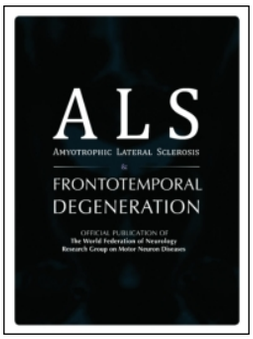

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THEME 7 IMAGING, ELECTROPHYSIOLOGY AND MARKERS OF DISEASE PROGRESSION

P156 FDG-PET STUDY IN AMYOTROPHIC LATERAL SCLEROSIS/PARKINSONISM-DEMENTIA COMPLEX OF THE KII PENINSULA OF JAPAN

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Keywords: FDG-PET, Kii ALS-PDC

Background: Amyotrophic lateral sclerosis/parkinsonism-dementia complex of the Kii peninsula of Japan (Kii ALS/PDC) is a unique fronto-temporal dementia. The purpose of this study is to reveal glucose metabolism in the brains of Kii ALS/PDC using FDG-PET.

Method: Seven patients with Kii ALS/PDC (one ALS, two ALS with dementia, four PDC) were submitted for the study. FDG-PET was performed on each patient and the data were statistically analyzed by 3D-SSP method.

Result: Glucose metabolism was decreased predominantly in the frontal lobe (frontal gyri, pars opercularis, orbital gyri) and temporal lobe (parahippocampal gyrus, temporal gyri, temporal pole, fusiform gyrus). In some patients, reduced glucose metabolism spread to the parietal lobe (parietal lobules, supramarginal gyrus, angular gyrus, precuneus). The decrease of glucose metabolism in the cingulated gyrus was seen in all patients.

Conclusions: In Kii ALS/PDC, the reduction of glucose metabolism was detected mainly in the frontal and temporal lobes, extending to the parietal lobe. The decrease of glucose metabolism in the cingulated gyrus was an essential finding in Kii ALS/PDC.

P157 EXECUTIVE FUNCTION AND FRONTOTEMPORAL DEGENERATION IN ALS AND ALS-FTD

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Keywords: FTD, imaging

Background: A sizable percentage of ALS patients possess a fronto-temporal syndrome. We therefore hypothesize that this reduced cognitive function reflects fronto-temporal degeneration across the spectrum of ALS and ALS-FTD.

Objectives: To correlate neuroimaging with neuropsychological testing in patients with ALS and ALS-FTD.

Methods: Twenty-one patients (10 ALS, 9 ALS-FTD, and 2 ALS with executive dysfunction) were studied with neuropsychological tests. Diagnoses of FTLD were made in a multidisciplinary team conference using Neary Criteria. MRI exams were performed on a 1.5T Scanner. Templates of lobar grey matter and white matter (determined from DTI connectivity) were registered to the patients' T1-weighted volumes ($1 \times 1 \times 1.5$ mm^3) and DTI data ($2.2 \times 2.2 \times 2.2$ mm^3) to determine grey matter volume (GV), white matter mean diffusivity (MD) and fractional anisotropy (FA), respectively. Anova ($p < 0.05$) were used for cross-sectional comparisons of MRI metrics between ALS and ALS-FTD cohorts after removing age and gender effects. Stepwise regression models of cognitive variables as functions of lobar GV, FA and MD with age and gender as covariates were performed.

Results: Frontal grey matter volume was decreased and right temporal white matter MD increased in ALS-FTD compared to ALS. For the MMSE and CVLT-SF there were no significant correlations. For verbal-fluency the following correlations were obtained: R Frontal-GV (RSQ: 0.31; $p < 0.05$), L Frontal-FA (RSQ: 0.41; $p < 0.008$), R Temporal-GV+FA (RSQ: 0.46; $p < 0.008$), and L Temporal-GV+FA (RSQ: 0.45; $p < 0.008$). For DKEFS-Trails the following correlations were obtained: R Frontal MD (RSQ: 0.52; $p < 0.05$), L Frontal-MD (RSQ: 0.45; $p < 0.008$), R Temporal-GV (RSQ: 0.67; $p < 0.001$), L Temporal-MD (RSQ: 0.36; $p < 0.008$). Boston naming and DKEFS-Stroop both correlated significantly with MD for the right frontal lobe and with GV for the left frontal and bilateral temporal lobes ($p < 0.008$).

Discussion and Conclusions: Phonemic verbal fluency correlated with grey and white matter abnormalities, as did a verbal inhibition task (Stