase in the number of MSCs at primary culture and a 4-6 fold increase in cell number at subsequent passages compared to those grown in FBS (n=4). The mean MSC yield after passage 3 was 28×10^8 for cells grown with PLUS compared to 7.7×10^8 for cells grown with FBS. Additionally, the median number of days to reach a clinical dose of 1×10^8 was 17.3 days for PLUS cultures compared to 32 days for FBS. At passage 3, the purity of MSCs grown in the presence of PLUS or FBS supplement was equivalent, measured by the presence of CD105/73 and absence of CD45/14 surface markers.

Conclusions: The replacement of FBS with 5% PLUS supplement represents a pathogen-free GMP-compliant alternative for clinical MSC production. Addition of PLUS supplement results in expansion of a similar phenotypic population of MSCs compared to traditional culture methods using FBS, and a clinical target dose is achieved in fewer passages and less time in culture. Further physiological and immunological studies should be performed on these cells before clinical use.

P902

Assessment of bone marrow-derived cell therapy in progressive multiple sclerosis (ACTiMuS)

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Background: We have recently completed a phase I trial of intravenous delivery of autologous bone marrow in progressive MS ('SIAMMS'). The possibility of repair was suggested by improvements in the neurophysiological secondary outcome measure.
Objectives: The 'Assessment of Bone Marrow-derived Cellular Therapy in Progressive Multiple Sclerosis' (ACTiMuS; NCT01815632) will examine the efficacy of intravenous delivery of autologous marrow in progressive MS.
Methods: The trial employs a prospective, randomised, double-blind, placebo-controlled, stepped wedge design at a single centre. Eighty patients with primary (20) or secondary (60) progressive MS will be recruited and randomised to either early (immediate) or late (1 year) intravenous infusion of autologous, filtered bone marrow. The placebo intervention is infusion of autologous blood. Participants will be followed up for a further year following the final intervention. The primary outcome measure is global evoked potential derived from multimodal evoked potentials. Secondary outcome measures include adverse event reporting, clinical (EDSS and MSFC) and self-assessment (MSIS-29) rating scales, optical coherence tomography (OCT) and brain and spine MRI. Outcomes will be analysed on an intention-to-treat basis. Laboratory studies performed in parallel with the clinical trial will further investigate the biology of bone marrow infusion in MS, including mechanisms underlying repair.
Results: Recruitment began in Spring 2014 and to date, bone marrow harvest and infusion of either autologous blood or marrow has been well tolerated as a daycase procedure.
Conclusions: We expect to present trial results in 2019.

P903

Clinical and immunological monitoring of autologous IL-10 modified dendritic cells transplantation in multiple sclerosis

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Background: Multiple sclerosis (MS) is postulated to be a T cell-mediated autoimmune disease with altered antigen presentation. Dendritic cells (DCs) are the most potent antigen-presenting cells of the immune system, and can modulate immune responses in MS to stimulatory or tolerogenic fashion. Previous experimental studies have demonstrated that the administration of immature IL-10 modified DCs can increase an anti-inflammatory immunity (Link H. et al., 2004).
Objectives: In order to estimate a tolerability, safety and immunological and clinical responses we investigated autologous immature IL-10 modified DCs treatment of 3 MS patients.
Methods: The 1st patient with secondary progressive (SP) without relapses MS (M, aged 46, duration 10 ys, EDSS 6.0) who earlier had no effect on glatiramer acetate and methylprednisolone (MP) treatment; 2nd - with SP with relapses MS (F, aged 53, duration 9 ys, EDSS 5.0) who earlier had positive effect on intravenous immunoglobulins and MP; 3d - with relapsing-remitting MS (M, aged 49, duration 22 ys, EDSS 3.0) who earlier had positive effect on MP. All patients were stable more than 1 month before and don't received MP less than 2 months before, immunosupressants anytime and DMT less than 3 months before enrollment in the study. DCs were generated from blood monocytic cells of the MS patient by culturing *in vitro* in the presence of GM-CSF and IL-4, followed by IL-10. The obtained autologous immature DCs ($CD11c^+HLA\ RD^+CD1a^+CD83^-CD80^-$) in the amount of $3x10^6$ were injected intracutaneously into the patient's back 6 times at intervals of a month.
Results: We found no system reactions. The 1st patient remained stable after treatment and then revealed disease progression on 0.5 point EDSS after 12 months. The 2nd patient after treatment in 2 months improved from 5.0 to 4.0 points EDSS and stabilized in that state for 12 months. The 3d patient after treatment improved from 3.0 to 2.5 points EDSS and was in that state for 3 months, then relapse occured and EDSS score returned to 3.0 point and remained in that level. Immunological monitoring of serum showed antibody titres decline to myelin basic protein, reduction of $CD3^+CD4^+$Th, $CD3^-CD16+NK^-$, $CD19^+$ Bs, IL-2 ($CD25^+$), $CD95^+$apoptotic cells and HLA DR^+ cells, and absolute and relative increase of T regulatory cells.
Conclusions: 6 single injections of autologous immature DCs are well tolerated and followed with the not long but clear anti-inflammatory immunological response in MS.

Symptom management

P904

Cingulum bundle alterations underlie subjective fatigue in multiple sclerosis

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Background: Subjective fatigue represents a frequent and ill-understood symptom of Multiple Sclerosis (MS). Despite its prevalence, however, published results regarding its neural bases are highly heterogeneous pointing toward frontal, parietal and deep grey matter areas as well as to callosal and associative fibers as the possible neural correlates of fatigue perception in MS. Here we propose that this heterogeneity could be re-framed in a coherent model under a connectionist approach, i.e. trying to identify a discrete neural network as the neural underpinning of subjective fatigue.
Objectives: To explore the neural basis of MS-related fatigue using a connectionist paradigm.
Methods: Seventy-seven patients with relapsing-remitting MS were included in this study and asked to undergo diffusion MRI and subjective fatigue levels evaluation with the Modified Fatigue Impact Scale (MFIS). MFIS scores were correlated with local Fractional Anisotropy (FA) values using a whole-brain voxel-wise approach. The JHU white matter atlas included in FSL was the used to identify if any of the included tracts presented with a significant overlap with the clusters identified in the voxel-wise analysis of diffusion data.

Lastly, we used the Network Modification (NeMo) package to identify the potential loss of connectivity due to damage in the aforementioned clusters (i) to confirm the results of the atlas-based evaluation of connectivity loss and (ii) to better characterize the pattern of loss of connectivity resulting from white matter damage
Results: The voxel-wise regression analysis with fatigue scores revealed a significant association between structural damage and fatigue levels in two discrete white matter clusters, both included in the left cingulate bundle. The connectivity analysis revealed that damage in these clusters was associated with loss of structural connectivity in the anterior and medial cingulate cortices, dorsolateral prefrontal areas and in the left caudate.
Conclusions: Our data point to the cingulum bundle and its projections as the key network involved in subjective fatigue perception in MS. More generally, these results suggest the potential of the connectionist framework to generate coherent models of the neural basis of complex symptomatology in MS.

P905

Long-term, sustained safety and efficacy of repeat onabotulinumtoxinA treatment in multiple sclerosis patients with neurogenic detrusor overactivity

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Background: Multiple sclerosis (MS) patients often have neurogenic detrusor overactivity (NDO), which can result in urinary incontinence (UI). Intradetrusor onabotulinumtoxinA (onabotA; BOTOX®; Allergan, Inc.) has been shown to be well tolerated and effective in the treatment of NDO due to MS or spinal cord injury in two phase 3, randomized, double-blind, placebo-controlled trials in patients who were not adequately managed by an anticholinergic. A large, multicenter, extension study has been conducted to investigate the long-term efficacy and safety of repeat onabotA treatments.
Objectives: To present the final long-term efficacy and safety results of repeat onabotA in the cohort of MS patients.
Methods: Patients who completed a phase 3 trial of onabotA were invited to continue to receive multiple intradetrusor onabotA injections (200U or 300U) in the extension study. Patients were treated on an 'as needed' basis (based on patient request and investigator assessment of predefined retreatment criteria), so the number of treatments needed during the study differed between patients. Change from study baseline (BL) in UI episodes/day, proportion of patients with 100% reduction in UI; duration of effect (time to request for retreatment), adverse events (AEs) and initiation of CIC were assessed.
Results: 231 MS patients received ≥1 onabotA treatment over 4 years. Repeat onabotA treatments consistently reduced the number of UI episodes/day; mean reductions from BL at week 6 following onabotA were consistent, ranging from -3.6/day to -3.9/day (onabotA 200U) over 5 treatments. 45.2-61.9% of patients achieved complete continence at week 6 following onabotA 200U over 5 treatments. Median duration of effect was 36.3 weeks in MS patients with complete treatment cycles (200U). Efficacy results for onabotA 300U were comparable. Most common AEs (onabotA 200U) were uncomplicated urinary tract infection (58.3, 47.4, 47.6, 40.2, and 34.4%; cycles 1-5, respectively) and urinary retention; rates were higher in the 300U group. De novo CIC rates were 28.8, 4.2, 7.1, 0, and 0% in the 200U group (cycles 1-5, respectively) and were higher with 300U. Annualized MS exacerbation rates ranged from 0.03-0.15 (200U). Discontinuation rates due to AEs/lack of efficacy were low (3.3%/1.7%).
Conclusions: Repeat onabotA treatment consistently reduced UI in MS patients with NDO who were not adequately managed by an anticholinergic, with no new safety concerns identified over 4 years' follow-up.

P906

Prolonged-release fampridine enhances physical activity during everyday life in patients with multiple sclerosis (FAMPKIN)

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Background: Treatment with prolonged-release fampridine has shown beneficial effects on walking ability in a subset of patients with multiple sclerosis (MS). Not only walking speed (Goodman and colleagues), but also additional aspects of the gait such as endurance and coordination were found to be improved by prolonged-release fampridine (FAMPKIN-study; NCT01576354).
Objectives: In the present sub-study of FAMPKIN, we were interested whether fampridine influences the physical activity of patients with MS in their everyday life.

Methods: This phase II study with cross-over design enrolled 55 patients with MS (34 woman, 21 men; age: 48.4 +/- 9.7 years; median EDSS = 4.5). Physical activity of patients was examined during two double-blind treatment phases (6 weeks each), in which the patients were either treated with fampridine (10mg twice daily) or placebo. Patients were equipped with an actimeter device fixed on their more impaired leg. The device recorded acceleration data with a sampling rate of 32Hz and averaged the data over 15 second intervals. Physical activity was measured over a period of 14 days during both treatment phases. The study population was classified into fampridine responders (n=17) and non-responders (n=30) based on criteria defined by Goodman and colleagues (Lancet, 2009).
Results: Physical activity was not altered by fampridine in the total study population (n=55). In contrast, the group of fampridine responders revealed a significant increase in the summated daily activity (P = 0.0261, paired Student's t-test) and in the average activity count per 15 second interval (P = 0.0179). These fampridine-related changes in physical activity were not observed in the group of non-responders (P = 0.8288 and P = 0.9139 respectively). The total time of activity during the waking phase was not different between the fampridine and placebo treatment phase for any group, thus implying that the intensity rather than the time of activity is primarily modified by fampridine in patients with MS.
Conclusions: These results demonstrate for the first time that fampridine is able to enhance the physical activity of patients with MS during everyday life. This data emphasizes the clinical relevance of the fampridine treatment for daily living of patients with MS-associated gait deficits. Furthermore, this data indicates that the treatment-induced improvements observed in the FAMPKIN study (walking speed, endurance, coordination etc.) might positively affect the quality of life of patients with MS.

P907

Thalamic dysfunction is associated with fatigue in patients with multiple sclerosis: a graph theory study

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Background: Fatigue affects a large proportion of patients with MS. The definition of its physiopathology might contribute to develop tailored treatments.
Objectives: In this study, we explored abnormalities of large-scale brain networks (connectome) in MS patients with fatigue, using resting state (RS) functional MRI (fMRI) and graph theory.
Methods: Graph theoretical analysis was applied to RS fMRI data from 64 MS patients with fatigue (F) according to the Fatigue Severity Scale. As control groups, 60 MS patients without fatigue (NF) matched for disease duration and brain T2 lesion volume with F-MS patients and 59 gender and age-matched healthy controls (HC) were included. Functional connectivity between 116 cortical and subcortical brain regions was estimated using a bivariate correlation analysis. Small-worldness properties were tested by comparison with matched random networks. Hubs were defined as regions having either degree or betweeness centrality one standard deviation greater than network average. Between-group differences of global and local network metrics were investigated using ANOVA models.
Results: Small-worldness (i.e., high clustering and short paths) was verified in all study groups. All global network parameters were significantly altered in F-MS patients and NF-MS patients compared with HC, with no significant differences between F- and NF-MS patients. The cerebellum (right lobule VI and bilateral crus I), and bilateral middle and inferior temporal gyri were hubs in all study groups. F- and NF-MS patients lost hubs in the bilateral anterior cingulate cortex and cerebellar regions (lobule VII-VIII, crus II). F-MS patients also lost hubs in the thalami and middle cingulate cortex. Compared to HC, F- and NF-MS patients had a decreased degree in the bilateral caudate nucleus. F-MS patients also experienced a decreased degree in the bilateral thalamus when compared to HC and in the left thalamus when compared to NF-MS patients. In MS patients, nodal degree of the left thalamus was significantly correlated with FSS score (r=-0.19, p=0.04). No correlations were found between regional network measures and clinical (disability and disease duration) and conventional MRI (T2 lesion volume and normalized brain volume) variables.
Conclusions: Fatigue in MS is related to a functional disruption of the thalamic connector, which should be the target of potential therapeutic interventions.

P908

Kinematic analysis of fampridine-induced modifications of the gait pattern in patients with multiple sclerosis

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Background: Although multiple sclerosis (MS) often leads to an impairment of ambulatory function, the options to treat these signs are restricted to physiotherapeutic and anti-spastic therapies. Previous studies demonstrated the beneficial effect of prolonged-release fampridine on walking speed in patients with MS.
Objectives: We aimed to elucidate the modifications of the gait pattern which underlie the improved walking function under fampridine treatment.
Methods: Many gait parameters of coordination, stability and range of motion were examined by detailed kinematic and kinetic gait analysis. 55 patients with MS (34 women, 21 men; age: 48.4 +/- 9.7 years; median EDSS = 4.5) were enrolled in a phase II, double-blind, placebo-controlled study with cross-over design. Kinematic and kinetic data of gait were assessed during two double-blind treatment phases (6 weeks), in which patients were treated either with placebo or fampridine (twice daily 10mg).

According to the criteria defined by Goodman and colleagues (Lancet, 2009), the study population was classified into responders (n=17) and non-responders (n=30) based on the timed 25 foot walk test (T25FW).
Results: For all patients, clinical gait tests (T25FW, 6 minute walk test [6MWT], timed up and go test [TUG]) showed an

improved gait performance during treatment with fampridine as compared to placebo. This effect was augmented in the group of fampridine-responders (paired Student's T-Test: T25FW: P=0.008; 6MWT: P< 0.0001, TUG: P=0.0164).

Detailed kinematic and kinetic gait analysis demonstrated heterogeneous modifications of the gait pattern. Gait profiles including parameters such as stride length, step width, and range of motion of different joints revealed substantial fampridine-induced gait modifications in most responders, however, the changes were different between the individual subjects. Summarized, modifications of the gait pattern were most frequently found in proximal leg joints (hip and knee), whose range of motion was increased under fampridine. The motion of the trunk during walking was smaller during the fampridine treatment, implying an increased dynamic stability.

Conclusions: The kinematic and kinetic data imply that fampridine induces differential gait modifications that are likely to depend on the individual deficits of a given patient. We hypothesize that the observed modifications of the gait pattern underlie the clinical improvement induced by the fampridine treatment.

P909

Clinical features of patients presenting with seizures at the time of multiple sclerosis diagnosis: a 12-year retrospective case-control study

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Background: Seizures occur at about a rate of 2-4% in the MS population. The proportion of patients who have a seizure as part of the first clinical manifestation leading to an MS diagnosis varies substantially among different studies and is inconsistently reported.

Objectives: To compare the clinical features of patients who experienced a seizure as part of the initial clinical presentation leading to an MS diagnosis to those who developed seizures after the diagnosis of MS.

Methods: Restrospective chart review of patients diagnosed with both Multiple Sclerosis and seizures or epilepsy. Patients were diagnosed with MS based on the McDonald criteria.

Results: 138 charts were identified over a 12-year old period (01/2000-12/2011). 83 were excluded due to not fulfilling the inclusion criteria. 55 cases were included in the final analysis. 37 were female (female-to-male ratio of 2:1). 34 (62%) patients had RRMS, 17 (31%) had SPMS and 4 patients (7%) had PPMS. Group 1 comprised 38 cases that developed seizures following a diagnosis of MS. Group 2 consisted of the remaining 17 patients (31%) who had a seizure that led to the diagnosis of MS. Among the Group 2 patients, 10 experienced a seizure as part of the initial clinical presentation, while the other 7 cases (13%) were determined to have had their first MS-related symptom prior to seizure onset by a median time of 42 months. Average age at MS diagnosis did not differ between Group 1 and 2 (30 vs 34 years, respectively, p = 0.11). No significant difference was detected in gender distribution (11/38 males in group 1 vs 7/17 males in group 2, p = 0.38) nor in the proportion of patients with stable MS on last follow up (18 in group 1 vs 11 in group 2, p = 0.23). Further comparisons of disease characteristics will be presented at the meeting.

Conclusions: Seizures at the onset of MS are not uncommon and occurred in almost one fifth of MS patients who developed seizures in our cohort. The 2:1 female-to-male ratio of seizure incidence in MS was equal to the gender ratio of MS incidence in the general population suggesting that there is no gender-specific epileptogenic factor in MS patients with seizures. Our study is the first to compare the clinical features of MS patients presenting with seizures at the time of their MS diagnosis versus those who develop seizures subsequent to their MS diagnosis. Large-scale prospective studies following MS patients and evaluating them for seizure onset are needed to shed more light on the intriguing intersection of MS and seizures.

P910

I. A Multi-center investigation of elevated body temperature in RRMS patients vs. healthy controls

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Background: Elevated body temperature in people with multiple sclerosis (MS) relative to healthy individuals was recently reported for the first time (Sumowski & Leavitt, 2014). This body temperature difference was observed in the absence of environmental manipulations (e.g., hot baths and exercise), importantly suggesting endogenous processes that may reflect disease-related inflammatory events.

Objectives: Here, our aim was to replicate the finding of elevated body temperature in MS patients relative to healthy controls in a large sample comprising data from three separate MS centers in two countries.

Methods: Core body temperature was measured in 111 relapsing-remitting MS (RRMS) patients and 84 age-matched healthy controls with an infrared aural thermometer (Braun ThermoScan IRT 4520). Both groups were 70% female in composition. Average disease duration for the MS group was 12.7± 8.2 years.

Results: MS patients had higher core body temperature than matched healthy controls (p=.003). Average body temperature for MS was 37.00°C ± 0.31, and for HC was 36.87°C ± 0.32. The group temperature difference remained after controlling for age, sex, and recruitment center (p< .001). All effect sizes were medium.

Conclusions: Here, we replicate the finding of elevated body temperature in RRMS compared to HC in a large, multi-center sample comprising data from three sites and two countries. Elevated body temperature in MS was recently shown to be linked to increased fatigue (Sumowski & Leavitt, 2014), therefore supporting the use of antipyretics and cooling garments as effective treatments for fatigue. Future investigations of antipyretics in the treatment of fatigue are warranted. Body temperature fluctuations in RRMS may signify fluctuations in disease-related inflammatory processes, including subclinical exacerbations. As such, monitoring body temperature may represent a simple means of detecting quiescent disease activity, a link that remains to be tested in future work.

P911

Detailed characterization of the effects of prolonged-release fampridine on walking function in patients with multiple sclerosis (FAMPKIN)

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Background: Recent studies demonstrated the beneficial effects of fampridine (4-aminopyridine) on gait velocity in a subset of patients with multiple sclerosis (MS). Fampridine blocks voltage-gated potassium channels leading to enhanced signal conduction in demyelinated nerve fibers.
Objectives: The FAMPKIN study (http://clinicaltrials.gov) aimed to characterize the effects of prolonged-release (PR) fampridine on different modalities of ambulatory function including muscle strength, stability and coordination. FAMPKIN designed as a phase II, double-blind, randomized and placebo-controlled cross-over study assessed gait function during two double-blind fampridine or placebo treatment phases (each 6 weeks).
Methods: Walking was investigated using different clinical tests, questionnaires and detailed kinematic-kinetic gait analysis. 55 patients with relapsing-remitting, primary- and secondary-progressive MS completed the study (34 women, 21 men; age 48.4 +/- 9.7 years; median EDSS = 4.5). Patients were classified into responder and non-responder according to the criteria used by Goodman *et al.* (Lancet, 2009). 31% (n=17) of all participants were fampridine-responder, 5% (n=3) were placebo-responder, and 9% (n=5) met the responder criteria in both phases.
Results: During fampridine treatment, gait velocity (timed-25-foot walk) was increased by 5.8% in the total population (n=55), 12.3% in the pure fampridine-responder subgroup (n=17), and by 3.3% in non-responders (n=29) compared to the placebo treatment. Walking endurance (6-minute walk test) was improved by 8.5% in the total population, 13.5% in responders, and 6.1% in non-responders. The timed-up-and-go showed an improvement of 2.6% in the total population, of 11.2% in responders and a worsening of -2% in non-responders. Stationary and dynamic measures of stability did not reveal significant changes induced by fampridine. Gait profiles consisting of multiple kinematic and kinetic parameters demonstrated heterogeneous, fampridine-induced gait modifications. The differential changes in the gait pattern among patients most probably reflect individual improvements of the gait pattern depending on the specific deficits of each patient.
Conclusions: The present study demonstrates beneficial effects of fampridine on different aspects of locomotion and indicates the clinical relevance of this treatment for a proportion of patients with MS.

P912

Prevalence of pain in multiple sclerosis: a multicenter Italian study

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Background: Multiple Sclerosis (MS) is a chronic inflammatory demyelinating disease of central nervous system (CNS). In MS pain represents one of the most common symptoms, estimated to occur in 29% to 86% of patients. Pain syndromes are mainly classified into neuropathic pain, cause by injury anywhere in the nervous system and somatic pain, due to an appropriate physiological response when nociceptor are activated.
Objectives: The aim of the study is to assess the presence of pain in MS patients, in a multicentre cross-sectional study and its relationship with a specific pain questionnaire (DN4).
Methods: Data was collected in a multi-centre, cross-sectional study involving 6 italian MS centres using a face-to-face structured questionnaire compiled by a neurologist. 200 patients/centres with a diagnosis of MS or CIS over a period of 6 months were interviewed. The only exclusion criterion was a relapse in the last month before the beginning of the study. The questionnaire included demographic data, year of symptom onset and diagnosis, Expanded Disability Status Scale (EDSS), clinical course, Beck Scale, QoL36, Disease modifying treatment, pain therapy, the DN4 questionnaire for differential diagnosis of pain syndromes associated to neuropathic or nociceptive pain. In subjects with DN4 greater than 4 NPSI scale were administered. We considered only symptoms present at the time of the interview. All data were registered in an ad-hoc database.
Results: Out of 1253 subjects interviewed, were 835 female (66,6%) and 418 male (33,4 %), mean age was 33,9 years, mean disease duration 8 years, 916 (73,1%) subjects had relapsing remitting disease course, 248 (19,8%) were secondary progressive, 55 (4,4%) were primary progressive and 30 (2,4%) were CIS. Mean EDSS score was 3.2. 458 subjects (36,6%) reported at least one painful symptom, of whom 173 with DN4≥4 nociceptive pain and 284 with DN4< 4 neuropathic type (Lhermitte, Trigeminal Neuralgia, Lower limb). Multivariated analysis showed a significant difference for for age, EDSS, disease duration and disease course and specific scale score for pain between pain and no pain group. In patients with DN4 >=4, NPSI had a mean of 41 with values ranging between 5 and 97.
Conclusions: This study underlines the relevance of pain in the clinical history of MS and the importance to use a specific pain questionnaire such as DN4.

P913

Restless legs syndrome, sleep quality and depression in relapsing remitting multiple sclerosis and correlations with cervical spinal cord damage

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Background: There is a growing interest in restless legs syndrome (RLS) and sleep disorders in multiple sclerosis (MS). Spinal cord (SC) damage represents a significant risk factor for RLS in MS patients in several studies. However, the relationship between RLS and SC lesions in MS is still unclear.
Objectives: The aim of this study is to investigate the sleep quality (SQ) and the prevalence of RLS and depression in patients with relapsing remitting multiple sclerosis (RRMS).
Methods: A total of 93 RRMS patients (67 females, 26 males, mean age 34,5 ± 8,6) were enrolled in the study. The diagnosis of

RLS was based on International Restless Legs Syndrome Study Group Criteria. SQ was assessed by the Pittsburgh Sleep Quality Index (PSQI), excessive daytime sleepiness (EDS) by the Epworth Sleepiness Scale (ESS), depression by the Beck Depression Inventory (BDI) and clinical disability by the Expanded Disability Status Scale (EDSS). The severity of depression, SQ and the prevalence of RLS were compared between the patients with and without cervical SC lesions. The correlation of RLS with SQ, depression and clinical disability was also investigated.

Results: Patients aged 18 to 49 years with disease duration of 1 to 20 years (mean 4,7 years ± 3,7) were evaluated. Forty patients (43%) had only brain lesions, whereas 53 patients (57%) had coexistent cervical SC lesions. Patients with SC damage exhibited poorer sleep (P = 0.03) and tended to have greater depressive symptoms (P = 0.06). Thirty subjects (32,3%, mean age 34,1 ± 8,5) met the criteria for RLS and RLS was significantly higher in patients with cervical SC lesions (P = 0.04). RLS was also associated with poorer sleep (P = 0.0001) and higher rates of depression (P = 0.0001). By comparing the RLS group with the group without RLS, no significant differences were found in MS duration, gender and the presence of upper motor neuron signs. But RLS group presented higher EDSS scores. EDS was detected in only 5 subjects (5,3%) and was not associated with any of the studied parameters.

Conclusions: RRMS with the cervical SC involvement was significantly associated with RLS. Changes in the distal A11 neuron synapses, main source of dopaminergic input into the SC and damage of this neural pathway due to MS lesions may lead to spinal network changes consistent with RLS, but larger studies are needed to clarify the pathological mechanism. RLS has a negative impact on SQ and mood. High awareness of RLS in MS is required.

P914

Reassessment of Lhermitte's sign in multiple sclerosis

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Background: The reliability and diagnostic value of Lhermitte's sign in multiple sclerosis (MS) has not been fully established.

Objectives: The purpose of this study was to determine the clinical, neurophysiological and neuroradiological correlations of Lhermitte's sign in a cohort of MS patients and reassess the relevance of this phenomenon in the clinical history of the disease.

Methods: A prospective study of 694 patients with MS and 110 age-matched healthy adults were evaluated by a structured questionnaire that included basic demographic data, age of onset, clinical characteristic of the disease, and the inquiry of Lhermitte's sign. Cranial and spinal magnetic resonance imagings (MRI) and median and tibial somatosensory evoked potentials (SSEP) were performed at the same time.

Results: One hundred-twelve (16%) patients reported to have Lhermitte's sign, 582 (84%) patients did not experience Lhermitte's sign during their disease duration ($P < 0.026$). No correlation was found between Lhermitte's sign and age, gender, EDSS, and disease duration. In 88% out of patients with Lhermitte's sign had a demyelinating lesion on the cervical MRI. In negative Lhermitte's sign group, 64% patients had a positive MRI. SSEP conductions were delayed in 92% of patients with positive Lhermitte's sign and in 70% of patients with negative Lhermitte's sign. Regarding the data, a significant correlation was found between MRI lesion and Lhermitte's sign ($P < 0.000$), and between SSEP abnormality and Lhermitte's sign as well ($P < 0.000$).

Conclusions: This is the first study investigating the reliability of Lhermitte's sign in MS patients with cervical MRI and SSEP in a large, controlled cohort. This study underlines the relevance of this phenomena with neuroradiologic and neurophysiologic abnormalities.

P915

Restless leg syndrome and multiple sclerosis

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Background: Multiple sclerosis (MS) is one of the diseases that may cause disability in younger age group.

Objectives: In this study, relationship between the frequency and presence of RLS, which affect life quality in MS patients with the symptoms of disease, such as depression and fatigue, and with other clinical characteristics has been investigated.

Methods: 177 patients with final MS diagnosis according to McDonalds criteria revised in 2005 and 163 healthy volunteers were included in this study. Clinical and demographic characteristics of MS patients were taken from patient anamnesis and MS outpatient clinic registration system. Complete blood count, iron, ferritin, free T3, free T4, TSH, creatinine, vitamin B12 and folic acid values of patients for last 6 months were taken from the automation system of our hospital and the patient records in the MS outpatient clinic. Patients and control group were assessed according to fatigue severity scale, Beck depression scale and international RLS severity assessment scale, for those who meet international RLS diagnosis criteria.

Results: RLS was found in 59 (33.3%) of MS patients and 21 (12.9%) of control group. Difference between MS and control groups were statistically significant regarding the presence of RLS and average RLS scores. ($p< 0.001$). Fatigue was higher in patient group ($p< 0.001$). Number of cases with significant depression were higher in patient group according to Beck depression scale ($p=0.003$). When MS patients with and without RLS were compared; EDSS averages, average number of attacks, fatigue and depression were significantly higher in the MS-RLS + group ($p< 0.05$). For clinical involvement, the presence of optic neuritis, tetraparesis, paresthesia and tremor was significantly higher in MS-RLS + patients. There was no significant difference between two groups regarding the presence and number of lesions determined in brain MRI and spinal MRI, GUP abnormality, presence of CSF oligoclonal band, immumodulator treatment used and serum biochemical laboratory findings. Mean Beck depression

scores and mean fatigue scores were significantly higher in MS-RLS + cases compared to control RLS + cases.
Conclusions: RLS was frequent in MS patients. RLS was associated with pyramidal, cerebellar, optic and sensual involvement that may be observed in MS patients. High attack frequency, high EDSS and fatigue were associated with RLS. Fatigue and depression were frequent in MS patients as seen in previous studies.

P916

The relationship between objectives parameters of sleep and measures of fatigue, depression and cognition in multiple sclerosis

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Background: People with multiple sclerosis (MS) report more difficulty with poor sleep, fatigue, sleepiness, mood disturbance and cognitive dysfunction compared to healthy controls. Additionally, some studies have reported sleep disturbances including reduced sleep efficiency (SE), periodic limb movements of sleep (PLMS), and obstructive sleep apnea (OSA) in MS patients. The inter-relationship between symptoms and objective measures of sleep in MS patients is poorly understood.
Objectives: The objectives of this study were to document objective parameters of sleep measured by polysomnography (PSG) and multi-sleep latency tests (MSLT) in a population of MS patients experiencing fatigue or sleepiness and to determine whether they correlate with measures of fatigue, sleepiness, mood or cognition.
Methods: Fatigue, sleepiness, depression, and cognition were assessed in 37 patients by performance of the Modified Fatigue Impact Scale (MFIS), Fatigue Severity Scale (FSS), Epworth Sleepiness Scale (ESS), Visual Analog Scale - Fatigue (VAS-F), Beck Depression Index (BDI) and NeuroTrax™ cognitive tests during the screen visit. Within one month, 32 patients reporting fatigue or sleepiness underwent PSG followed by MSLT.
Results: This MS patient cohort averaged an SE of 75.1%, Wake after Sleep Onset (WASO) of 66.2 minutes, Sleep Onset Latency (SOL) of 43.36 minutes, and Multi-Sleep Latency of 10.43 minutes. No Stage 3 sleep was recorded in 10 subjects and no Rapid Eye Movement (REM) sleep was recorded in 4 patients. An elevated PLMS Index was observed in eight of the 32 patients. An Apnea Hypopnea Index (AHI) > 5 was observed in 12 of the 32 patients. Neither SE nor WASO correlated with fatigue or sleepiness. On cognitive tests, SE correlated with the global score as well as the executive function and information processing subscales. The duration of WASO negatively correlated with the verbal function subscale.
Conclusions: Overall, 30 of the 32 MS patients reporting fatigue or sleepiness had objective evidence of a sleep disturbance on either PSG or MSLT. Over 70% of the patients experienced more than one disturbance. PSG should be considered in MS patients reporting either fatigue or sleepiness to rule out a treatable sleep disturbance.

P917

A retrospective description of the use of nabilone in UK clinical practice

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Background: Nabilone, a synthetic cannabinoid analogue, is licensed in the UK for chemotherapy induced nausea and vomiting but is thought to be frequently used off-label for intractable chronic pain and spasticity in conditions such as multiple sclerosis (MS).
Objectives: To describe nabilone use in the UK for licensed and non-licensed indications.
Methods: This multicentre observational study was undertaken in two phases; a 'pilot study' (3 NHS Trusts) and an 'extension study' (5 Trusts). Sites were selected following identification by the manufacturer as high-prescribing centres. Data were collected retrospectively from medical records of 250 patients prescribed nabilone between 1st January 2005-March 2013, mean observation period 31.1 (standard deviation (SD) 22.8) months.
Results: MS was the most common distinct condition for which nabilone was prescribed (19%, 48/247 patients with data recorded). Results are presented here for these 48 patients. Where n< 48, data were missing from medical records. 67% (32/48) patients were female. At nabilone initiation mean age was 51.9 (SD 9.0) years and prior symptom duration 6.9 (6.7) years (n=33); 76% (28/37) patients had been prescribed ≥3 other classes of medication for the same symptoms. Nabilone was most commonly prescribed for pain (81%, 29/36) and spasticity (61%, 22/36). 50% (21/42) patients started on 1mg and 38% (16/42) on < 1mg daily. 52% (25/48) patients were recorded as benefitting from nabilone; most common benefits were pain relief (n=19), improved sleep (n=15) and spasticity relief (n=7). 31% (15/48) patients were recorded as experiencing adverse effects, most commonly drowsiness (n=8), fatigue (n=5) and dizziness (n=2). The estimated mean cost of nabilone was £3,296 (SD £3,300)/patient/year (n=42).
Conclusions: Almost all nabilone use was for unlicensed indications. Although most patients prescribed nabilone for MS had experienced symptoms for many years and had been prescribed a number of other medications, over half derived benefit and adverse effects were no different to other CNS-active medications. The cost of nabilone should be considered in light of numbers of prior treatments and the comparatively high cost of other MS medications. Incomplete recording was a problem for many data fields collected in the study. Careful and complete documentation is important, particularly when a drug is used off-license.

P918

Effect of fampridine (4-aminopyridine) on the manual functions of patients with multiple sclerosis

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Background: Patients with Multiple Sclerosis (MS) may exhibit a variety of symptoms, among them limb functions impairment. While over 40 % of MS patients report spasticity and related gait problems as their main complaint, relatively few studies examined hands dysfunction and related pharmacotherapies. Fampridine (4-aminopyridine), a potassium ion channels blocker, has been approved for use in patients with ambulatory deficits. Anecdotal evidences concerning the possible therapeutic effects of fampridine on manual abilities of patients with MS (PwMS) raise the need for thorough investigation.
Objectives: To assess the effect of fampridine on manual functions of PwMS.
Methods: 17 Relapsing Remitting MS patients with ambulatory and manual function deficits treated at the MS Clinic, Carmel Medical Center, Haifa, Israel, were recruited for this study. Lower and upper limbs functions were assessed before, as well as 1 month and 3 months after treatment initiation with fampridine.

Walking ability was evaluated using timed 25-foot walk. Evaluation of manual function was evaluated by: Jamar Dynamometer (grip strength), Pinch Meter (pinch strength in the hand), Nine Hole Peg test (coordination and fine motor skills) and Arthritis Hand Function test (daily activities). In addition, the Computerized Penmanship Evaluation Tool (ComPET) was used to evaluate writing capabilities of the participants. ComPET results and further analysis will be presented at the conference.
Results: 37% of participants presented an improvement of 25% or more in walking speed after one month of treatment, similarly to the observed in previous studies. Statistically significant improvement ($p<0.05$) was found in the manual function tests following 3 months of treatment. Jamar Dynamometer and Pinch Meter showed increase of 20% in grip and pinch strength measures, respectively. Arthritis Hand Function test demonstrated improvement of 17% in daily activities.
Conclusions: These preliminary results, which are part of a broader study with additional patients, suggest that in addition to the known beneficial effects of fampridine on ambulation, also improvement of manual functions, in terms of both muscle strength and daily functions may be expected in PwMS. Results of the overall study group, including analysis also of the ComPET findings, will be presented at the conference.

P919

Involvement of the Obersteiner-Redlich zone of the trigeminal nerve in an exacerbation of multiple sclerosis (MS)

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Background: Facial pain and other sensory abnormalities are frequently seen in patients with MS. The Obersteiner-Redlich zone of the trigeminal nerve root entry zone refers to the area of transition between the myelin-producing oligodendrocytes of the central nervous system and the Schwann cells of the peripheral nervous system in the trigeminal nerve. MRI findings that involve the central intrapontine tract or the Obersteiner-Redlich zone of the trigeminal nerve are seen in approximately 3% to 38% of patients with MS, although imaging abnormalities do not always reliably correlate with clinical symptoms.
Objectives: We present the case of a MS patient with facial sensory symptoms and corresponding imaging findings.
Methods: A 33-year-old woman with a history of relapsing-remitting MS presented with two days of left facial numbness and incoordination. Examination revealed diminished facial sensation, impaired taste sensation on the left anterior tongue, and dysmetria on the left. She underwent MRI brain imaging.
Results: MRI brain showed T2/FLAIR hyperintensity and enhancement of the Obersteiner-Redlich zone that extended to the transcisternal portion of the left trigeminal nerve. Imaging also showed increased T2 signal of the left trigeminal nuclei, particularly the main sensory nucleus in the pons and the spinal nucleus in the medulla. She demonstrated clinical and radiological improvement after treatment with intravenous steroids and plasmapheresis.
Conclusions: Although the immunogenic target of MS is considered to be myelin of the central nervous system, there can also be involvement of the Obersteiner-Redlich transition zone and of the peripheral cranial nerves in MS. This is demonstrated by our patient's clinical and radiological findings.

P920

Efficacy, safety and response rate of nabiximols assessed in an Italian monocentric cohort

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Background: Multiple Sclerosis (MS) patients often suffer from limb spasticity and pain that are not adequately controlled by common antispastic drugs. Nabiximols is a cannabinoid-based drug that proved effective in reducing spasticity and pain in MS.
Objectives: To assess nabiximols efficacy and safety in clinical practice, by evaluating an Italian cohort of MS patients followed at the San Raffaele Hospital in Milan, and to identify clinical features associated with response.
Methods: All patients that started nabiximols at our MS center were enrolled. The following clinical data were collected prospectively at baseline and after 4 and 14 weeks: expanded disability status scale (EDSS), Ambulation index (AI), 10mt walk test (10MWT), numerical rating scale for spasticity (sNRS) and pain (pNRS). After 4 weeks, responder status was defined by at least a 20% reduction in sNRS compared to baseline.
Results: Since September 2013, 74 patients started nabiximols, 32 being females and 42 males (F:M ratio 0.76). Mean age at therapy start was 48,5 years (22,4-76,9) and mean disease duration was 17,5 years (5,4-42). Ten patients had a

relapsing remitting course while 64 had a progressive disease, either primary (11) or secondary (53). At baseline median EDSS was 6.5 and median AI was 6. All patients complained of moderate to severe spasticity, with a mean sNRS of 7,5. Fifty-nine patients reached a 4-week follow-up: 18 subjects (30,5%) were classified as non-responders with a mean 3% increase in sNRS after one month. Two patients (3,4%) were responders based on evidence of reduced spasticity but discontinued treatment because of adverse effects (fatigue, confusion). Thirty-nine patients (66,1%) were full responders, with a mean sNRS reduction of 30% and no relevant tolerability issues. Responder group also showed a significant decrease in pNRS (-30%) and AI (≥1 point improvement in 6 patients). No significant differences in baseline variables were observed between the two groups.

Conclusions: This study confirms nabiximols efficacy in reducing MS-related spasticity and pain; no major adverse events were reported. We observed a higher responder rate (66%) compared to other cohorts described in the literature. This is maybe because many patients started nabiximols based on the evidence of benefit from previous administration of cannabis infusions. We didn't found significant baseline clinical differences in the two response groups, perhaps due to the limited sample size.

P921

Changes in functional impairment in persons with multiple sclerosis treated with dalfampridine

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Background: Functional capacity can be defined as a person's ability to perform social and occupational activities. Impairments which limit capacity may impact functional performance and quality of life in persons with multiple sclerosis (pwMS). Although dalfampridine extended release (D-ER) has been available for clinical use since 2010, its relationship to functional impairment in a clinical setting has not been reported.

Objectives: Accordingly, the objective of the present study was to evaluate D-ER's relationship with functional impairment in pwMS using outcomes feasible for use in a clinical setting.

Methods: PwMS newly prescribed D-ER for routine clinical care were invited to participate in this prospective, observational study. During the 14 week study, participants were monitored immediately prior to starting D-ER (baseline) and at four follow-up time points. Functional measures included the Barthel Index, Patient Determined Disease Steps Scale (PDDS), total Performance Scale score (PS), number of falls in the past two weeks and the use of assistive devices. Fifty-two participants were enrolled and 31 met criteria for analysis. In addition to the analysis of all 31 participants, the data were subdivided into timed walk responders (TWRs; n=21) and timed walk non-responders (TWNRs; n=8) to assess whether the relationship between D-ER and functional outcomes differs between the responder groups.

Results: Examination of participants taking D-ER for the 14 week study period (n=31) showed improvement on the PS (p=.032) and a trend towards improvement on the PDDS (p=.085). Specific, PS improvements and trends observed on mobility (p =.039), fatigue (p =.013), bladder/bowel (p =.058), and tremor/coordination (p=.004) subscales. No changes were found on the Barthel Index, number of falls or use of assistive devices. TWRs also showed improvement on the PDDS (p=.019) and overall PS score (p=.028), fatigue (p=.046), tremor/coordination (p=.030) subscales and trends towards improvement were observed on mobility (p=.066). TWNRs showed a trend towards improvement on PS sensory (p=.102) and tremor/coordination (p=.102) subscales.

Conclusions: Our study assessing the relationship of D-ER and functional outcomes in pwMS showed improvement in the PDDS and overall PS score. Mobility, fatigue, and tremor/coordination subscales showed the most improvement suggesting D-ER is related to improvments in function in a subset of pwMS.

P922

Walking ability and balance in patients with multiple sclerosis treated with prolonged-release fampridine: randomized, double-blind MOBILE study

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Background: Walking impairment is a common disability in multiple sclerosis (MS) and negatively impacts patients' quality of life (QoL).

Objectives: To evaluate the impact of prolonged-release (PR) fampridine tablet (dalfampridine extended release in US) on self-assessed walking ability, dynamic and static balance, and QoL in patients with MS.

Methods: MOBILE was a randomized, placebo-controlled, double-blind, multicenter study. Patients (18-70 years) with progressive or relapsing-remitting MS and EDSS score of 4-7 were treated with PR-fampridine 10mg tablets or placebo twice daily for 24 weeks. Efficacy endpoints assessed at baseline and at Weeks 2, 4, 8, 12, 16, 20 and 24 included change from baseline in the 12-item MS walking scale (MSWS-12), Timed Up and Go (TUG) test, Berg Balance Scale (BBS), 29-item MS impact scale (MSIS-29) physical subscale, and the EuroQol (EQ-5D-5L) VAS and utility index scores. Post hoc statistical analyses compared multiple thresholds of mean improvement over 24 weeks between treatment groups for each of MSWS-12 and TUG using logistic regression adjusted for baseline.

Results: 132 patients were randomized and received study treatment (PR-fampridine n=68, placebo n=64). PR-fampridine treatment vs placebo resulted in greater and sustained mean improvements over 24 weeks (median treatment difference [95% CI]) on the MSWS-12 (-3.27 [-7.59, 1.19]), TUG speed (9.64% [2.05%, 16.48%]), BBS (1.50 [0.00, 2.93]), and MSIS-29 physical subscale (-3.30 [-7.68, 0.98]). After treatment discontinuation at Week 24, benefits returned to baseline levels. No clear differences between treatment groups were observed for

the EQ-5D VAS and utility index results. Over 24 weeks, a higher proportion of patients randomized to PR-fampridine vs placebo experienced clinically meaningful improvements on the MSWS-12 (≥8 point mean improvement): 48.5% vs. 28.1% (P=0.015); and TUG speed (≥15% mean improvement): 47.1% vs. 30.2% (P=0.026), respectively. Safety findings were similar to post-marketing experience.

Conclusions: PR-fampridine treatment vs placebo resulted in sustained improvements in walking, balance, and impact of MS on the patient; these benefits were apparent at Week 2 and maintained throughout the 24-week treatment period until treatment discontinuation. These findings demonstrated additional benefits to those of the Phase 3 studies, with a longer treatment period and a broader range of objective and patient-reported measures of walking ability and balance.

P923

The effectiveness and safety of the intrathecal baclofen for the multiple sclerosis-related spasticity

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Background: Multiple sclerosis (MS) patients may develop spasticity during the course of their disease. The spasticity might impose new functional limitations on the patients and impede their care.

Objectives: We aim to present our 14-year experience on implementing the intrathecal baclofen therapy (ITB) for the symptomatic treatment of spasticity in MS patients.

Methods: A retrospective study was conducted at Evangelismos Hospital to enroll MS patients who had undergone ITB pump implantation from 2000 to 2013. MS patients who had failed oral anti-spasticity treatment (due to undesired side effects and/or unsatisfactory control of their spasticity) were submitted to an intrathecal trial injection of 50mcg baclofen. A pump for the intrathecal administration of baclofen was implanted to the patients who responded favorably to the baclofen trial. (Medstream, Depuy Synthes; Synchromed II, Medtronic) (Archimedes, Codman; IP 2000 V, Tricumed).

Results: Thirty patients (19F, 11M) with a mean age of 47.8±9.79yo (range:23-65) were enrolled. The mean pre-op MAS (Modified Ashworth Scale) was 2.86 (range 2-4), the mean best MAS after the baclofen trial was 1.18.

Data presented here concern 14 patients who fulfill all follow-up criteria. The mean post-op MAS was 0.375. The mean final baclofen dose was 104 mcg/24h (range: 30-320). An average of 5.7 dose adjustments were required to achieve a relative stable dose. One patient died during the follow up from aspiration pneumonia due to MS related generalized paralysis. One patient opted to remove the pump, because his spasticity had improved over time to the point that he did not require intrathecal baclofen. Three patients were reoperated for hardware related complications.

Conclusions: Intrathecal baclofen is a viable option for the symptomatic treatment of MS-related spasticity. Close collaboration with a neurosurgical center that would handle any surgical or hardware related complications is of essence.

P924

Safety, quality of life, and walking ability with PR-fampridine treatment in clinical practice in France: interim results of the LIBERATE study

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Background: Impaired walking negatively impacts patients' lives and is associated with reduced quality of life (QoL) in multiple sclerosis (MS). Prolonged-release (PR) fampridine tablet (dalfampridine extended release in the United States) is the only drug indicated to improve walking in MS.

Objectives: To collect long-term, real-life data on safety, patient-reported QoL, and physician-reported walking ability in MS patients treated with PR-fampridine.

Methods: LIBERATE is a prospective, noninterventional, multicenter, observational study in patients beginning treatment with PR-fampridine 10 mg tablet twice daily in a routine clinical setting. Study enrollment began April 16, 2012 with 128 current sites primarily in Germany and France. Data were collected at the enrollment visit (baseline) and during follow-up visits according to local routine clinical practices; data collected between Day 136 and 270 are reported as Month 6 follow-up. Patients who discontinued treatment were encouraged to stay in the study (patients off treatment). Endpoints included incidence of adverse events (AEs), physician-assessed Clinical Global Impression of Improvement (CGI-I) of walking ability and patient's assessment of impact of MS using the Multiple Sclerosis Impact Scale-29 Items (MSIS-29). Reported here are interim results from the French study sites.

Results: Of the 989 patients enrolled in the French sites, 765 patients with a median EDSS of 5.5 were treated with PR-fampridine. As of January 2014, 403 treated patients had completed the 6-month follow-up (patients on treatment, n=274; patients off treatment, n=129). Of all treated patients (n=765), 265 (35%) discontinued treatment: 167 (22%) due to lack of efficacy and 88 (12%) due to AEs. Patients treated with PR-fampridine demonstrated improvement (mean change from baseline to Month 6 [SD]) in MSIS-29 physical subscale (-10.06 [18.16]; n=218) compared with patients off treatment (-1.10 [17.03]; n=85). CGI-I scores improved in 78%, remained stable in 14%, and worsened in 8% of patients treated with PR-fampridine (n=172) at Month 6, compared with 14%, 63% and 24% of patients off treatment (n=80), respectively. The most common AEs were insomnia (n=68 [7%]), headache (n=38 [4%]), and vertigo (n=30 [3%]) among enrolled patients.

Conclusions: LIBERATE interim data demonstrate that PR-fampridine treatment is well tolerated and associated with improvements in QoL measures over 6-months in a routine clinical practice in France.

P925

Special challenges of neuro palliative care in multiple sclerosis patients

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Background: Palliative care is a special challenge in the treatment of multiple sclerosis patients and an unmet need at present. In contrast to palliative care units, which commonly treated oncological patients the range of symptoms as well as characteristics of patients are different.
Objectives: In previous studies of our neuropalliative care (NPC) treated patients with multiple sclerosis (MS) in our center (MSK) we could demonstrate strong differences compared to primary oncological palliative care units patients (PCU). Especially the high intake rate of other drugs point to a more specialised NPC treatment of MS patients beside pain control.
Methods: Using the HOPE (HOspice and Palliativ Evaluation) database the medical treatment taken at admission, EDSS and demographic data of MS patients of our center collected from January to December 2013 were analysed.
Results: Data of 110 patients (female = 64; mean age = 55 years ± 11.1 years, range 24 - 83 years) of MSK could be analysed. The mean EDSS was 8.4 ± 0.5 and the mean number of active pharmaceutical ingredient (API) per patient was 6.1 ± 3.3, maximum 14 drugs/patient. In the group of the 110 patients an amount of 145 different API was given. Beside the standardised used drug classification of the HOPE database additional classes of drugs, e.g. antispastic (87 patients, 8 different API), antiepileptic (87 patients, 16 different API), urological drugs (86 patients, 13 different API), or antidepressant /neuroleptic drugs (59 patients, 15 different API) were necessary to describe the range of treatment. Additionally a part of these API was given off-label for treatment of special neurological symptoms like ataxia or bladder dysfunction.
Conclusions: Based on the detailed analysis of medical treatment in NPC patients with MS our previous results, which pointed to strong distinctions in MSK patients compared to "classical" PCU patients could be confirmed. In contrast to common used API in PCU, like opioids or laxatives a broad range of other neurological API was given frequently like antispastic, antiepileptic or neurourological API. Our findings clearly reinforced the unmet need of a special training and expertise in NPC of MS patients. Therefore special NPC units under the direction of neurological management are necessary.

P926

Dystonic features are common in MS-related tremor and contribute to its severity

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Background: Multiple Sclerosis-related tremor is common, its presence significantly adds to disability while its treatment is very challenging. Furthermore, the underlying structural and functional correlates of MS tremor are poorly understood. Detailed phenomenological studies are needed to characterize MS-tremor and investigate factors that may predict its severity.
Objectives: To investigate clinical factors that contributes to MS-tremor severity. To determine if dystonia is more common in MS tremor patients than non-tremor controls.
Methods: Fifty-four MS patients, with and without disabling upper limb tremor (uni- or bilateral), matched for age, sex and disability (EDSS and disease duration) were recruited resulting in 39 limbs per group. Cerebellar function was rated using the SARA score. Severity of tremor (including writing and drawing) was rated using the Bain score and dystonia (Global Dystonia Scale (GDS) for proximal and distal upper limbs) using standardized video and handwriting recordings. Samples were shuffled and rated by a blinded movement disorder expert. Dystonic features (including dystonic posturing, mirror movements, geste antagonistes and writer's cramp) were scored for presence and severity using a five-point scale.
Results: Significant associations ($p < 0.05$) between EDSS, distal more than proximal GDS, SARA scores and Bain scores were found. Upper limb GDS were significantly ($p < 0.001$) worse in patients with tremor than those without. Dystonic features including geste antagonistes, mirror dystonia and dystonic posturing were more frequent and more severe ($p < 0.001$) in tremor patients. In a multi-variable model, a 1-unit increase in distal GDS predicted: 0.52-unit (95% CI 0.08 to 0.97), $p = 0.022$) increase in tremor severity; 0.8-units (95% CI -0.07 to 1.6, $p = 0.070$) increase in writing severity and a 1-unit (95% CI 0.48 to 1.6, $p = 0.001$) increase in Bain drawing scores. A 1 unit increase in proximal GDS predicted: 0.93-unit increase (95% CI 0.45 to 1.41, $p < 0.001$) in tremor severity score; 0.99-units (95% CI 0.00 to 1.99), $p=0.050$) increase in writing scores and 1.5-units (95% CI 0.62 to 2.41, $p = 0.002$) increase in Bain drawing score.
Conclusions: The results of this study demonstrate the importance of recognizing dystonia as part of the complex movement disorder that represents MS tremor. The presence of dystonia points to injury in the cerebello-pallido-thalamo-cortical network and could explain the efficacy of onabotulinumtoxin-A therapy in the treatment of MS tremor.

P927

Efficiency sulbutiamine for treatment of fatigue in patients with multiple sclerosis

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Background: Multiple sclerosis is the most common autoimmune inflammatory disease in young adults. One of the most disabling sequelae of this health condition is fatigue, which is reported to affect 53-92% of persons with MS.
Objectives: Increase the effectiveness of diagnosis and treatment of fatigue in multiple sclerosis.
Methods: Twenty-two patients (8 male and 14 female), aged 21 to 45 years, with definite relapsing-remitting MS (McDonald´s criteria 2010) were included in the study. Depression was excluded using Beck´s Depression Inventory (BDI). Sleep quality was assessed using Epworth Sleepiness Scale (ESS). Disease severity was evaluated using the Kurtzke´s expanded disability status scale (EDSS). The activity of process was assessed using magnetic resonance imaging of the brain with contrast Gadolinium. For detecting fatigue was used Krupp´s Fatigue Severity Scale (FSS).

To identify the effectiveness of treatment of fatigue in MS was used Modified Fatigue Impact Scale (MFIS). Sulbutiamine was dosed up to 600 mg/day during 4 weeks. Assessments were performed at baseline and after 2 and 4 weeks. Control group consisted 15 people without treatment fatigue.
Results: From March to October 2013 were treated 22 patients with relapsing-remitting MS. According to the FSS in all patients was diagnosed with fatigue (4.51±0.24). The degree of disability by EDSS scale ranged from 1,5 to 6,5 points. Revealed a reliable correlation between the severity of disability and severity of fatigue (r = 0,4, p ≤ 0,01). Using correlation analysis revealed that the severity of fatigue in patients with multiple sclerosis increases significantly with age. There was no significant relation between the severity of symptoms of fatigue and gender (r = 0,2, p ≤0,05). Fatigue was detected in both patients during the exacerbation and in remission. According to the MFIS average indicators fatigue before treatment were 42.3±0.45. Severity of fatigue after treatment Sulbuthiamine according to the MFIS was significantly lower (32.4±0.21, p≤0.001) compared with those in the control group.
Conclusions: These results demonstrate that a Sulbutiamine treatment may significantly improve fatigue in relapsing-remitting MS.

P928

Pilot randomized control trial of a brief multidisciplinary consultation intervention for treating sexual dysfunction in multiple sclerosis

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Background: Sexual dysfunction (SD) in multiple sclerosis (MS) patients is a common but often overlooked symptom. Estimates of the prevalence of SD in the MS population range between 40-80%. Pharmacological treatment trials have had only limited efficacy; thus, behavioral treatments need to be explored.
Objectives: To conduct a pilot randomized control trial of a brief two-visit multidisciplinary behavioral intervention utilizing a consultation model for adults with MS and sexual dysfunction. Further, to test the hypothesis that the intervention group will experience significant improvements compared to a control group in sexual functioning as measured by the Multiple Sclerosis Intimacy and Sexuality Questionnaire-19 (MSISQ-19).
Methods: Participants were recruited from the Holy Name Medical Center MS Center. A total of 50 participants were recruited, 25 randomized to the intervention group and 25 to the control group. Those in the control group received educational materials on the topic of sexuality and intimacy in MS. Those in the intervention group received two consultation intervention sessions along with their sexual partner, each separated by one month to allow for completion of practice assignments between sessions. In both groups, participants completed baseline as well as 2-month outcome measures. The primary outcome measure is the MSISQ-19. The secondary outcome measure is the Multiple Sclerosis Quality of Life Inventory.
Results: Data were analyzed using a mixed-model ANOVA with MSISQ-19 as the criterion variable. An interim analysis of 24 of the 50 participants (data collection to be completed 7/2014) shows a significant main effect for timepoint, F (1, 22) = 4.76, p = .04. Regardless of treatment group, there was a significant reduction in sexual symptoms from baseline to follow-up. However, there was neither a main effect for treatment group F (1, 22) = 1.38, p = .253, nor an interaction effect (1, 22) = 0.01, p = .920. Thus, preliminary results indicate that both intervention sessions and educational materials reduce sexual symptoms significantly but that there is no difference in their efficacy. Additionally, qualitative reports from both intervention and control participants reveal improved sexual satisfaction and reduced sexual symptoms.
Conclusions: Behavioral and educational interventions have the potential to improve sexual functioning and sexual satisfaction for people living with MS.

P929

Long-term effects of fampridine on walking ability in patients with multiple sclerosis: a phase II, open-label, uncontrolled, monocenter study

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Background: The beneficial effect of fampridine, a blocker of voltage-gated potassium channels, on walking speed in patients with multiple sclerosis (MS) was demonstrated previously in two Phase III trials. Our phase II core study (FAMPKIN), preceding the present study, showed that fampridine not only improved walking speed, but also other aspects of locomotion, including walking endurance and coordination in patients with MS.
Objectives: As the two treatment phases (fampridine or placebo) in the FAMPKIN study were limited to 6 weeks, the present study (FAMPKIN-EXT) aimed to investigate the long-term effects of fampridine on ambulatory performance in patients with MS.
Methods: 53 patients previously enrolled in the FAMPKIN study were included in the extension study (33 women, 20 men; age: 49.7 +/- 9.2 years; EDSS 5.3 +/- 1.2) and treated with prolonged-release fampridine (10mg twice daily; open-label). The results presented here include data from the first year of the extension study. Study visits and measurements were performed at the beginning of the extension study (Screening (S), no fampridine), after 11.5 months of continuous fampridine treatment (V2), and after a 2 week washout period (V3) with washout starting the day after V2. Gait assessments included the timed-25-foot walk (T25FW) investigating walking speed, the 6-minute walk test (6mWT) assessing endurance, and the 12-item multiple sclerosis walking scale (12MSWS) assessing the patients' perspective.
Results: Gait velocity was unchanged between S and V2, however, patients were slower (-15%) at V3 compared to V2 (S: 8.1 +/- 0.9s; V2: 8.2 +/- 0.9s; V3: 9.4 +/- 1.1s; V2 *vs.* V3, P< 0.01). Walking endurance revealed no difference between S and V2, but was reduced by 9% from V2 to V3 (S: 355 +/- 24m; V2: 352 +/- 25m; V3: 320 +/- 25m; V2 *vs.* V3, P< 0.001). The 12MSWS did not reveal a significant difference between S and V3, but showed a significant improvement in self-reported walking ability after 11.5 month of continuous fampridine treatment (S: 38.4 +/- 1.8 pts; V2: 33.4 +/- 1.8 pts; V3: 40.6 +/- 1.7 pts; V1 *vs.* V2 and V2

vs. V3, P< 0.001). The beneficial long-term effects of fampridine assessed with clinical gait tests were accompanied by specific modifications of the gait pattern, as demonstrated by kinematic and kinetic gait analysis.
Conclusions: FAMPKIN-EXT demonstrated positive long-term effects of prolonged-release fampridine in patients with MS.

P930
II. A multi-center investigation of elevated body temperature in relapsing remitting vs. secondary progressive MS
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Background: Higher body temperature in persons with relapsing-remitting multiple sclerosis (RRMS) relative to secondary progressive MS (SPMS) was recently reported for the first time (Sumowski & Leavitt, 2014). Elevated body temperature during the relapsing-remitting stage may reflect disease-related inflammatory processes that characterize RRMS, in contrast to the relative paucity of inflammatory events seen in SPMS.
Objectives: Here, we aim to replicate our finding of elevated body temperature in RRMS compared to SPMS in a large, multi-center sample. This would support elevated core body temperature as an indication of inflammatory processes taking place in persons with RRMS, whereas we expect SPMS to have lower body temperature, given that disease-related inflammatory processes have abated in this disease stage.
Methods: Participants were 98 RRMS patients and 35 SPMS patients. Core body temperature was measured with an infrared aural thermometer (Braun ThermoScan IRT 4520). RRMS group was composed of 34% male, 66% female. SPMS group was 46% male/54% female.
Results: The RRMS group had higher core body temperature than the SPMS group (p=.008). Body temperature for the RRMS group was 36.96°C±0.35, and for the SPMS group was 36.77°C ±0.38. This difference remained significant after controlling for age, sex, and recruitment center (p=.050).All effect sizes were considered medium.
Conclusions: Body temperature differences between RRMS and SPMS has now been shown in a large, multi-center sample comprising data from two countries. Core body temperature is an easily obtained metric that may hold important implications regarding disease-related inflammatory processes. Moreover, the decreased body temperature in SPMS observed here may signify the abatement of inflammatory processes during this disease stage.

P931
Case report: a patient with residual cognitive sequelae after post-malaria acute demyelinating encephalomyelitis (ADEM)
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Background: ADEM is an acute monophasic inflammatory encephalopathy, often precipitated by viral infections and vaccinations.
Objectives: We report a case of severe ADEM two months after a malaria infection, resulting in significant cognitive sequelae. We review the literature on the occurrence of ADEM following *Plasmodium* sp. infections and discuss a possible association between ADEM and post-neurological malarial syndrome (PNMS), an uncommon complication after recovery of malaria.
Methods: A 55 year old man, who was diagnosed and treated for *P. falciparum* malaria infection two months previously, presented with ataxia and confusion.
Results: MRI brain showed multi-focal inflammatory lesions affecting the cortex and subcortical white matter with Gadolinium enhancement. A diagnosis of post-infective acute demyelinating encephalomyelitis (ADEM) was made and he was treated with intravenous methylprednisolone. He recovered gradually thereafter but was left with significant cognitive sequelae.
Conclusions: This case report, alongside with other similar reports, has suggested the distinctions between ADEM and PMNS are unclear. Some cases previously diagnosed as PMNS can actually be explained by ADEM.

P932
Prolonged-release Fampyra® post-marketing experience in the Alsace
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Background: Prolonged-release fampridine (Fampyra®) is an oral form of the potassium channel-blocking, licensed in European Union (EU) for the improvement of walking in patients with multiple sclerosis (MS).
Objectives: In this study, we provide a descriptive analysis of the post-marketing efficacy and safety of Fampyra® in MS patients in our region.
Methods: Descriptive analysis data were extracted from the EDMUS (European database for Multiple Sclerosis, Alsace, France) since the product is available in clinical practice. The efficiency of the fampridine for every patient is expressed in walking time to make the same distance at J0 and J15, and we calculate the average of this walking time.
Results: Four hundred twenty-three patients treated by Fampyra® were included in this study, The patients was aged 28 - 80 years and had a mean EDSS score of 5.9 (SD 0.9) at baseline. The number of patients who met the responder criterion was 295 of 389 (75.8%).

To responder the average change from baseline in walking time 15 days after Fampyra® initiation (J15) was 31.3% (17.8 sec at J15 versus 25.9 sec at Baseline). The most frequent adverse effects seen in our patients were: insomnia 14%, asthenia 12%, and balance disorder 11%.
Conclusions: Our results suggest that 76% response to Fampyra®, perfectly correlated with the MSWS-12 score. The most commonly reported events were that were also identified in the clinical trials.

P933

Long-term treatment of intrathecal baclofen in multiple sclerosis: frequency and severity of withdrawal syndrome

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Background: Spasticity is a frequent disabling symptom in patients with multiple sclerosis (MS). Intrathecal baclofen (ITB) delivered by programmable pump devices represents an important treatment modality for long-term treatment of spasticity.
Objectives: One of a serious adverse event is a withdrawal syndrome after sudden interruption of ITB delivery. We analyzed frequency and severity of this complication in group of MS patients.
Methods: Fifteen MS patients (11 females, age ranged 21-56 years) with implanted 23 ITB pump systems (Synchromed II, Medtronic), representing 86 pump years, were enrolled in this study. Mean follow-up time was 68 months (range 18-118 months).
Results: Four patients developed an ITB withdrawal syndrome on total daily dose of ITB range of 90-180 μg/day. Three patients had catheter-related complications (occlusion, kink and break) and in one patient, pump corrosion was identified after an expertise. Patients developed mostly mild symptoms of ITB withdrawal (increase of spasticity, agitation, pruritus). All patients were monitored with vital functions at emergency unit and immediately received oral baclofen and parenteral diazepam. ITB withdrawal syndrome occurred once per 21,5 years of ITB treatment.
Conclusions: ITB withdrawal syndrome is a life threatening event with relatively low frequency. In our study, the reported events were mostly mild due to prompt treatment regime and relatively low dose of ITB. A prerequisite for the successful application and follow-up of intrathecal pump systems is comprehensive professional training, knowledge of complications and safe administration in the hands of multidisciplinary team in the specialized centers.

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P934

Dalfampridine and MS: clinical benefit and what else? A multimodal approach for an early evaluation of effectiveness

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Background: Dalfampridine (4-AP) is a potassium-channel blocker able to enhance walking speed in Multiple Sclerosis (MS) by improving the action potentials of demyelinated axons in which internodal Kv channels are more exposed.
Objectives: We present results in 20 MS patients (pts) treated with 4-AP and evaluated with clinical, neurophysiological and imaging tools to investigate responsiveness to 4-AP and its mechanism of action.
Methods: Clinical (EDSS, Timed 25-Foot Walk-T25FW, Timed Up-And-Go Test-TUG), neurophysiological (Motor-Evoked Potentials-MEPs) and imaging (Diffusion Tensor Imaging-DTI) evaluations were performed before (T0) and after (T1) 14 days treatment with 4-AP 10 mg bid. MEPs were recorded from Abductor Hallucis of both legs. Changes in DTI parameters, i.e. Fractional Anisotropy (FA), Axial, Mean, and Radial Diffusivities (AD,MD,RD), were investigated with Tract-Based-Spatial-Statistics (TBSS); DTI results were corrected for multiple comparisons by threshold-free cluster enhancement ($p< 0.05$). Wilcoxon test was used for statistical analysis. Pts were clinical responders (CR) when T25FWT decreased by >=20%. MEPs were scored assigning 1 point for each abnormal value in Central Motor Conduction Time (CMCT) and/or mean Amplitude (Amp) in either side [(1)] and pts classified as neurophysiological responders (NPR) when the score improved.
Results: We enrolled 20 pts, median EDSS 5.75 (range 4.0-6.5). Improvement between T0 and T1 was found in T25FW ($p< 0.001$), TUG ($p< 0.001$), CMCT ($p=0.009$), Amp ($p=0.009$) and MEPs score ($p=0.002$) in the whole pts group. Whole WM TBSS analysis revealed MD and RD reduction ($p< 0.05$) in corticospinal tracts (CST), not confirmed by a Wilcoxon test on mean values of DTI metrics in CST. 10 pts were classified as CR and 10 as NPR. CR patients showed T0-T1 differences in CMCT ($p=0.047$), Amp ($p=0.019$) and MEPs score ($p=0.008$). The same results were not found in non-CR pts.
Conclusions: According to previous studies, 50% of clinical responsiveness to 4-AP treatment was found. The effect of 4-AP on demyelinated axons can explain the improvement in MEPs in our pts. Since the increase of RD values is usually considered a measure of myelin loss, its slight but significant reduction after treatment may suggest an early amelioration in water molecules motion on which a potential 4-AP role has to be further investigated.

1. Ruck T et al. Long-term effects of dalfampridine in patients with multiple sclerosis. J Neurol Sci 2014; 337:18-24.

P935

PR-Fampridine improves walking and quality of life in people with MS related severe walking impairment: a pragmatic observational study

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Background: Prolonged release Fampridine has been shown to increase walking speed in ~40% of people within 2 weeks of commencing treatment. Consensus deems a 20% increase in walking speed as clinically meaningful. Although licensed widely this is

conditional in Europe pending long term outcome and quality of life data. In the UK the drug in not routinely prescribed.
Objectives: To assess the effect of PR-Fampridine on walking speed, quality of life and functional goal attainment in peolple with severe MS related walking impairment.
Methods: People with a diagnosis of Multiple Sclerosis and persistent walking impairment were identified and assessed in the MS ambulation clinic at the NHNN, London UK. Baseline demographics, clinical disease course, concomitant medication, lower limb power, spasticity, walking aids, timed 25 foot walk (T25FW), Multiple Sclerosis Walking Scale 12, EQ5D-5L, VAS (Visual analogue scale) walking and a falls log were completed and functional goal(s) of treatment identified. Those eligible were offered a trial of PR-Fampridine 10 mg BD. Subjects were treated for 2-4 weeks and reassessed. Those with a 20% or more increase in walking speed were deemed responders. Outcome measures, other benefits and side effects were recorded at each visit and goal attainment at 3 months.
Results: 137 subjects were assessed of whom 76 were deemed eligible for trial of PR-Fampridine. Mean age 55, 61% female, mean EDSS 6.3.

To date 56 subjects have completed a treatment trial, 39 (69%) were deemed responders (20% increase in walking speed). Responders walking speed increased from 0.8 ft/s to 1.2 ft/s (50% increase) vs 0.94ft/s non-responders ($p < 0.001$). MSWS12 scores decreased by 30 points (~33%) in responders vs 17 non-responders ($p< 0.001$). The EQ5D-5L VAS increased from 53 to 67.5 (27%) ($p< 0.001$), VAS walking increased from 2.4 to 5.2 on treatment ($p< 0.001$). Response was sustained during follow up. Subjects achieved many of their identified goals of treatment and reported reduced fatigue, improved spasticity and memory.
Conclusions: In this cohort with severe MS related walking impairment PR-Fampridine is a useful adjunct improving speed and quality of gait and quality of life measures - EQ5D -5L and VAS walking in clinical responders. In this severely affected cohort the proportion of responders was higher than in clinical trials. Providing an ambulation service also offers non-responders alternative treatments to improve gait and is an excellent model to meet complex care needs.

P936

Spasticity in multiple sclerosis: the relationship with other neurological impairments and overall quality of life

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Background: Previous research has shown that spasticity negatively affects physical functioning and health status, however information on its impact on overall quality of life (QOL) in multiple sclerosis (MS) is limited. Furthermore, qualitative studies indicate that spasticity may affect a number of MS-associated conditions such as fatigue, depression, anxiety, pain and sleep. However these relationships have not been examined in the quantitative studies.
Objectives:

1) To determine the effect of spasticity on overall QOL.
2) To investigate the relationships between spasticity and other neurological impairments associated with MS.

Methods: Demographic details were obtained and a questionnaire pack containing the World Health Organization Quality Of Life-BREF (WHOQOL-BREF), Leeds MS QOL scale (LMSQOL), World Health organization Disability Assessment Schedule (WHODAS), Multiple Sclerosis Spasticity Scale-88 (MSSS-88), Numerical Rating Scale (NRS 0-10) for spasticity, Neurological Fatigue Index - MS (NFI-MS), Hospital Anxiety and Depression Scale (HADS), SF-Qualiveen and Neuropathic Pain Scale (NPS) was given to patients at three UK neuroscience centres.
Results: 260 patients (64.8% response rate) completed the questionnaire pack. 84.8% reported spasticity. 56.1% had moderate (NRS 4-6) or severe (NRS 7-10) spasticity. Patients with spasticity were more likely to be disabled, suffer from depression, have higher levels of fatigue and report more pain, bladder and sleep problems ($p< 0.001$). An association between anxiety and spasticity was weak ($r=0.23, p< 0.05$). Older age, progressive type of MS, higher Extended Disability Status Scale (EDSS) score, unemployment were associated with increased severity of spasticity. Spasticity was found to be a significant predictor of WHOQOL-BREF physical health after adjusting for sociodemographic variables, anxiety, depression, fatigue, bladder dysfunction and pain. Psychological health, social relationships and environment were not predicted by spasticity after controlling for the same factors ($p>0.05$). Depression and fatigue were the strongest predictors of poor QOL, which is consistent with current literature.
Conclusions: Spasticity is very common in MS and is often severe and disabling. There is a strong association between spasticity and fatigue, depression, pain, sleep and bladder problems. The findings suggest that spasticity might directly and indirectly influence overall QOL.

P937

Radiosurgical thalamotomy for treatment of severe multiple sclerosis associated tremor and its impact on quality of life

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Background: Patients with multiple sclerosis (MS) frequently suffer from disabling tremor. Pharmacological treatment has a modest efficiency. Surgical treatments, such as deep-brain stimulation and thalamotomy diminish tremor in patients with parkinsonian or essential tremor. These represent a novel option, but the procedures can be risky and expensive. Radiosurgery provides an alternative option for traditional surgery with potentially less complications. We share our experience with radiosurgical thalamotomy for the management of patients suffering disabling tremor due to MS.
Objectives: To describe the use of thalamotomy as a palliative measure in MS-induced tremor.

Methods: We studied 4 patients with secondary progressive multiple sclerosis. All of them presented important tremor that significantly affected their basic life activities.

A 4-mm isocenter was used to deliver a dose of 120 Gy to the centromedian nucleus of the thalamus. Clinical outcome was assessed with EDSS, quality of life measures, Fahn-Tolosa-Marin (FTM) scale.

Results: All patients experienced an improvement in the severity of tremor. In all patients, this change allowed them to have a better control of their arms and some effective use of them. Mean basal EDSS was 8.6 and after the procedure changed to 8.3. All patients and caregivers reported an impromevent in quality of life, due to a better use of arms. Mean FTM results were 79 points. Two patients (50%) developed contralateral hemiparesis and spasticity after the procedure which required physical therapy.

Conclusions: Radiosurgical thalamotomy is a palliative measure that can be useful in patients with highly-disabling tremor due to MS.

P938

Impact of bladder symptoms on quality of life in multiple sclerosis

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Background: Neurogenic bladder dysfunction is a common feature of multiple sclerosis (MS) but the effect of bladder dysfunction on quality of life in MS remains under-reported. The King's Health Questionnaire (KHQ) is well validated for the assessment of bladder symptoms and their effect on quality of life (QOL). The KHQ contains 2 single item questions addressing general health perception and impact of bladder symptoms; six multi-item QOL domains covering role limitation, physical limitation, social limitation, personal relationships, emotions and sleep/energy. Two further domains score symptom severity and frequency.

Objectives: In this prospective observational study we aimed to apply the KHQ to assess the impact of bladder symptoms on the QOL of patients with MS.

Methods: .41 patients with MS (20 relapsing MS, 21 primary progressive MS) with a mean age of 44.4 (SD 9.4) years (26F, 15M) and a mean disease duration of 6.0 (SD 5.7) years were recruited from a tertiary MS clinic. Patients were clinically evaluated with the Expanded Disability Severity Score (EDSS) and the KHQ. A one sample t-test was used to assess the impact of bladder symptoms on each of the six QOL domains within the KHQ. Linear regression models were used to examine relationships between QOL scores and symptom severity and frequency, when adjusting for age, gender and EDSS.

Results: 37 of 41 patients (90%) experienced some form of bladder symptom when directly questioned. Bladder symptoms negatively impacted on performance of normal roles ($P < 0.001$), physical function ($P < 0.001$), social interactions ($P < 0.001$), personal relationships ($P = 0.007$), emotions ($P< 0.001$) and sleep quality ($P< 0.001$). When correcting for age, gender and EDSS, symptom severity predicted QOL scores in the emotions (standardised regression coefficient (β) = 0.37, P= 0.04), personal relationships (β=0.53, P=0.04), social functioning (β=0.71, $P< 0.001$) and physical (β= 0.46, P=0.004) domains. Symptom frequency predicted scores in the emotions (β= 0.42, P=0.02), sleep (β= 0.68, $P< 0.001$), physical (β=0.38, P=0.02), and role limitation (β=0.54, P=0.002) domains.

Conclusions: Bladder symptoms in MS are common and affect QOL, independently of age, gender and global disability. Bladder symptoms in MS are often amenable to treatment; early recognition of symptoms, leading to early treatment could therefore have a positive impact on patients' QOL.

P939

Sustained release fampridine treatment impacts on the daily physical activity amount in MS people with walking disability

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Background: Sustained-release fampridine (fampridine-SR) is the first drug approved for the improvement of walking in adult MS patients with walking disability (EDSS 4-7). Efficacy is defined according to the percentage of change in walking speed by the Timed 25-Foot Walk (T25FW). Speed improvements of ≥20% are associated with clinically significant changes in self-reported walking ability, using the MSWS-12 score. Nonetheless, the impact of the drug on real daily physical activity has not been evaluated.

Objectives: The aim of this study was to evaluate the impact of fampridine-SR on the daily physical activity amount.

Methods: Patients with a diagnosis of MS, with EDSS between 4 and 7 were proposed to initiate treatment with fampridine-SR (10 mg twice daily). Physical activity was measured using the International Physical Activity Questionary (IPAQ™) which splits patients in 3 categories according to the amount of physical activity (low, moderate and high). T25FW, MSWS-12 and IPAQ™ were measured at baseline, before starting treatment, and 2 weeks later.

Results: Forty-four patients (70.5% female; mean [SD] age, 51 [9.7] years) have been included at the time of the abstract elaboration. Two patients dropped out the study. Thirty four patients (80.9%) were considered responders to fampridine-SR (mean percent change in T25FWT: 30.6%; range: 20%-50.8%). At baseline, the physical activity amount was low in 25 patients (59.5%) and moderate in 17 (40.5%). After 2 weeks on fampridine-SR, physical activity was low in 19 patients (45,3%), moderate in 22 (52,3%) and 1 (2,4%) patient achieved a high level on IPAQ™ score. Statiscally significant differences in scale IPAQ before and after treatment were found in the responder group (p-value=0.005) but not in non-responders (p=0.317).

Conclusions: Increasing the speed walking in MS people seems to impact on the amount of total daily physical activity. Responders to fampridine-SR are more physically active.

P940

Balance disorders and fatigue in multiple sclerosis: dysfunction in different central sensory integration areas

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Background: Balance disorders and fatigue are the most common disabling symptoms in Multiple Sclerosis (MS). Both may occur even at the earliest stage of the disease. Balance depends on the integration of the somatosensory, vestibular and visual information in the central nervous systems (CNS). Central sensory integration is an essential component of effective balance. Fatigue in MS is also associated with central sensory integration deficit.
Objectives: Our objective was to investigate if balance disorders and fatigue are related to the central sensory integration dysfunction.
Methods: We evaluated 44 MS consecutive outpatients with relapsing-remitting disease. We measured balance by modified Sensory Organization Test (mSOT) in the dynamic posturography, Pro Balance Master (NeuroCom). This test provides information about the integration of the proprioceptive, visual and vestibular systems. It consists of four sensorial conditions: eyes open or closed with stable or unstable surface. The sway of patients during the test was converted into a percentage value called Composite Equilibrium score (CE).The CE is calculated based on the weighted average of the percentage for each condition and represents the function of central sensory integration. Fatigue symptom was measured by Fatigue Status Scale (FSS). Statistical analysis was performed by the Spearman correlation test, continuous data was shown by mean (SD) and non continuous data by median (range).
Results: The median EDSS score was 1 (0 - 4.5) and FSS score was 4.6 (1 - 7.5). The CE score was 72(±11.2). A low correlation was found between FSS and CE (rs= -0.407, p=0.006). We also found a low correlation between FSS and each sensorial condition: C1 (rs= -0.376, $p < 0.05$), C2 (rs= -0.364, $p< 0.05$) and C4 (rs= -0.371, $p< 0.05$). There was no correlation between FSS and C3 (rs= -0.296, p= 0.05).
Conclusions: Balance disorders and fatigue had a weak correlation in this study. Our patients showed poor performance in SOT test and important fatigue symptom. Both are related to central sensory integration dysfunction, but most likely in different areas in CNS. Previous studies showed a correlation between imbalance and fatigue when brainstem and cerebellar regions were impaired. The lack of correlation could be explained by the fact that neither of our patients presented dizziness nor cerebellar symptom. We concluded that balance disorders and fatigue are associated with impaired in different sensory integration areas in CNS.

P941

Effects of long-term treatment with prolonged-release fampridine on cognitive functioning in patients with multiple sclerosis

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Background: Non-pharmacological interventions, such as ergotherapy, psychotherapy and cognitive rehabilitation may at least transiently improve cognition, but probably do not prevent the long-term decline of cognitive functions in subjects with multiple sclerosis (MS). In FAMPKIN, the original study of the EXTENSION described here, we found that treatment with prolonged-release fampridine (4-aminopyridine), a potassium-channel blocker, is associated with improvements in walking function, as well as fatigue. Despite increasing awareness of cognitive dysfunction in MS patients, past efforts to develop treatments for cognitive impairment in MS have largely been minimal or ineffective.
Objectives: The aim of the present neuropsychological part of FAMPKIN EXTENSION is to study neuropsychological functions in patients with MS under fampridine therapy.
Methods: 32 MS subjects of FAMPKIN EXTENSION were included and received continuous and unblinded treatment with fampridin PR (twice a day with 10mg) for 11.5 months. Cognitive functioning was assessed 2 times within one year using an established neuropsychological test battery as well as questionnaires to examine depression and fatigue. Tests were administered during continuous treatment with prolonged-release fampridine (V2), and within a 2 week treatment-free interval (V3). Changes between V2 and V3 were evaluated using the Wilcoxon signed-rank test.
Results: There was a statistically significant difference in test-retest z scores for four of six target cognitive measures assessing attention (tonic alertness: V2: Mdn=264.50, V3: Mdn=271.50, z=-2.23, $p< 0.05$; selective attention, errors: V2: Mdn=1.00, V3: Mdn=0.00, z= -2.88, $p< 0.01$), psychomotor speed (Digit Symbol: V2: Mdn=43.00, V3: Mdn=49.50, z=-2.42, $p< 0.05$) and executive function (design fluency: V2: Mdn=18.50, V3: Mdn=13.00, z=-2.80, $p< 0.05$), always indicating better test performance under fampridine therapy. These cognitive effects were paralleled by patients' perception of reduced fatigue, evaluated with a standardized questionnaire (WEIMUS: motor fatigue, V2: Mdn=15.00, V3: Mdn=20.00, z=-2.57, $p< 0.01$; cognitive fatigue, V2: Mdn=10.50, V3: Mdn=14.00, z=-2.57, $p< 0.05$).
Conclusions: Our data show that fampridine PR therapy positively impacts cognitive performance in MS patients by enhancing attention, psychomotor speed and executive function skills. These findings suggest that fampridine PR may reduce fatigue in this patient population.

P942

Individualized dosing of a novel oral Δ9-THC formulation improves subjective spasticity and pain in patients with progressive multiple sclerosis

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Background: Cannabinoids have been shown to improve symptoms of Multiple Sclerosis (MS) including muscle spasticity and pain through modulation of neuronal excitability via presynaptic cannabinoid receptors. Previous formulations of Δ^9-THC are notorious for variable pharmacokinetic profiles, thereby

demanding cumbersome uptitration. The current formulation was developed to overcome this and improve clinical application of Δ^9-THC in the treatment of spasticity and pain in MS.
Objectives: The aim of the present study was to evaluate the efficacy of a novel oral formulation of Δ^9-THC (ECP002A) to treat spasticity, pain and improve functional outcome measures in 24 patients with primary or secondary progressive MS.
Methods: This was a two-phase study consisting of a dose-finding phase utilizing pharmacokinetic- pharmacodynamic (PK-PD) modeling and a treatment phase. In the dose-finding phase, the effect of an escalating oral dose of ECP002A on pharmacodynamic outcome variables was assessed in a randomized placebo-controlled, two-way cross-over trial design. Patients visited the outpatient clinic on two occasions and received an escalating oral dose of ECP002A or placebo. Plasma concentrations of Δ^9-THC and metabolites were measured to generate an individual treatment regimen based on PK and PD. In the 4-week treatment phase, the individual dose was administered three times daily in a randomized placebo-controlled, parallel fashion. During the treatment phase muscle spasticity (Ashworth and subjective spasticity), pain and clinical outcomes (e.g. EDSS, 25Ft Timed walk, GNDS) were measured at baseline, week 2 and 4.
Results: Pain was significantly reduced when measured directly after administration of ECP002A in the clinic, but not when measured in a daily diary. A similar pattern was observed in subjective muscle spasticity. Other clinical outcomes were not significantly different between active treatment and placebo. Cognitive testing indicated there was no decline in cognition after 2 or 4 weeks of treatment due to ECP002A compared to placebo.
Conclusions: ECP002A appears to be effective in reducing subjective symptoms including muscle spasticity and pain in patients suffering from MS when measured immediately after dosing in the clinic. This effect could not be shown when measured retrospectively using a diary.

P943
Heterogeneous etiology of fatigue syndrome in multiple sclerosis patients
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Background: Fatigue is one of the most common and disabling symptoms in multiple sclerosis (MS). It can profoundly disrupt the occupational and social functioning of patients and have a detrimental effect on their quality of life. The etiology and pathophysiology of MS-related fatigue remains unknown.
Objectives: The aim of this study was to evaluate fatigue syndrome in various clinical phenotypes of MS and to study the correlations between fatique and MS clinical parameters as well as immunomodulating therapy.
Methods: We examined 106 patients with clinically defined MS, according to the McDonald's criteria; 82 female; mean age: 38 ±10 years; mean disease duration: 11 ± 7.0 years; mean EDSS: 3.0 ± 1.5. In 63 patients MS course was relapsing-remitting, in 23 - progressive relapsing, in 20 - secondary progressive. Forty-one patients were treated with interferon beta, the other 11 - glatiramer acetate, 18 -mitoxantrone and 6 with natalizumab. All patients completed a questionnaire consisting of: Modified Fatigue Impact Scale (MFIS), Beck Depression Inventory (BDI) and EQ-5D - standardised instrument for use as a measure of health outcome. Patients with severe depression were excluded.
Results: Fatigue syndrome was confirmed in 96% patients. There was no correlation between fatigue and following parameters: patient age, gender, number of relapses and MS course. Fatigue was significantly associated with depression ($P< 0.01$) and pain severity ($P< 0.01$). We found a statistically significant correlation between fatigue and MS duration ($P=0.03$). The study also confirmed the beneficial impact of the immunomodulatory therapies on fatigue syndrome ($P< 0.01$).
Conclusions: Fatigue is very frequent MS symptom independent of the level of disability or clinical disease subtype. Our study shows that the immunomodulatory treatment can improve fatigue in MS patients.

P944
Abobotulinumtoxin a for detrusor overactivity in patients with multiple sclerosis: effect on quality of life and urodynamic parameters
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Background: Neurogenic detrusor overactivity (NDO) is common in patients who suffer from multiple sclerosis (ME). We assessed the efficacy, safety and effects on quality of life of abobotulinumtoxinA (ABX-A) in patients with NDO resulting from multiple sclerosis.
Objectives: Assess the efficacy, safety and effects on quality of life of abobotulinumtoxinA (ABX-A) in patients with NDO resulting from multiple sclerosis.
Methods: Twelve patients with MS underwent their first ABX-A injection for refractory NDO. They had clinical and urodynamic cystometry assessment before and three months after injection. and Incontinence Quality of life total Score were evaluated. The patients were divided in three groups according to treatment efficacy: Full success (total urinary continence, no overactive detrusor), improvement, or total failure (urge incontinence and overactive detrusor).
Results: Incontinence Impact Questionaire 7 scores showed considerable improvement 12 weeks after treatment. 83% of patients had clinical improvement or full success of the treatment with a reduction of their urgency and incontinence. Significant urodynamic improvement after treatment was show on diferente parameters: Maximum cystometric capacity, maximunm detrusor pressure and volume during the first involuntary detrusor contration ($p < 0,05$).
Conclusions: Detrusor Abobotulinumtoxin A injections for refractory neurogenic detrusor overactivity in patients with multiple sclerosis have a consistente effect on bladder control, resulting in sustained improvement in quality of life.

P945
Incidence of hypogonadism and fatigue in male multiple sclerosis patients
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Background: A clinical trial of oral testosterone to treat multiple sclerosis-related fatigue (MSRF) demonstrated a high rate of screen failures due to low baseline testosterone levels, something not previously characterized in this population.
Objectives: This study was designed to determine the frequency of low testosterone and low-normal testosterone levels in male MS patients and assess if testosterone levels correlate with self-reported fatigue.
Methods: Screening data from a clinical trial of testosterone for MSRF were used and consecutive male patients were recruited during clinic visits. Patients on testosterone or anti-androgen therapy or with a relapse in the past month were excluded. The co-primary outcomes were to: (1) determine the percentage of patients with low or low-normal testosterone levels and (2) detect any correlation between testosterone level and MSRF as measured by either the Modified Fatigue Impact Scale (MFIS) or visual analog scale (VAS). Exploratory outcomes assessed for associations with testosterone levels were age, type/duration of MS, disease severity (Expanded Disability Status Scale [EDSS], Timed-25-foot Walk [T25W] and Nine-Hole-Peg-Test [9HPT]) and depression (Center for Epidemiologic Studies-Depression [CESD] scale).
Results: Halfway through the planned recruitment period, 56 patients have been enrolled. The mean (SD) age and duration of MS were 45 (12) and 13 (8.5) years, respectively. 72% of patients had relapsing-remitting MS, 20% secondary progressive MS and 8% primary progressive MS. The cohort had a moderate burden of disability (mean [SD] EDSS 3.5 [2.0]). Testosterone levels were normal in 44%, low-normal in 36% and abnormal in 20%. 46% of patients were fatigued using the standard MSIF cutoff. After accounting for other covariates, testosterone levels were not associated with MSRF (partial-regression coefficients -0.594, p=0.203 for the MSIF and -0.062, p=0.397 for the VAS).
Conclusions: Hypogonadism is common in male MS patients. While an interim analysis does not demonstrate any association between testosterone and MSRF, recruitment is ongoing and final results will be available in September 2014. Analysis of the completed dataset will determine if low or low-normal testosterone levels are associated with fatigue or any other demographic or clinical features of MS. If hypogonadism is common in MS this might reflect an unappreciated important comorbidity and/or another cause of fatigue or reflect a neuroendocrine manifestation of MS itself.

P946

Urinary dysfunction in multiple sclerosis; frequency, characteristics and management in standard care

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Background: Multiple sclerosis (MS) is the most frequent central nervous system demyelinating disease which affects young people, mostly women. Impaired bladder function impacts patient's quality of life in MS.
Objectives: The aim of the study was to determine frequency, characteristics, and management of urinary dysfunction in a population of MS patients, in standard care.
Methods: Our retrospective study concerns two hundred and thirty three MS patients followed in neurologic consultations of the Amiens University Medical Center. Demographic and clinical characteristics, lombar puncture findings and resonnance magnetic imaging (RMI) were analysed. For patients with bladder function impairment, clinical and urodynamic patterns were studied.
Results: In our sample group, the average Expanded Disability Disease Score (EDSS) was 2,5± 2,29. Urinary symptoms occur in 54,1% of our sample group. The age of the patients, duration and seriousness of illness, and progressive forms of MS are associated with urinary symptoms. We didn't find any association between a specific kind of urinary symptoms and MS type.Cerebral RMI matching the Barkoff criteria, greater lesion load and presence of three or more periventricular lesions increase the probability of bladder impairment. Abnormalities of medullar RMI are not associated with bladder impairment. There is no association between clinical and urodynamic findings. Prescription of urological treatment is not systematic and its efficiency is difficult to evaluate because the expected benefits are outweighed by illness progression.
Conclusions: Urinary symptoms are frequent in MS, even in a population with minor disabilities. In a sample group followed in consultations, more than half of them are affected. Diagnosis is based on urodynamics.

P947

Central pain in multiple sclerosis: what we know and what we don't

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Background: Central pain has been defined as "pain initiated or caused by a primary lesion or dysfunction in the central nervous system" by the International Association for the Study of Pain, as opposed to peripheral neuropathic pain or nociceptive pain. Once considered rare, central pain syndromes in multiple sclerosis (MS) are now recognized as highly prevalent and clinically significant.
Objectives: Our objective was to review the literature on the epidemiology, phenomenology, pathophysiology and treatment of central pain in MS and to identify knowledge gaps.
Methods: A review of literature was undertaken using the MeSH terms, "pain" and "multiple sclerosis," "central pain," and "multiple sclerosis" and "hyperalgesia."
Results: Most research has focused on several central pain syndromes in the MS population, including dysesthetic limb pain, L'hermitte's sign, painful tonic spasms, and trigeminal neuralgia. It is widely acknowledged that pain has a significant impact on health-related quality of life in MS patients. Prevalence estimates for any pain syndrome, however, vary substantially - from 26% to over 80%. It remains unclear if prevalence increases with worsening disability and duration of MS. Such variability is in part attributable to the lack of systematic classification for pain syndromes - studies include a wide variety of pain categories (i.e. neuropathic, inflammatory, nociceptive, mixed, psychogenic, etc.). Mechanisms of pain in MS are complex and poorly understood, but new diagnostic procedures including quantitative sensory testing and pain-related evoked potentials may lead to better characterization and classification of central pain in MS. Although a

variety of pain medications are used to treat pain in MS, evidence-based recommendations are lacking as there are only a handful of small randomized controlled studies for treatment of central pain syndromes in MS.
Conclusions: Central pain syndromes are common and significantly reduce quality of life in MS patients. A comprehensive, widely accepted classification of pain syndromes analogous to the International Classification of Headache Disorders would advance epidemiologic studies in the field. There remains a significant need for well-designed studies to clarify the mechanisms, comorbidities, and treatment of central pain in the MS population.

P948

Increased arrhythmic incidence in multiple sclerosis is associated with the location of the demyelinating plaques in the spinal cord

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Background: Arrhythmias have been linked with mortality and morbidity in Multiple Sclerosis (MS). However very few data exist concerning the incidence of serious arrhythmias in MS.
Objectives: Aim of this study was the detection of the arrhythmic burden in MS.
Methods: We enrolled 108 consecutive patients that fulfilled the 2005 revised McDonald criteria for MS. Age at disease onset, disease duration, disease subtype, medication and EDSS score at the time of examination were recorded. MRI and CSF data were collected. Cardiac ultrasound, 24hour-ambulatory ECG recording (Holter), electrocardiography and clinical examination were performed to identify patients with arrhythmia. Ventricular arrhythmias were classified according to Lown criteria; classes 3 and above were classified as complex ventricular arrhythmias (CVA). Patients with history of arrhythmias, use of antiarrhythmics or implanted pacemaker or defibrillator were excluded.
Results: Mean age of the patients was 42.3±9.5 years and 46 (42,59%) males; 65 patients (60,19%) had a Relapsing-Remitting type of MS, 5 (4,63%) had a Primary Progressive type while the rest 38(35.18%) had Seconary Progressive MS. Mean disease duration was 12±13 years and mean EDSS score was 2.5 ± 1.5. All patients had normal echo findings with ejection fraction 59.4±7.5% and no wall abnormalities. 22 patients (20,37%) had abnormal holter with 8 (7,4%) patients having atrioventricular block and 14 (12,96%) had CVA. Premature ventricular beats were detected in 76 (70,37%) patients, of whom 14(12,96%) were classified as CVA 3 having non sustained ventricular tachycardia. A common finding of the abnormal holter patients was the presence of demyelinating lesions in the lower cervical (C5-7) and upper thoracic (Th1-4) spinal cord. No differences were observed in type of MS, disease duration, EDSS and treatment approaches.
Conclusions: Our study shows a high incidence of arrhythmias in the MS population. This finding might be due to the presence of lesions in lower cervical and upper thoracic spinal cord possibly interfering with ANS function. Our findings might have implications for treatment strategies and help to distinguish patients at higher risk.

Treatment biomarkers, pharmacology, and MOA

P949

Natalizumab-induced circulating hematopoietic stem cells (HSC) have higher expansion capacity in MS patients who show significantly increased HSC count

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Background: We have previously shown that different HSC mobilization responses among natalizumab-treated MS patients are associated with differential clinical response to the treatment. We also found natalizumab-induced circulating HSC to be predominantly quiescent, consistent with recent mobilisation from the bone marrow and to have intact progenitor capacity.
Objectives: To further assess the functionality of natalizumab-induced HSC in MS patients by evaluating their clonogenic potential. MS patients were prospectively followed during the first two months of treatment or evaluated cross-sectionally at one time point during treatment. Comparisons were made among MS patients stratified according to HSC mobilization status.
Methods: Lin-/CD34+ HSC were magnetically sorted from natalizumab-treated MS patients and healthy donors (HD) for comparison; Colony forming unites (CFU) assay was performed to measure the growth of progenitors colonies from HSC cultured in methyl cellulose. Both un-manipulated PBMC or purified HSC were seeded in 35mm Petri dishes in duplicates. CFU were enumerated at a transmitted light inverted microscope after 14 days of incubation, to calculate absolute number and proportion of CFU (CFC), CFC number x10^6 per sample and CFU test efficiency (CFUe %). 5 HD and 10 MS patients (2 followed prospectively) were assayed.
Results: CFC proportion and number x10^6 were higher in the MS patients compared to HD (T test $p < 0.05$), further suggesting the overall higher expansion potential of natalizumab-induced circulating HSC over HD.
When stratifying the MS populations according to HSC mobilization status (Mobilizer status: fold change of HSC absolute count after two infusions ≥ 2.35 compared to baseline) we observed that: in the two patients followed prospectively, CFC absolute number, proportion and test efficiency were significantly increased only in the Mobilizer patient after two natalizumab infusions compared to baseline ($p < 0.05$); among the total natalizumab-treated population, Mobilizer patients (n=4 of 10) showed a significantly higher expansion potential according to all four CFC variables, as compared to Non-Mobilizer patients (n=6 of 10; $p < 0.05$).
Conclusions: Natalizumab-induced HSC of MS patients are 'healthy' and expand at higher rate than HDs' circulating HSC; the overall higher clonogenic capacity of Mobilizer natalizumab-induced HSC over Non-Mobilizer patients samples further suggests that the previously observed variable peripheral HSC increase has biological relevance.

P950

IL-2Rβγ-signaling contributes to regulatory T cell maintenance and stability in daclizumab HYP-treated RRMS patients

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Background: Regulatory T cells (Treg) mediate immune tolerance and depend on interleukin-2 (IL-2) for maintenance and lineage stability. Treg deficiency, instability and dysfunction are implicated in the pathogenesis of numerous autoimmune diseases, including relapsing-remitting multiple sclerosis (RRMS). Daclizumab HYP (DAC HYP) is a humanized monoclonal antibody that binds the IL-2 receptor alpha chain (CD25) and prevents its association with IL-2. DAC HYP inhibits high affinity IL-2Rα-dependent IL-2 signaling while leaving intermediate affinity IL-2Rβγ-signaling intact. DAC HYP has demonstrated clinical efficacy in patients with RRMS.

Objectives: To determine the *in vivo* impact of CD25 blockade on the phenotype and stability of human Tregs, and to evaluate associations between circulating Tregs and treatment response or adverse events in DAC HYP-treated RRMS patients.

Methods: Tregs from DAC HYP- and placebo-treated RRMS patients from the SELECT phase II clinical trial were analyzed at baseline and post-treatment over the 52-week study. Phenotypic and functional properties were measured using flow cytometry. Lineage stability was assessed by epigenetic analysis of the *FOXP3* promoter. IL-2 signaling was quantitated using STAT5 PhosFlow. Associations between Treg measurements and treatment response or cutaneous adverse events were determined by a negative binomial model and Wilcoxon Rank-sum test, respectively.

Results: Tregs in placebo-treated RRMS patients remained stable over the 52-week study. DAC HYP-treated patients had a 45.8% decline in activated Tregs and a 29.7% decline in resting Tregs. Tregs in DAC HYP-treated patients retained a demethylated *TSDR* in the *FOXP3* promoter, maintained active cell cycling and had minimal production of the pro-inflammatory cytokines IL-2, IFN-γ, and IL-17. In the presence of DAC HYP, IL-2 serum concentrations increased and IL-2Rβγ signaling induced STAT5 phosphorylation and sustained FoxP3 expression. No associations were observed between circulating Treg numbers and treatment response or adverse events.

Conclusions: FoxP3-expressing cells maintained during DAC HYP treatment retain phenotypic and functional characteristics of Tregs. Increased serum IL-2 and IL-2 signaling via the intermediate affinity IL-2Rβγ in Tregs contributes to Treg maintenance. These data suggest that Treg lineage stability can be maintained in the face of CD25 blockade and that DAC HYP-treated patients retain a population of functional Tregs.

P951

Pharmacogenomics of interferon-beta treatment in Caucasian MS patients

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Background: In the absence of a definite cure for MS, disease modifying drugs (DMD) are administered to reduce the number of relapses and slow disease progression. Current first-line DMDs include 3 interferon-beta (IFNβ) products. While these agents have been shown to be effective, up to half of treated patients do not respond well to these treatments and determination of response status requires clinical follow-up of 1-2 years. This may lead to prolonged patient suffering and delays the administration of other effective treatments in non-responsive patients. Hence it is imperative to identify biomarkers that can predict the outcome of IFNβ treatment prior to the initiation of therapy for MS management.

Objectives: This project aims to identify genetic biomarkers associated with response to IFNβ treatment.

Methods: To explore the genetic variants associated with interindividual response variability to IFNβ treatment we conducted a two-stage GWAS. In the discovery stage, Australian MS patients from the Australia & New Zealand Multiple Sclerosis Genetics Consortium (ANZgene) and Wellcome Trust Case Control Consortium 2 (WTCCC2) were used to identify responders (R) & non-responders (NRs) to IFNβ treatment by applying extreme phenotype criteria in patients followed up for 2 years (R: No relapse & no change in EDSS. NR: ≥ 2 relapses or increase 1 point EDSS). Stringent GWAS quality control procedures were employed using PLINK software. Samples with < 90% call rate and SNPs with call rates of < 90%, Hardy Weinberg equilibrium $P < 10^{-4}$ and

minor allele frequency < 0.01 were excluded. Possible cryptic relatedness or population stratification among the subjects were evaluated using PLINK and EIGENSTRAT respectively.

Discovery stage analysis was performed on a total of 284,577 SNPs obtained from 127 Australian IFNβ treated patients (R=51 & NR=76). Prioritization of SNPs for the validation stage was based on P values generated by combination of efficiency robust statistical testing strategies such as MAX4 and proportional odds model. P values were also corrected to account for variable baseline covariates.

Results: 339 statistically significant SNPs from the discovery stage were selected for replication in the validation cohort comprising of 479 IFNβ treated MS patients (R=273 & NR=206) from Australia, Italy & Spain. Analysis of the complete data will be presented at the conference.

Conclusions: Conclusions will be presented at the conference.

P952

Fingolimod promotes regulatory phenotype and function in B-cells of multiple sclerosis patients

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Background: Fingolimod is a recently approved treatment for multiple sclerosis, a chronic inflammatory disease of the CNS, aimed at reducing the number of potentially autoreactive lymphocytes in the peripheral blood and thereby limiting their immigration into the CNS. The influence of Fingolimod treatment on B cell subsets of multiple sclerosis patients and its potential clinical relevance have not been evaluated, so far.

Objectives: The aim of our study was to evaluate the impact of Fingolimod treatment on B cell subset composition in the periphery and the cerebrospinal fluid (CSF) and B cell functions such as cytokine production and migratory capacity.

Methods: We analyzed B cell subset composition and cytokine production of B cells derived from peripheral blood mononuclear cells from multiple sclerosis patients under Fingolimod treatment in comparison to untreated multiple sclerosis patients and healthy controls, by flow cytometry and ELISA. Further, lymphocyte migration across primary human brain microvascular endothelial cells was evaluated in an *in vitro* transmigration assay under homeostatic as well as under inflammatory conditions, and B cell subsets were evaluated in CSF and peripheral blood by flow cytometry. Finally, regulatory B cell frequencies were correlated with parameters of disease stability.

Results: Within the peripheral B cell compartment of Fingolimod-treated patients, the proportion of regulatory B cells ($CD38^+$ $CD27^-$ $CD24^+$ $CD5^+$) was significantly increased when compared to treatment-naïve multiple sclerosis patients and to healthy controls, and significantly more regulatory B cells produced Interleukin-10. Fingolimod treatment enhanced the capacity of regulatory B cells to transmigrate across brain endothelial cells in an *in-vitro* model of the blood-brain-barrier. In line with this, the CSF/blood ratio of total B cells and regulatory B cells was strongly increased by Fingolimod treatment, and patients exhibited increased regulatory B cell frequencies in the CSF. Finally, elevated regulatory B cell frequencies in the periphery significantly correlated with clinical and paraclinical disease stability, thus pointing to a functional relevance of regulatory B cells under Fingolimod treatment.

Conclusions: Taken together, these data point to a novel and as yet unrecognized role of Fingolimod in correction of impaired B cell functions in multiple sclerosis by enhancing regulatory B cells.

P953

Vitamin D3 administration to MS patients leads to increased serum levels of TGF-beta

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Background: Vitamin D is an environmental risk factor for MS. It has immunomodulatory effects, including promotion of T-cell differentiation into T-regulatory cells, which produce regulatory cytokines including TGFβ. Increasing serum vitamin D levels have been associated with decreased disease activity in MS patients, but there are only few studies concerning the immunological effects of vitamin D supplementation in MS.

Objectives: In this study we investigated the effect of weekly supplementation of vitamin D3 or placebo on serum levels of multiple cytokines in patients with relapsing remitting MS.

Methods: The study was conducted on the patient cohort of The Finnish Vitamin D study. All patients were using IFN-beta-1b and were randomized to add-on treatment with either cholecalciferol 20000IU/week or placebo. Concentrations of LAP (TGF-beta-1a), INF-γ, IL-2, IL 17A, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, TNF-alpha and IL-1β were determined at screening and at 12 months using commercial fluorescent bead immunoassay kits.

Results: LAP (TGF-beta-1a) levels increased significantly in the vitamin D treated group from a mean of 47 (SE 11) nmol/l to 55 (SE 14) nmol/l in 12 months (p=0,0249). Placebo treatment had no significant effect on LAP levels. The levels of the other cytokines did not change significantly in either group.

Conclusions: We showed increased TGF-beta levels in MS patients treated with vitamin D3. Our results support augmented regulatory T cell function in MS patients supplemented by vitamin D3.

P954

Reduction and reconstitution of B-cells in peripheral blood and lymphoid issues in cynomolgus monkeys following administration of ocrelizumab

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Background: Ocrelizumab (OCR) is a humanized monoclonal antibody designed to selectively target CD20+ B-cells. In a previous Phase 2, randomized, placebo and active-controlled, multicenter trial in patients with relapsing-remitting multiple sclerosis (RRMS), the number of gadolinium-enhancing (Gd+) lesions and annualized relapse rates were significantly reduced with OCR. OCR is currently in Phase 3 clinical development for relapsing MS (RMS) and primary progressive MS (PPMS).
Objectives: To determine the kinetics of B-cell reduction and reconstitution in cynomolgus monkey peripheral blood and lymphoid tissues following intravenous (IV) administration of one or two cycles of OCR.
Methods: OCR was administered to cynomolgus monkeys at doses of 0, 10, 50 and 100 mg/kg over one or two treatment cycles. Cycle 1 consisted of two IV infusions given two weeks apart. Following a 14 week observation period, a subset of cohorts were given a retreatment cycle (cycle 2) consisting of two IV infusions administered two weeks apart. Peripheral blood B-cells were periodically monitored by flow cytometry throughout the study. At two necropsy time points, B-cells from select lymphoid tissues were quantified by flow cytometry and immunohistochemistry (IHC).
Results: Following cycle 1, peripheral blood B-cells (CD3-CD40+) were rapidly suppressed to undetectable levels for all cohorts with repopulation beginning at week 6 (10 mg/kg) and week 14 (50 and 100 mg/kg). Following the 2nd cycle there was near complete B-cell recovery by week 43. At 100 mg/kg, the rate of B-cell recovery was similar for cohorts receiving either one or two cycles. At week 20 (2 weeks after cycle 2), there was a consistent reduction of B-cells in spleen, lymph nodes (LN) and Peyer's patches as measured by ICH with reduction most prominent in spleen. By the study termination (week 43), repopulation of lymphoid B-cells was similar to controls. By flow cytometry, mean lymphoid tissue B-cells at week 20 were reduced to 55/52% (bone marrow), 0.5/0.8 % (spleen), and 3/3% (LN) relative to controls at the 50/100 mg/kg doses. Upon recovery (week 43), values were comparable to controls.
Conclusions: Ocrelizumab induced a rapid depletion in blood B-cells in cynomolgus monkeys, but only partial depletion in lymphoid tissue B-cells following two cycles of administration. The effects were reversible and following either one or two cycles, the rate of peripheral blood B-cell reconstitution was similar.

P955

Laquinimod reduces CNS autoimmunity by activation of natural killer cells

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Background: Laquinimod, a new orally active immunomodulator, is currently under investigation for the treatment of relapsing-remitting multiple sclerosis (MS). In previous clinical trials laquinimod considerably reduced the annualized relapse rate, disability progression and brain atrophy in MS patients. Experimental data provided evidence for immunomodulatory effects of LAQ on innate and adaptive immune cells. As such, LAQ reduced the immunogenicity of dendritic cells (DCs) and induced type II myeloid cells. Since recent immunological studies emphasized a strong crosstalk between DCs and natural killer cells (NKs), we analyzed whether LAQ modulates NK cell responses.
Objectives: This study investigated the effects of laquinimod on NK cell phenotypes in MOG_{35-55} immunized C57BL/6 mice.
Methods: We characterized activating and inhibitory receptors on NK cells in LAQ-treated, MOG_{35-55} immunized animals by flow cytometry. Functional studies (cytotoxicity, cytokine production, inhibition of T cell proliferation) were performed with ex vivo purified NK cells from MOG_{35-55} immunized and LAQ-treated mice. Treatment efficacy of LAQ was analyzed in EAE animals depleted of NK cells by PK136 antibody administration.
Results: LAQ increased the frequency of CD69+ NK cells ($p < 0.001$) and upregulated the expression of the activating receptors TACTILE ($p < 0.001$), DNAM-1 ($p< 0.01$) and NKG2D ($p < 0.001$) specifically on NK cells. Additionally, LAQ improved classical NK cell effector functions, as NK cells derived from LAQ-treated mice killed B16F10 melanoma cells *in vitro* more efficiently than NK cells derived from vehicle controls ($p< 0.05$). In addition, they produced more IFNγ in response to IL-12 and IL-18 stimulation ($p< 0.05$). In a triple co-culture system with 2D2 T cells and bone marrow derived DCs, NK cells derived from LAQ-treated mice significantly inhibited T cell proliferation in response to MOG_{35-55} ($p< 0.05$). Depletion of NK cells in vivo did not change the LAQ-induced reduction of DCs ($CD11c^{high}$ $MHCII^{+}$) in the spleen but impaired the efficacy to suppress EAE.
Conclusions: Treatment with LAQ induced NK cell activation, which contributes to the efficacy of LAQ to suppress CNS autoimmunity. In MS, NK cell functions are impaired and treatment with LAQ might restore NK cell function.

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How do Treg cell function and related cytokines levels change in alemtuzumab treated patients? A 24 months immunological study

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Background: Alemtuzumab, a highly effective treatment in relapsing remitting multiple sclerosis (RRMS), is associated with a long-standing lymphopenia, particularly of T CD4+ subset.
Objectives: To determine:

1) T-cell phenotypic and functional analysis;
2) cytokine, chemokine, and chemokine receptor mRNA levels after alemtuzumab in RRMS:24-months follow-up.

Methods: Multicenter 24 months follow-up of 29 alemtuzumab-treated RRMS patients in CAMMS 323 or 324 trials. Fluorescence-Activated Cell Sorting (FACS) analysis of Treg, Thelper (Th)1 and Th17 cells. Immunological molecule mRNA levels (chemokines:CCL-11,CXCL-10;chemokine receptors:CCR-4,CCR-6; cytokines:IFNγ,TGFβ,TNFα,IL-1β,IL-2,IL-6,IL-10,IL-12p35,IL-17A,IL-17F,IL22,IL-23,IL-26,IL-27;and T-bet,RORγt,Foxp3) quantified by TaqMan® low density array (TLDA) real-time polymerase chain reaction in whole blood. Treg suppressor activity assessed at M12 and M24 by ELISPOT on peripheral blood mononucleate cells (PBMC) depleted of CD25high T cells and activated with myelin basic protein (MBP) or anti-CD3-CD28. Timepoints: Baseline (before first alemtuzumab course);Month (M) 6, M12, M18, M24: respectively 6,12,18, 24 months after the first course. M12 was before second alemtuzumab course. The relative mean difference between baseline and subsequent timepoints estimated through the formula: (Timepoint-Baseline)/Baseline, and significance of the differences tested.
Results: Twenty-nine patients from 6 European sites. After alemtuzumab, CD4+ lymphocytes decreased from 45 to 14%.Th17 increased at M18; no significant variation were observed in Th1 cells. T-bet and IL-23 decreased; Foxp3, TGFβ, IL-10 and IL-27 increased from M6 to M24. Treg cell specific suppressive function on MBP auto-reactive Th1 and Th17 cell increased only at M24, whereas the number of Treg cells and their overall suppressor function did not significantly change during the follow-up. Six relapses were observed in 5 patients. No occurrence of severe autoimmune diseases or dysthyroidism.
Conclusions: The overall alemtuzumab-induced CD4+ lymphocyte depletion might relate to the reduction of MS disease activity. The increase of suppressor cytokines IL-27,IL-10,TGFβ and of MBP-specific Treg suppressor function could have resulted in the decrease of T-bet, modulating the anti-CNS pathogenicity of Th1 and Th17 cells. The further 2 year long-term follow-up could provide information on the timing of subsequent courses of alemtuzumab in individual patients.

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Fingolimod selectively affect antigen-presenting cells ex vivo and *in vitro* in multiple sclerosis

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Background: Fingolimod (FTY) is known as S1P receptor analog that mediates inhibition of lymphocyte egress from lymph nodes into peripheral blood. Beside these effects also directed modulation of immune cell populations is discussed. Dendritic cells (DC) and monocytes (Mo) are professional antigen presenting cells (APC) orchestrating relevant processes of innate immunity and suggested to be directly involved in multiple sclerosis (MS) pathogenesis. The most frequent proinflammatory human blood DC is the so-called slanDC. It is assumed that immunomodulatory MS treatment strategies including FTY evoke indirect effects on T and B cell populations by specific modulation of APC function.
Objectives: To evaluate effects of FTY on the innate immune system in MS.
Methods: As ex vivo project, blood was drawn from MS patients before and during FTY therapy. T cells, B cells, NK cells, slanDC, CD1+DC and Mo were analyzed by FACS at different timepoints. As detailed *ex vivo-in vitro* project, slanDC, CD1+DC and Mo were isolated by immunomagnetic cell sorting of healthy controls (HC) and FTY treated MS patients. After stimulation with TLR-2/4 ligands in presence or absence of FTY or FTY-phosphate (FTY-P), APC were analyzed regarding cytokine release, expression of surface markers, phagocytosis activity and capacity to promote proliferation and differentiation of T-cells.
Results: In ex vivo analysis cell number of CD4, CD8 and CD19 lymphocytes decreased after treatment initiation, whereas slanDC, CD1+DC and Mo kept stable during 24 month follow up. FTY and FTY-P decreased proinflammatory cytokine release of IL-6, IL-1b and TNF-a in slanDC, CD1+DC and Mo after LPS or PGN stimulation. Compared to HC, slanDC of FTY treated patients showed a decreased IL-6 and IL-1b secretion after LPS, but an increased IL-10 secretion. Expression of surface maturation and activation markers including CD83 and CD86 were down regulated in slanDC and CD1+DC after FTY challenge. FTY but not FTY-P pre-treatment inhibited slanDC and CD1+DC depending CD4 and CD8 T cell proliferation. FTY and FTY-P reduced the ability of LPS-stimulated slanDC and CD1+DC to promote differentiation of naïve CD4 T-cells into IFN-g-expressing Th1 cells. Mo and slanDC decreased in phagocytosis capacity after FTY and FTY-P.
Conclusions: For the first time we present direct immunomodulatory effects of FTY on different APC subpopulations in contrast to known impact on T and B lymphocyte trafficking. These results suggest the possibility of impact on T cell programming by FTY.

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Steady state pharmacokinetics and blood lymphocyte responses in healthy subjects dosed with XP23829, a novel fumaric acid ester for multiple sclerosis

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Background: XP23829 is an innovative prodrug of monomethyl fumarate (MMF), the same active metabolite produced by dimethyl fumarate (DMF). Reductions in blood lymphocytes are known effects of fumaric acid esters.
Objectives: To investigate the steady state pharmacokinetics (PK) and safety of two formulations of XP23829 and DMF, including dynamic changes in lymphocyte counts.
Methods: This randomized, double-blind, placebo-controlled, multiple ascending, oral-dose study of Formulations 1 (delayed release) and 2 (extended release) of XP23829 was conducted in

healthy adults enrolled into 5 sequential cohorts of 12 active and 3 placebo subjects. Each cohort received 7 days at maintenance dose following up to 4 days of up-titration: Formulation 1 at 200 mg BID or 400 mg BID (fasted); Formulation 2 at 800 mg QD (fed or fasted) and 500 mg BID (fed). An additional cohort received DMF (Tecfidera®, BG-12) 240 mg BID (fasted; fed on Day 7). PK of MMF in blood and plasma were determined on Day 7 (Days 6 and 7 for DMF). Clinical labs were assessed at screening, baseline, Day 8 (1 day after last dose) and follow-up (8-10 days after last dose).

Results: Mean area under the curve over 24 hours ($AUC_{ss,24}$) values in plasma MMF were 3670 and 6910 ng*hr/mL for 200 and 400 mg BID Formulation 1, respectively and 7010 ng*hr/mL for DMF (fasted). Mean MMF $AUC_{ss,24}$ in plasma was 4490, 5080, and 5510 ng*hr/mL for 800 mg QD (fasted), 800 mg QD (fed) and 500 mg BID Formulation 2, respectively.

Both XP23829 and DMF reduced blood lymphocytes at Day 8 compared to baseline, but reductions with XP23829 were more pronounced when compared to DMF. The mean change from baseline in lymphocytes (x 10^9 /L) ranged from -0.07 to -0.44 for Formulation 1 and -0.84 to -1.16 for Formulation 2 (compared to -0.32 for DMF and -0.23 for placebo). Lymphocytes did not drop below 0.5 x 10^9 /L in any subject. Lymphocytes returned to near baseline levels at follow-up.

Conclusions: XP23829 provided similar (Formulation 1) or more sustained (Formulation 2) MMF exposure compared to DMF in healthy subjects. After dosing for 11 days, XP23829 resulted in greater reductions from baseline in blood lymphocytes compared to DMF. The clinical activity of XP23829 will be explored in an upcoming phase 2, dose-ranging clinical study in moderate-to-severe plaque psoriasis.

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Teriflunomide mechanism of action: linking preclinical evidence to clinical efficacy and safety

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Background: Teriflunomide is a once-daily oral immunomodulator approved for relapsing-remitting multiple sclerosis (MS). It inhibits dihydroorotate dehydrogenase, the key mitochondrial enzyme in de novo pyrimidine synthesis in rapidly dividing lymphocytes, thus limiting proliferation of activated lymphocytes.

Objectives: To present the proposed mechanism of action (MoA) of teriflunomide based on *in vitro*, preclinical and clinical data.

Methods: The following were analyzed: *in vitro* human lymphocyte proliferation and viability; *in vitro* mouse T-cell responses to cognate peptide variants in the presence/absence of various teriflunomide concentrations; teriflunomide impact in a rat experimental autoimmune encephalomyelitis (EAE) model (disease severity, central nervous system [CNS] immune cell infiltration, neuronal conduction); antibody responses in mice and in two clinical studies; and pivotal clinical trials in patients with MS.

Results: *In vitro*, teriflunomide inhibited proliferation of human T and B cells stimulated with anti-CD3 or CpG, respectively, and strongly inhibited proliferation of mouse T cells stimulated with high-affinity peptides; it had no impact on cell viability. Teriflunomide-treated EAE rats had reduced CNS T- and B-cell infiltration, reduced disease severity and preserved neuronal conduction in somatosensory and motor tracts. Teriflunomide-treated mice developed effective but slightly delayed (< 2 weeks) memory and primary antibody responses to adenovirus. Teriflunomide-treated patients with MS mounted effective memory responses to influenza vaccine (TERIVA, NCT01403376), and teriflunomide-dosed healthy subjects made seroprotective antibody responses to rabies vaccine (neoantigen), albeit at lower titers than those given placebo. The TEMSO (NCT00134563) and TOWER (NCT00751881) trials showed significant decreases in annualized relapse rates and CNS lesion accumulation (TEMSO only; not tested in TOWER) vs placebo, consistent with an anti-inflammatory MoA for teriflunomide. There was no evidence of immunosuppression in TEMSO, its extension (NCT00803049) or TOWER; infection and malignancy rates were similar to placebo. Mean leukocyte counts were reduced by < 15% from baseline, but remained in the normal range.

Conclusions: Teriflunomide modulates immunity through selectively inhibiting proliferation of activated T and B cells. Our observations provide insight into the mechanism by which teriflunomide treatment reduces disease severity while preserving protective immunity in patients with MS.

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Enhanced axonal metabolism in multiple sclerosis patients treated with natalizumab

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Background: The anti-inflammatory effect of natalizumab, second-line therapy for relapsing-remitting multiple sclerosis (RRMS) in most countries, leads to a dramatic reduction in the formation of white matter (WM) lesions, a decrease in the whole-brain atrophy rate as well as improved rates of clinical progression and relapses. Previous spectroscopy studies have shown that patients treated with interferon beta-1b (IFNb) or glatiramer acetate (GA) have increased total N-acetylaspartate over total creatine (tNAA/tCr) ratios, whereas the effect of natalizumab on brain metabolite concentrations remains unknown.

Objectives: To investigate the longitudinal effect of natalizumab on absolute concentrations of brain metabolites in RRMS patients using proton magnetic resonance spectroscopic imaging (MRSI).
Methods: 25 RRMS patients initiating natalizumab treatment were included and scanned every six months for 18 months. Control groups included 18 RRMS patients with similar disease duration and lesion volumes, continuing IFNb or GA and 12 healthy controls, matched for age and sex. Imaging included short echo-time 2D-MRSI with absolute metabolite quantification of tNAA, tCr, choline-containing compounds (Cho), myo-inositol (Ins) and glutamate (Glu). Concentrations were determined for whole regions of lesional white matter (LWM), normal appearing white matter (NAWM) and grey matter (GM).
Results: At baseline in both patient groups, lower concentrations of tNAA and tCr were found in LWM compared to NAWM and additionally lower Glu in LWM of natalizumab patients. In natalizumab patients only, significant metabolite increases were found for LWM tNAA (7%, $p< 0.001$), tCr (6%, $p=0.042$) and Glu (10%, $p=0.028$), while lesion volumes did not change. In IFNb/GA patients, no significant change was measured in LWM for any metabolite, while lesion volumes increased. NAWM and GM metabolite concentrations remained stable in both patient groups and the healthy control group.
Conclusions: Only patients treated with natalizumab showed an increase in tNAA, tCr and Glu in lesional white matter. These increasing metabolite concentrations might be a sign of enhanced axonal metabolism due to the anti-inflammatory effect of natalizumab.

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Characterization of CD56bright NK cells in daclizumab HYP-treated RRMS patients

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Background: Daclizumab HYP (DAC HYP) is a humanized monoclonal antibody that binds the alpha chain (CD25) of the high affinity IL-2 receptor and prevents its association with IL-2. DAC HYP inhibits high affinity IL-2Rα-dependent IL-2 signaling while leaving intermediate affinity IL-2Rβγ signaling intact. $CD56^{bright}$ natural killer (NK) cells express low levels of IL-2Rα but high levels of IL-2Rβγ and are expanded in DAC HYP-treated relapsing-remitting multiple sclerosis (RRMS) patients. It is hypothesized that the increased numbers of $CD56^{bright}$ NK cells contribute to the therapeutic efficacy of DAC HYP. Previous work has reported that $CD56^{bright}$ NK cells act either through the production of immunoregulatory cytokines or through direct cytolytic activity.
Objectives: To characterize the impact of CD25 blockade on $CD56^{bright}$ NK cells and to investigate the contribution of $CD56^{bright}$ NK cells to the therapeutic efficacy of DAC HYP.
Methods: Banked cryopreserved peripheral blood mononuclear cells (PBMCs) from DAC HYP- and placebo-treated RRMS patients enrolled in the SELECT phase II clinical trial were analyzed. Phenotypic characterization of NK cells was performed by flow cytometry. Transcriptional profiling of FACS sort-purified NK cells subsets from pre-treatment baseline and post-treatment week 24 and week 52 was performed. *In vitro* functional studies were conducted to determine the effects of high- and intermediate-affinity IL-2R signaling on both healthy donor NK cells and NK cells from DAC HYP-treated RRMS patients.
Results: DAC HYP-treatment differentially affected NK cell subsets, including expression of IL-2R components and NK cell receptors. Mechanistic studies demonstrated a unique role for high-affinity IL-2Rαβγ signaling in driving GM-CSF and IFN-γ production by $CD56^{bright}$ NK cells.
Conclusions: We provide evidence that expanded NK cell subsets in DAC HYP-treated patients have partially overlapping, yet distinct phenotypic and functional profiles. Furthermore, in the context of DAC HYP treatment, expanded $CD56^{bright}$ NK cells are skewed toward an immunoregulatory phenotype.

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Modulation of circulating CD39-expressing T regulatory cells from MS patients by fingolimod

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Background: Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system resulting from an imbalance between pro-inflammatory and regulatory immune processes. CD39 is an ectonucleotidase that cleaves ATP to AMP and has been suggested as a novel marker for regulatory T cells (Treg). As ATP has numerous pro-inflammatory effects, its degradation by CD39 could be beneficial. Fingolimod, used for the treatment of relapsing-remitting (RR) MS, interferes with T cell egress from lymph nodes. Its effect on Tregs is only partially described.
Objectives: The purpose of this study was to explore whether regulatory mechanisms are induced in fingolimod-treated MS patients.
Methods: Peripheral Blood Mononuclear Cells (PBMC) were collected from healthy controls and RRMS patients before starting fingolimod therapy and 3 months after treatment initiation. Natural Treg frequency was assessed by the novel technique of methylation-specific qPCR of the first intron of *FOXP3*. *Ex vivo* cytokine expression in PBMCs was analyzed by qPCR. *Ex vivo* CD39 expression was analyzed by flow cytometry in $CD4^+$, $CD8^+$, $CD19^+$ cells as well as in $CD4^+Foxp3^+CD25^{hi}$ Tregs. CD39 activity was assessed by ATP luminometry.
Results: Natural Treg frequency was similar between MS patients and healthy controls. B cell, $CD4^+$ T cell and Treg proportions were largely reduced by fingolimod. *Ex vivo* CD39 mRNA levels were however increased in MS patients, in comparison to healthy controls. Fingolimod treatment further increased CD39 and AHR mRNA levels in PBMCs, while IL-17A, IL-22 and FOXP3 mRNA were reduced. The proportion of $CD39^+$ Tregs was increased by fingolimod but not of $CD39^+CD4^+Foxp3^-$ T cells. Fingolimod reduced the proportion of $CD39^+CD19^+$ B cells and $CD39^+CD8^+$ T cells. We could not detect however any change in CD39 activity following treatment by fingolimod.
Conclusions: Fingolimod not only mediates $CD4^+$ T cell retention, but also decreases the proportion of $CD19^+$ circulating B cells. Interestingly, the proportion of circulating $CD39^+$ Tregs is specifically increased. This observation could have functional

relevance to the therapeutic effects of fingolimod, as CD39+ Tregs have been shown to suppress pathogenic Th17 cells[1]. Work is in progress to understand whether this effect is purely related to the cellular redistribution mediated by fingolimod or could be due to interference with sphingosine-1-P receptor signaling at the cellular level.

1 Fletcher JM et al. J Immunol. 2009;183:7602-10.

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Laquinimod regulates inflammatory gene induction in a human model of reactive astrogliosis

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Background: Laquinimod is an orally-delivered immunomodulator which includes actions on the innate immune system and is in Phase 3 clinical trials for relapsing-remitting multiple sclerosis. Laquinimod crosses the blood-brain barrier and enters the CNS, thus in addition to peripheral actions it may directly impact CNS-resident lineages including astrocytes and microglia, which increasing evidence implicates as key regulators of CNS inflammation. Interleukin-1beta (IL-1β) is implicated in lesion pathogenesis in MS, and is a strong inducer of astroglial reactivity in human cultures.
Objectives: Here, we report that laquinimod profoundly impacts proinflammatory gene expression in a primary human model of reactive astrogliosis *in vitro*.
Methods: Primary human astrocytes were isolated and treated with IL-1β in combination with laquinimod. Cells were examined via microarray analysis, QPCR, and multiplex ELISA.
Results: We found that IL-1β 10ng/ml induced a pro-inflammatory transcriptional pattern in primary human astrocytes which encompassed induction of inflammatory cytokines, reactive nitrogen species, chemokines, adhesion molecules, matrix metalloproteinases, and inducers of endothelial plasticity and blood-brain barrier permeability, as shown by microarray analysis, QPCR and multiplex ELISA. Importantly, at therapeutic concentrations, laquinimod dose-dependently abrogated IL-1β induction of cytokines including TNFα, IL-6, IFNα, IL-12 and IL-23, inducible nitric oxide synthase, the matrix metalloproteases MMP7 and MMP10, and the permeability factor VEGF-A, implicated as a driver of BBB disruption. Suggesting immunomodulation rather than suppression, laquinimod differentially regulated IL-1β-induced expression of CXC and CC chemokines, inhibiting induction of CXCL1,2,5,6,8 and 10 and CCL8 while potentiating CCL5 and CXCL14. IL-1β signals via the transcription factor NF-κB, and suggesting mechanism, laquinimod treatment delayed IL-1β-induced IκBα degradation and NF-κB p65 nuclear translocation.
Conclusions: Collectively, these data reveal laquinimod as a regulator of the proinflammatory phenotype in a human model of reactive astrogliosis, and suggest that it may act on resident cells to reduce production of cytotoxic mediators, restrict inflammatory cell chemotaxis and migration, and rescue blood-brain barrier integrity. Laquinimod therapy may restrict lesion pathogenesis in RRMS and other inflammatory CNS conditions, via direct actions on immune-competent CNS-resident cells.

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The effect of immune therapeutic agents on the T-cell receptor repertoire in MS-patients

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Background: In healthy individuals the V-beta element lengths of the TCR represent a Gaussian distribution, whereas MS patients exhibit shifted patterns or oligoclonal expansions of several V-beta elements. The impact of different immune therapies on the TCR repertoire distribution has not been explored so far.
Objectives: To study the effects of interferon (IFN)-beta, glatiramer acetate (GA), natalizumab (Nat) and fingolimod (Fingo) on the expression of the T cell receptor (TCR) repertoire in peripheral venous blood of patients with multiple sclerosis (MS).
Methods: The TCR repertoire of CD4+ and CD8+ T cells in peripheral mononuclear cells was assessed by longitudinal complementary determining region 3 spectratyping of patients with relapsing-remitting MS before and while being treated with IFN-beta, GA, Nat or Fingo. Healthy individuals severed as controls.
Results: Untreated patients with relapsing remitting MS revealed a disease typical shifted or oligoclonal expansion in the TCR repertoire distribution compared to healthy controls with a Gaussian V-beta element distribution. While IFN-beta and GA do not show a significant effect on the TCR repertoire, Fingo leads to an increase of peripheral T cell expansion, while the treatment with Nat for >18 months seems to normalize the TCR repertoire.
Conclusions: Our data demonstrate distinct effects of immune therapeutics for treatment of MS on the TCR repertoire. The analysis of the TCR repertoire seems to mirror their specific mode of action: While immunomodulators like IFN-beta or GA are not expected to show a variation in the TCR repertoire, the increase of the peripheral T cell expansion under treatment with Fingo could be a result of the retention of specific T cells in the lymph nodes. The 'normalizing' effect on the peripheral TCR repertoire under treatment with Nat may reflect an altered immune surveillance of the CNS, which leads to an inhibition of a secondary oligoclonal T cell expansion in the periphery.

P965

Reduction in microglia activation measured by [11C] PK11195-PET in patients treated with natalizumab

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Background: The primary innate immune cells in multiple sclerosis (MS) consist of infiltrating macrophages/monocytes and resident microglia for which function to cause CNS injury both through direct effects on neighboring cells, such as oligodendrocytes, and through generation of soluble proinflammatory mediators that have distant effects on cells, such as neurons. [C11]-PK11195 (PK) is a Positron Emission Tomography (PET) radioligand known to bind to the translocator protein expressed by activated microglia and macrophages. Natalizumab, approved for treatment of relapsing-remitting (RR), has a primary mechanism thought to be mediated through the blockade VLA-4 integrins, which diminishes the migratory capacity of peripherally derived immune cells. However, the effect of natalizumab on innate immune inflammatory burden in within the CNS is unknown.
Objectives: To determine the effect natalizumab on whole-brain binding of [C11]-PK11195 as well as within lesions at 3 and 6 months of treatment.
Methods: Twelve RRMS patients starting natalizumab therapy were included in the study. Dynamic images of brain [C11]-PK11195-PET were acquired over a period of 60 minutes at baseline, 3 months and 6 months post Natalizumab treatment. Quantification of PK uptake was done by volume of distribution (V_T) calculation using image-derived input function. Input time activity curves were computed using the carotid artery using fine reconstruction of 60 frames of 3 seconds each that allows capturing the peak in activity. Full pharmacokinetic quantification was done using a segmented MRI including the caudate, putamen, cerebellum, cortical gray matter, white matter, whole brain and total lesion.
Results: At the time of this abstract, quantification of [C11]-PK11195-PET uptake at each brain region was completed in 10 patients. After the start of natalizumab treatment, half of the patients (5) demonstrated a significant decrease in VT (>16%, $P < 0.001$) in all regions by 6 months. Moreover, among all the 10 patients, the average VT of all regions significantly decreased by 4.4% ($P< 0.05$).
Conclusions: These results suggest that PK-PET may be a useful tool to quantify the therapeutic reduction of the innate immune response in MS patients. In patients treated with Natalizumab, the beneficial effect may expand beyond the reduction of migratory capacity of immune cells and reduce innate immune burden within lesions as well as in other brain regions indicating a global reduction in CNS immune burden.

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High dose corticosteroid treatment alters transcripts of susceptibility genes in peripheral blood of multiple sclerosis patients

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Background: To date, more than 100 genetic risk loci associated with multiple sclerosis (MS) have been detected in two recent genome-wide association studies (GWAS), which are mainly related to genes with key functions in the immune system. The role of these risk genes in patients with established disease, however, remains largely elusive.
Objectives: In this study, we aimed to investigate the gene expression profiles in whole blood samples taken from relapsing-remitting MS patients (RRMS) during relapse, remission and during corticosteroid treatment with a special focus on genes implicated in MS susceptibility.
Methods: Using Illumina® whole genome expression arrays and quantitative RT-PCR, we measured overall gene expression in whole blood samples from six MS patients using three samples each: The first sample was taken during an acute clinical relapse confirmed by MR-tomography before administration of glucocorticosteroids, the second sample two days later after having started intravenous methylprednisolone (1000 mg/d) therapy. At least four weeks after relapse, a third sample was taken in clinical remission.
Results: We couldn´t detect any difference in gene expression between patients experiencing an acute relapse (sample 1) and patients in remission (sample 3). However, treatment with methylprednisolone (sample 2) yielded 1908 genes significantly regulated as compared to samples 1 and 3. Focusing on the 110 non-HLA risk loci that had been identified in two recent GWAS studies, we found 25 susceptibility genes to be significantly regulated during corticosteroid treatment. Interestingly, most of them (20 of 25) were downregulated - among these, the well-established risk genes IL7R and CXCR5.
Conclusions: This study demonstrates that treatment of a relapse with high dose steroids has an important impact on the regulation of genes recently implicated in MS susceptibility. The downregulation of many MS risk genes that we have observed may be related to the mechanism of action of the drug.

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Normalization of abnormal MicroRNA expression in monocytes of relapsing-remitting MS patients treated with fingolimod

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Background: MicroRNAs (miRNAs) are short ribonucleic molecules and post-transcriptional regulators of gene expression. Initially identified over a decade ago, miRNAs are estimated to target approximately 60% of the mammalian genome, including genes known to influence both innate and adaptive immunity. In recent years, miRNAs have been suggested as potential biomarkers and biological targets of emerging drug therapies in immune disorders and neurodegenerative diseases. In multiple sclerosis (MS), miRNAs are abnormally expressed in cells comprising the immune and central nervous systems, including T cells, B cells, and macrophages/microglia. Together with novel bioinformatic and molecular approaches, we have previously described the biological and disease-relevant functional significance to abnormal microRNA expression in MS.
Objectives: The objective of the present study was to determine how fingolimod could influence miRNA expression in circulating monocytes of MS patients.
Methods: qPCR was used to measure the expression of disease-relevant miRNAs in peripheral blood CD14+ monocytes of clinically stable, untreated relapsing-remitting MS (RRMS) patients.

MiRNA expression levels were then compared to both fingolimod-treated patients and age-matched healthy subjects.
Results: Compared to healthy subjects, several miRNAs were abnormally expressed in RRMS patients, including mirs-155, -146a, -124a, -132, and -34a. In patients treated with fingolimod, the abnormal miRNA expression levels were normalized and displayed similar levels to healthy subjects. To determine whether these results were due to a direct effect of fingolimod on human monocytes, CD14+ cells were isolated from healthy subjects, pre-exposed to fingolimod, and stimulated with LPS. *Ex vivo*, fingolimod did not alter the LPS-induced changes in miRNA expression.
Conclusions: These findings suggest that the mechanism by which fingolimod normalized miRNA levels *in vivo* was not due to a direct pharmacological effect, but rather reflects monocytes with less inflammatory potential as a result of decreased circulating pro-inflammatory lymphocytes.

P968

Effect of natalizumab treatment on circulating CD4+CD62L+ T-cells in multiple sclerosis patients

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Background: L-selectin or CD62L is an adhesion molecule involved in rolling and transmigration of leukocyte cells. Recently, Schwab and colleagues showed a correlation between a low expression of CD62L on $CD4^+$ T-cells and the development of progressive multifocal leukoencephalopathy (PML) in Natalizumab(NTZ)-treated Multiple Sclerosis (MS) patients.
Objectives: To verify the effect of NTZ treatment on the expression of CD62L on $CD4^+$ T cells and the possible correlation with PML.
Methods: Patients were recruited by CRESM, San Luigi, University Hospital. We conducted a flow cytometry analysis on cryopreserved, viable peripheral blood mononuclear cells from 14 healthy donors (HD) and 127 patients with MS: 74 had been treated with NTZ (1-86 months), 42 received other treatments (22 with Interferon beta, IFNb; 20 with Glatiramer acetate, GA) and 10 were untreated (T0). We also analyzed one PML case in a NTZ-treated patient from the Multiple Sclerosis Center of the University of Chieti.
Results: The percentage of L-selectin on $CD4^+$ T cells was significantly lower in patients treated with NTZ (34.98% ± 13.39; mean ± SD) when compared to patients treated with GA (49.30% ± 14.03; p=0.0004), IFNb (45.71% ± 19.22; p=0.0237) or HD (45.41% ± 10.08; p=0.0057). There are no significant differences between NTZ-treated and T0 patients (42.09% ± 9.36). Interestingly, we found two outliers for the expression of $CD62L^+$ on $CD4^+$ T-cells in GA-treated group and in T0 patients. In the NTZ-treated group three patients had values lower than the calculated risk threshold 13.57% (mean-2x SD of IFNb+GA group). The effect of NTZ was already evident during the first year of treatment, and persisted in the following years (p=0.0048, compared to IFNb+GA group). In the three high risk patients two CD62L serial evaluations were performed, during NTZ treatment and four months after withdrawal: during treatment 2 out of 3 had low values of $CD4^+CD62L^+$ which increased to normal values 4 months after discontinuation. We also had the opportunity to analyze a patient in the acute PML/IRIS phase, 4 months after NTZ withdrawal and under steroid treatment, CD62L expression was 30.48%.
Conclusions: NTZ treatment decreases the expression of CD62L on circulating $CD4^+$ T cells. This effect is already evident in the first year of treatment and disappears after withdrawal of treatment. The study of the effect of NTZ on $CD4^+CD62L^+$ cells can be a valuable tool to personalize NTZ therapy.

P969

Distinct effects of fingolimod on gene expression of T-cell subsets in the blood of patients with multiple sclerosis

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Background: Fingolimod is an oral disease-modifying agent approved for the treatment of multiple sclerosis (MS). It is a sphingosine-1-phosphate receptor modulator that prevents the egress of CD4+ T helper cells and CD8+ cytotoxic T cells from lymphoid tissues. In particular, the percentages of naive T cells and central memory T cells, which express the homing receptor CCR7, are reduced in the peripheral blood of treated patients. However, the precise molecular effects on CD4+ and CD8+ T cells have so far not been investigated at the transcriptome level.
Objectives: To study the gene expression patterns of T cells before and during fingolimod treatment in order to better understand the molecular mechanisms of the drug and to identify biomarkers of the individual biological response to therapy.
Methods: With informed consent, blood samples were drawn from 10 patients with relapsing-remitting MS at 3 different time points: before fingolimod treatment initiation (baseline) as well as after 24 hours and 3 months. CD4+ and CD8+ T cells were separated by magnetic-activated cell sorting. Cellular RNA was isolated and then analyzed with Affymetrix Human Transcriptome Arrays (HTA) 2.0, which contain >6.0 million distinct probes covering >70.000 coding and non-coding transcripts. Genes were considered as differentially expressed genes during therapy compared to baseline if they showed a fold-change>2.0 and a t-test p-value< 0.001.
Results: No gene was expressed at significantly higher or lower levels already 24 hours after the first dose of fingolimod. However, after 3 months, 979 genes and 172 genes were differentially expressed in CD4+ and CD8+ T cells, respectively. The levels of a subset of 37 genes were altered in both cell types, e.g. FCGR3A, FCGR3B (up-regulated) and CCR7 (down-regulated). Average CCR7 mRNA levels were in particular decreased in CD8+ T cells. An analysis of gene functions revealed that common and distinct biological processes and pathways are affected in both T cell subsets.

Conclusions: Treatment with fingolimod leads to a marked reduction of lymphocytes in the peripheral blood. Here, we present the first study that used high-resolution gene expression microarrays to analyze how the molecular properties of two different T cell subsets are modulated during therapy. T cell activation genes were differentially expressed in both cell types, but in general distinct biological processes were affected in response to treatment in CD4+ and CD8+ T cells.

P970

Immunoglobulin family genes are associated with clinical response to natalizumab

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Background: Natalizumab, a humanized monoclonal antibody prevents extravasation of T and B cells into the Central Nervous System and reduces inflammatory immune reaction in multiple sclerosis (MS). Therapy with Natalizumab is highly effective in relapsing remitting MS (RRMS), however individual patients´ clinical response may exhibit variability. Understanding the underlying molecular mechanisms of optimal and sub-optimal clinical response may lead to better patients´ management.
Objectives: Evaluate blood gene expression biomarkers for monitoring clinical response to Natalizumab treatment in JC virus negative RRMS patients.
Methods: A total of 132 gene expression microarrays ((HU-A133A-2 Affymetrix Inc.,) were obtained from 66 RRMS patients, 50 females, age 38.9±1.1 years, disease duration 12.7±0.6 years and EDSS 3.8±0.2. Each patient was sampled before initiation of Natalizumab and one year thereafter. Optimal responders were defined as relapse free and disability progression free patients and a gene expression profile of this group was evaluated by paired t-test. Genes with $p < 0.05$ after Bonferroni correction were defined as most informative genes (MIG´s). Gene expression differences between optimal and sub-optimal responders over 1 year and functional analysis using Ingenuity software were performed.
Results: Seventy eight percent (n=52) of patients under Natalizumab treatment were relapse and disease progression free. In the responders group EDSS improved by -0.2±0.1 at 1 year as compared to suboptimal responders in which EDSS increased by 0.7±0.1 (p=1.3E-10). Natalyzumab respoders were characterized by 508 MIGs enriched by mechanisms of B cells activation including B cells development (p=8.7E-08), PI3K Signaling in B lymphocytes (p=6.1E-06), and BCR signaling (p=3.4E-04). Sub-optimal responders were differed in gene expression of 345 genes ($p < 0.05$ by T-test). Interestingly, this profiles was also enriched by genes related to B cells development (p=2.8E-04), however, based on completely different pathway components. Sub-optimal responders were characterized by activation of immunoglobulins family genes including IGHD, IGHM, IGKC, IGLC1, CD43.
Conclusions: At one year of Natalizumab treatment patients with sub-optimal clinical response demonstrated over-expression of specific subset of immunoglobulins related genes. These results have clinical implications that may lead to better management of Natalizumab treated RRMS patients.

P971

Differential effects of fingolimod and copaxone upon FoxP3+ regulatory B-cells in MS

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Background: B cells are implicated in the immune pathogenesis of multiple sclerosis (MS) by secreting antibodies and cytokines as well as by their efficient antigen presentation. **Fingolimod** is a molecule chemically related to sphingosine, causes internalization of sphingosine-1-phosphate (S1P) receptors on lymphocytes, thereby blocking their egress from lymph nodes and thymus thereby reducing these cells in the periphery and their recruitment into sites of inflammation. **Glatiramer acetate (GA)**, is composed of four amino acids (L-glutamic acid, L-alanine, L-lysine, and L-tyrosine). Studies showed that B cells from Copaxone-treated mice increased production of IL-10 and reduced expression of CD80 and CD86.
Objectives: To asses in 13 RRMS patients treated with Fingolimod and 13 RRMS treated with Copaxone if there is any difference on the proportion of circulating B cells subsets in relation to their treatment.
Methods: We analyzed by flow cytometry the proportion of circulating B cell phenotype; total CD19+, CD19+Fox, and CD19+CD25+Fox in the RRMS patients before and after at 3 and 6-months of Fingolimod and Glatiramer acetate treatment.
Results: The total B lymphocytes significantly decreased at 3 and 6-months p=0.05 and 0.03, respectively for fingolimod and p=0.02 and 0.05, respectively for Copaxone. By contrast, Fingolimod showed significantly increased regulatory B lymphocytes (Breg) expressing the nuclear transcription factor associated with suppressive activity FoxP3+ at 3 and 6-months with respect to baseline (p=0.02 and 0.01). A trend to higher proportions of CD19+CD25+FoxP3+ was also apparent at 3-month of fingolimod (p=0.06). The Copaxone did not show any changes in Breg after therapy. Preliminary data showed suppressive effects by CD19+CD25+ BReg on CD4+ T cells proliferation mediated by IL-10 at 3-months of fingolimod, which was not apparent after Copaxone.
Conclusions: Our data may extend on the different mechanisms of fingolimod and copaxone in MS immunoregulation.

P972

Rapid, sustained and reversible pharmacodynamics of DAC HYP in MS patients supports mechanism of action via modulation of the IL-2 pathway

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Background: Daclizumab high-yield process (DAC HYP), a humanized IgG1 monoclonal antibody, binds IL-2 receptor α chain (IL-2Rα or CD25) and blocks association of IL-2, inhibiting high affinity but not intermediate affinity IL-2Rβγ IL-2 signaling.

This modulation of IL-2 signaling results in expansion of CD56^{bright} NK cells and decline of T-regulatory (Treg) cells.
Objectives: Evaluate pharmacodynamic (PD) effects during early and extended phases of DAC HYP treatment in Multiple Sclerosis (MS) patients.
Methods: A subset of subjects initiating treatment with sub-cutaneous (SC) DAC HYP 150mg Q4W in the OBSERVE trial were intensively sampled for 2 weeks after the first dose. CD25 expression, CD56^{bright} NK cells (CD3-CD16^{+}CD56^{bright}), and Tregs (CD4^{+}CD127$^{low/-}$FoxP3^{+}), were measured by validated FACS assays. Serum IL-2 levels were measured by a validated immuno-PCR ELISA. Results were compared to subjects who had been on long-term treatment with DAC HYP 150mg in the SELECTED study, including a subset of subjects that experienced a 6 month washout during long-term treatment.
Results: Complete saturation of CD25 on T cells was seen by 8 hours after the first SC injection of DAC HYP 150mg and sustained through the monitored treatment period, up to 2 years. By day 4, Tregs declined by ≈25% and continued to decline and then plateaued at ≈-60% change from baseline after week 8. A notable ≈50% increase in CD56^{bright} NK cells was seen by day 8 and the expansion continued in year 1 of treatment resulting in ≈5-fold increase by week 52. Serum IL-2 levels increased ≈2-fold by 4 weeks and were stable thereafter. In subjects who received DAC HYP for 3 years, the expansion of CD56^{bright} counts plateaued after year 1 and counts were then maintained in years 2 and 3. After the initial decline up to week 8, Tregs were maintained at a constant reduced level in years 2 and 3. Furthermore, the observed effects of DAC HYP on CD56 ^{bright} NK cells and Tregs were reversible to baseline levels over 6 months of washout and after re-initiation of treatment mirrored the dynamics of the first year of treatment.
Conclusions: The PD effects of DAC HYP have an early onset of action and are sustained during treatment, yet reversible upon washout. The rapid and synchronous change in serum IL-2, Treg and CD56^{bright} NK cells supports the hypothesis that DAC HYP has an immunomodulatory mechanism via decreasing high affinity and increasing intermediate affinity IL-2 signaling.

P973

Fine tuning of Treg and iNKT cells after treatment with Fingolimod in relapsing-remitting multiple sclerosis patients

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Background: Altered numbers and functions of T regulatory (Tregs) and invariant Natural Killer T (iNKT) cells have been reported in Multiple Sclerosis (MS). Tregs play a role in autoimmunity and supposedly control autoreactivity. iNKT cells are potent cytokine producers and have immunoregulatory potential. Fingolimod (FTY) induces lymphocyte retention in lymph nodes, decreasing the severity of inflammatory phenomena. However, little is known on the dynamics of the cell populations that are involved in lymph node entrapment.
Objectives: To evaluate numbers of peripheral Treg and iNKT cells in Relapsing-Remitting (RR) MS patients before and after 3, 7, 14 days and 1, 6 and 12 months of treatment with FTY.
Methods: CD3+, CD4+ and CD8+ T cells were volumetrically counted on whole blood using a CyFlow Counter (Partec, Muenster, Germany). Isolated PBMC were stained with different mAbs and analyzed with a 16 parameter Partec CyFlow ML. Treg cells were identified using the following mAbs: anti-CD3, -CD4, -CD25, -CD127, Foxp3; iNKT cells were recognized using anti-CD3, -CD4, -CD8, CD161 and -Va24Ja18. Data was analyzed by FlowJo 9.7.4 and Stata 11.0 softwares using Ranksum and Anova test.
Results: Nine RRMS patients were enrolled and followed up for one year. The number of CD3+, CD4+ and CD8+ T cells decreased after 1 month of therapy (median number of CD3+ from 1281/ul to 267/ul, CD4+ from 737/ul to 39/ul and CD8 from 460/ul to 245/ul; CD4% from 61.5 to 20.75), and remained at low values, while the percentage of CD8 increased (from 30.90% to 55.20%). Moreover, FTY induced a significant increase in the amount of peripheral Tregs (from 3% to 7%). The number and percentage of iNKT did not change during treatment of FTY. However, we observed that within iNKT cells, the percentage of those expressing CD4+ decreased (from 53.5% to 27% among iNKT), while those that were CD4- and CD8- (i.e., double negative, DN) increased during the first 14 days of therapy (from 20.60% to 43.10% among iNKT).
Conclusions: FTY increased the circulating levels of Tregs; this is consistent with the capability of restoring Treg homeostasis in MS patients. Moreover, FTY causes a reduction in iNKT cells expressing CD4, that produce Th2-type cytokines, and an increase of DN iNKT, that are less cytotoxic than those expressing CD8+. Taken together, these data suggest a double beneficial role of FTY in MS through the negative modulation of inflammatory iNKT cells and the restoration of Treg homeostasis.

P974

Increased neutralization capacity of TNF-α in sera of MS patients may play a role in MS pathogenesis and is reversed by therapy with interferon-β

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Background: High TNFα is found in active MS lesions and in CSF of MS patients it correlates with disease activity. However, TNFα neutralizing agents induced worsening of disease activity in MS studies, and TNFα antagonist therapy, in other inflammatory diseases may cause demyelinative neurological adverse events. These suggest that TNFα neutralization may play a role in inflammatory demyelinating diseases.
Objectives: To study TNFα neutralization capacity and levels of anti- TNFα antibodies (Ab) in the sera of RRMS patients.
Methods: Twenty six untreated RRMS patients, 27 interferon β1a (IFNβ1a) treated RRMS patients and 28 matched healthy controls (HC) participated in the study. The capacity of sera to neutralize TNFα bioactivity was studied by a neutralization of

TNFα- induced death of L929 cell line. Briefly, 1ng/ml of rhTNFα was incubated with two fold dilutions of sera from donors for 2hr, 37°C. Then, 50µl/well of the serum-rhTNFα mixed solution was added to the cells, with1µg/ml Actinomycin D, for overnight, 37°C, 5% CO2. Cells survival was examined by XTT assay and OD_{450nm} measurement. TNFα neutralization was calculated by the percentage of blockade of the TNFα killing effect on the cell line. Serum levels of binding anti-TNFα were studied by ELISA and are expressed in OD_{450nm}.

Results: Sera of untreated RRMS patients had a higher TNFα neutralization capacity as compared to HC. For serum titer of 1:8 the average of neutralization of TNFα killing effect in untreated RRMS was 57.8±20.8% vs. 32.4±20.6% in HC, p=0.025. For titer of 1:16 the averages of neutralization of TNFα effect were 51.0±19.3% vs. 22.4±22.5%, respectively, p=0.017, and for titer 1:32: 36.4±12.8% vs. 21.7±16.1%, p=0.05. In IFNβ1a treated patients the TNFα neutralization capacity was similar to HC, e.g for titer 1:32 the average of neutralization of TNFα effect was 20.4±19.0%, p=0.027 in comparison to untreated patients. The anti-TNFα binding Ab levels were higher in HC (0.545±0.209) vs. untreated RRMS (0.436±0.118, p=0.021) and vs. IFNβ1a treated patients (0.372±0.097, p=0.037).

Conclusions: TNFα neutralization is known to induce MS-like lesions. We found that untreated RRMS patients have an increased TNFα neutralization capacity. These finding suggest that blockade of TNFα signaling may play a role in the pathogenesis of MS. No correlation was found between the sera TNFα neutralization capacity and the levels of anti TNFα binding Ab. IFNβ1a therapy normalized the TNFα neutralization capacity. This is an unreported mode of action of IFNβ1a.

P975

Natalizumab increases HLA-G expression in PDCs of multiple sclerosis patients

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Background: Multiple sclerosis (MS) is the most common autoimmune disease of the central nervous system (CNS). MS symptoms are consequence from interruption of nerve impulse on the myelinated tracts of the CNS. Plasmacytoid dendritic cells (PDCs) are the major producers of type 1 interferon and may express molecules like indoleamine-2-3-oxigenase (IDO) and HLA-g with immunosuppressive effect. As MS is a disease with inflammation-related symptoms, all medicines for MS aim modulating or suppressing the inflammatory response. In recent years several drugs based on monoclonal antibodies have been developed an attempt to ameliorate the symptoms of MS. Natalizumab (NTZ), an anti-VLA-4 monoclonal antibody which binds to the α4 subunit of α4β1 and α4β7 integrins expressed on the surface of activated T cells, avoids the migration across the blood-brain barrier toward to the CNS by endothelial receptors such as VCAM-1 and ICAM-1.

Objectives: In this work we aimed to investigate the behavior of PDCs from MS patients submitted to treatment with NTZ.

Methods: Male and female MS patients (N=16) and healthy controls between 20 and 40 years old were our group of study. For performing the experiments, PBMCs were separated from the blood and some cerebrospinal fluid was collected. Anti-BDCA-2 and anti-HLA-g antibodies (BD Biosciences) were used to mark the cells of interest and the samples were acquired in a FACS Calibur® flow cytometer. The results were analyzed using the software Kaluza. Mann-Whitney test was used for the statistical analyses and p-value < 0.5 was considered significant.

Results: In patients with relapsing-remitting MS treated with NTZ, we observed a significant increase of HLA-g expressing on PDCs from the peripheral blood and the presence of HLA-G in PDCs in cerebrospinal fluid.

Conclusions: Our results suggest that in addition to blocking the migration of activated T cells, NTZ may contribute to the modulation of the inflammatory response in patients with MS.

P976

Effects of alemtuzumab on selective immune cell subsets in the blood of patients with relapsing remitting multiple sclerosis

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Background: The monoclonal anti-CD52 antibody alemtuzumab mediates antibody and complement-depending cytotoxicity. The application of alemtuzumab results in an altered distribution of different immune cell subtypes. Little is known about differential CD52-expression and specific depletion and recovery of particular cell subtypes.

Objectives: To evaluate effects of alemtuzumab on different immune cell subtypes in multiple sclerosis patients.

Methods: Peripheral blood mononuclear cells from 12 relapsing-remitting multiple sclerosis patients were drawn before and after alemtuzumab infusion including baseline, month (M) 3, M6, M9, M12, M15, M18, M21 and M24. Surface and intracellular markers of T-cell, B-cell, NK-cell and APC subpopulations were characterized with fluorescence-labeled antibodies and evaluated on LSR-Fortessa.

Results: Regarding CD52 expression, CD8+ and Treg-cells presented lower CD52-baseline expression than CD4+ and Th17 T-cells. CD52-baseline expression did not differ in B-cells. Baseline CD52-expression displayed lowest amounts on NK cells, compared to other cell subsets. CD52-expression was similar low between all investigated APC subtypes.

Absolute numbers of CD4+ and CD8+ T-cells decreased after alemtuzumab persisting up to M24. Relative number of CD4+ lymphocytes remained depleted, whereas CD8+ lymphocytes reached baseline values at M6. In M3, CD4+ and CD8+ CD45RA+ cells decreased and returned to baseline after M12. CD4+ and CD8+ CD45RO+cells proceeded in the opposite way. CD4+CD25+FoxP3+ Treg-cells increased in M3. Th17 T-cells decreased persistently after alemtuzumab.. Alemtuzumab decreased the number of CD19+ B-cells which recovered until M12. Individual B-cell subsets including CD19+CD27+ B-cells showed a persistent decrease, whereas CD19+CD5+ B-cells increased again. Absolute numbers of NK-cells were not affected by alemtuzumab treatment. Percentage of CD3-CD56+ NK-cells

increased from baseline. There were no changes regarding frequency of monocytes, slanDC and CD1+DC. Only BDCA2+DC decreased after alemtuzumab.
Conclusions: Alemtuzumab lead to an effective depletion of most but not all investigated immune cell subtypes. Different reconstitution patterns and CD52-baseline expression may predict and account for individual cell depletion and immunosuppressive effects.

P977
Investigation of the effects of itraconazole, a probe CYP3A and P-glycoprotein inhibitor, on the pharmacokinetics of ceralifimod (ONO-4641)
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Background: Ceralifimod (ONO-4641) is an oral, selective, non-prodrug, sphingosine-1-phosphate receptor-1 and -5 agonist in clinical development for the treatment of relapsing-remitting multiple sclerosis. *In vitro* studies have shown that ceralifimod is slowly and partially metabolized, mainly by cytochrome P450 (CYP) 3A4 and CYP3A5, and that it is a substrate of the drug transporter P-glycoprotein (Pgp).
Objectives: The purpose of this mechanistic drug-drug interaction (DDI) study was to investigate the effects of repeated oral doses of itraconazole, a prototypic strong CYP3A and Pgp inhibitor, on the pharmacokinetics (PK) of ceralifimod, specifically the role of CYP3A in ceralifimod metabolism and the role of Pgp in the systemic exposure of ceralifimod.
Methods: 16 healthy volunteers (6 men, 10 women) were included in this open-label, sequential, two-period trial. In the first period, ceralifimod PK was assessed under fasting conditions and without itraconazole coadministration: subjects received a single 0.15-mg dose of ceralifimod on Day 1. In the second period, ceralifimod PK was assessed in combination with itraconazole: subjects received 200 mg itraconazole once daily for 14 days starting on Day 23 and a second single dose of ceralifimod 0.15 mg on Day 26 (fourth day of itraconazole administration). Serial blood samples collected over 312 hours post ceralifimod dosing in both study periods were assessed via validated high-performance liquid chromatography with tandem mass spectrometric detection to quantify ceralifimod PK assessments.
Results: Ceralifimod concentration-time profiles and PK parameters remained essentially unaltered when coadministered with itraconazole. The statistical analysis resulted in test/reference ratio estimates for peak concentration (C_{max}) and area under the curve ($AUC_{0\text{-}264h}$) close to 100% (99.7% and 103.5%, respectively). 90% confidence intervals derived from the residual error term (90.9-109.4% for C_{max}; 93.7-114.4% for $AUC_{0\text{-}264h}$) were within the usual interval (80-125%) for bioequivalence studies. Overall, 14 adverse events (AEs) occurred in 8 subjects (50.0%), with no relevant difference in the number or nature of AEs between the two treatment periods.
Conclusions: Itraconazole 200 mg/day did not affect the PK of ceralifimod, thus, no relevant DDI with CYP3A or Pgp inhibitors is to be expected. Ceralifimod can be coadministered with drugs known to inhibit the CYP3A isoenzyme and/or Pgp without dose adjustment.

P978
CD19 mRNA quantification improves rituximab treatment-to-target approach: a proof of concept study
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Background: Rituximab (RTX) is a chimeric monoclonal antibody directed against CD20, a B-cell surface antigen, used for treatment of non-Hodgkin's lymphoma and several autoimmune diseases, including demyelinating diseases of the Central Nervous System (CNS) such as Relapsing Remitting Multiple Sclerosis (RRMS) and Neuromyelitis Optica Spectrum Disorders (NMOSDs). Optimising the dosing and monitoring the regimen of RTX treatment is an urgent challenge for the management of patients with relapsing autoimmune disorders of CNS. This means maximizing the efficacy of the RTX treatment and reducing over-treatment, and the cost and risks of severe adverse events. Monitoring blood CD19+ B cells by Flow Cytometry (FC) is the common strategy to personalize RTX re-treatment (Treatment-to-target-approach), but relapses occur despite CD19 antigen remaining under the threshold (< 0.1% total lymphocytes), possibly due to a limited sensitivity of FC.
Objectives: Aim of the present study is to compare pre-amplification (PA) Real Time PCR (RT-PCR) blood CD19 mRNA quantification with FC.
Methods: Five series of blood samples from 3 NMOSD patients treated with RTX were studied (47 blood samples), analysing CD19+ B cells by FC and PA-RT-PCR; a positivity threshold for PA-RT-PCR was defined (CD19 mRNA Relative Expression > $1{,}739 \times 10^{-3}$).
Results: CD19+ B cells were quantified by PA-RT-PCR in all 47 samples, whereas they were < 0.1% of total lymphocytes in 31/47 samples analysed by FC. In all samples in which CD19+ B cells were above FC threshold, CD19 mRNA was also above its threshold. Interestingly, CD19 mRNA was also above PA-RT-PCR threshold in 8 other paired samples, which resulted negative by FC, and preceded the positivity of FC in 7 out 8 of them by 1 -3 months.
Conclusions: The present study indicates that the PA-RT-PCR method could be a more sensitive strategy than FC for the detection of CD19+ B cells, thus improving the treatment-to-target approach of RTX retreatment. This approach could be applied to other RTX- treated diseases and to other monoclonal antibodies directed against CD20+ B cells.

P979
Peripheral blood lymphocyte count: a possible immunological marker of fingolimod efficacy?
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Background: Fingolimod 0.5 mg (FTY) is an established oral drug for relapsing-remitting (RR) multiple sclerosis (MS). FTY inhibits lymphocytes egress from lymph nodes, interrupting their recirculation to the central nervous system. As a consequence, blood lymphocyte count (L) decreases to 20-30% of baseline. There are no data regarding immunological markers that can be used as a measure of FTY treatment efficacy.
Objectives: Our aim was to assess a possible relationship between L and clinical/MRI outcome in MS patients treated with FTY in a clinical setting.
Methods: All consecutive patients who started FTY in our Centre before January 1st, 2013 (n=61) were included; six were excluded due to inadequate number of blood tests. Observation period was 1 year for the whole cohort, 2 years for 29 patients. Neurological exam, blood neutrophil count (N) and L were performed at baseline (T0), after 1 month (T1), then quarterly. Brain MRI was done yearly. Other variables considered: gender, BMI, previous disease-modifying drugs and immunosuppressants (IS), number of relapses 1-2 years before FTY, age and MS duration. Possible confounding factors like steroid courses were recorded. Definition of active patients was: ≥1 new T2 lesions or ≥1 Gd-enhancing lesion at MRI and/or ≥1 relapse and/or ≥1 point worsening in EDSS score confirmed after 6 months.
Results: we compared active vs disease-free patients with respect to the following variables: L at T0 (L0), L at T1 (L1), mean L (mL) over 1 and 2 years of treatment, ΔL1 (L0-L1) and ΔL (L0-mL). No significant differences were found over 1 year of treatment. A significant difference was found for L0 (2084±392 vs 2428±428; p=0.050) and ΔL1 (1503±402 Vs 1830±314; p=0.049) over 2 years. L was influenced by gender (female vs male L0: 2113±753 vs 2436±607, p=0.053), previous IS use (L0: 1920±556 vs 2287±750, p=0.062) and steroid courses (L0: 1981±357 vs 2340±416, p=0.037; ΔL: 1342±269 vs 1683±398, p=0.012). This latter finding was not observed for mean N (1401±1486 vs 1151±1039, p=0.344), excluding a relevant direct effect of steroids on L. Multivariate logistic analysis (L0, ΔL1, gender, IS) abolished any statistically significant correlation.
Conclusions: Lower values of L0 and ΔL1 were associated with MS activity over 2 years of FTY treatment. However, such finding was not confirmed by multivariate model. Therefore, in our opinion, peripheral blood lymphocyte count cannot be used as a marker of FTY treatment efficacy.

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A two-years follow-up study of multiple sclerosis patients treated with interferon-beta: predictive value of NAbs at six months of treatment

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Background: Interferon beta (IFN-beta) is a first-line treatment of relapsing-remitting multiple sclerosis (MS); although the clinical efficacy of this therapy has been demonstrated consistently in large, randomized, placebo-controlled trials, a proportion of treated patients ranging from 7% to 49% show a poor clinical response. Furthermore, it has been observed consistently that a proportion of patients develop neutralizing antibodies (NAbs) directed against IFN-beta.
Objectives: To evaluate the possible predictive value of the determination of NAbs after only six months of IFN-beta treatment in the clinical response to this treatment after two-years of follow-up.
Methods: A total of 321 serum samples collected from 321 MS patients after only six months of interferon beta treatment were collected (136 received IFN beta-1b 250 μg subcutaneously [sc] every other day, 114 IFN beta-1a 22 μg or 44 μg sc three times weekly, and 71 IFN beta-1a 30 μg intramuscularly [im] once weekly). NAbs were measured through a cytopathic effect (CPE) assay using the encephalomyocarditis murine virus on human lung carcinoma cell line (A549). The titres were calculated according to Kawade's formula, and expressed in tenfold reduction unit (TRU). Samples were considered positives if titres were > 20 TRU. The following clinical data were collected for each one of the MS patients: EDSS variation between the basal visit (prior IFN-beta treatment) and the 24 months visit, and the relapse rate after two-years of IFN-beta treatment.
Results: A total of 40 (12.5%) serum samples were positive after six months of IFN-beta treatment; only 7 (2.2%) serum samples had titres >100 TRU. After two-years of follow-up, those MS patients with a positive NAbs serum sample after six months of IFN-beta treatment had a relapse rate of 0.89 vs. 2.1 for MS patients with a positive NAbs serum sample >100 TRU ($p< 0.01$) vs. 0.65 for those MS patients without NAbs after six months of IFN-beta treatment ($p>0.05$ and $p< 0.01$, respectively); the EDSS variation among those MS patients with a positive NAbs serum sample after six months of IFN-beta treatment was +0.2 vs. +1 for MS patients with a positive NAbs serum sample >100 TRU ($p< 0.01$) vs. -0.2 for those MS patients without NAbs after six months of IFN-beta treatment ($p< 0.01$ and $p< 0.001$, respectively).
Conclusions: A positive NAbs serum sample after only six months of IFN-beta treatment could be a good marker of poor clinical response, overall for positive NAbs serum samples >100 TRU.

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Stress induced angioplasticity is protective in experimental autoimmune encephalomyelitis

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Background: In the brain, tissue survival is dependent on maintenance of metabolic homeostasis. In response to stress cells of the blood brain barrier make fine tuned regulatory adaptations that result in a continuous matching of tissue oxygen with capillary density (adaptive angioplasticity) that promotes survival. Loss of adaptive angioplasticity may underlie the pathophysiology of a number of neurodegenerative diseases including multiple sclerosis (MS). We hypothesize that treatment strategies that restore vascular homeostasis will improve tissue repair, ameliorate inflammatory activity, mitigate secondary injury and improve clinical outcome in myelin oligodendrocyte glycoprotein (MOG)

peptide-induced experimental autoimmune encephalomyelitis (EAE).

Objectives: Identify new molecular targets that promote repair in neurodegenerative disease.

Methods: C57BL/6 mice were immunized with MOG35-55. Following development of clinical symptoms mice were placed in normobaric hypoxia chambers calibrated to 10% oxygen for up to 3 weeks. Clinical scores and weight were evaluated daily and spinal cords were harvested. Lumbar spinal cord sections were stained using Hematoxylin-Eosin(H+E) to visualize leukocyte infiltration and for the glucose transporter protein (Glut-1) to determine vascular density.

Results: Exposure to chronic mild hypoxia induced angioplasticity in immunized and sham immunized control mice. Mild hypoxia ameliorated the signs and symptoms of chronic EAE during the treatment period. Induction of angioplasticity was associated with induction of hypoxia inducible factor-1 alpha (HIF-1a) and VEGF as well as increased vascular density and decreased evidence of leukocyte migration to the spinal cord. In addition, endothelial activation markers VCAM and uPAR were decreased in the spinal cords of hypoxia exposed EAE mice.

Conclusions: Stress induced adaptive angioplasticity promotes recovery of EAE. Mechanistic insight may lead to the identification of novel therapeutic targets that will promote tissue repair. Current knowledge of pathophisiology and treatment of neurodegenerative diseases requires further research in this area. The intent of the study is to identify new molecular targets that promote repair in neurodegenerative diseases including multiple sclerosis. EAE with stress induced angioplasticity is the key model for that study.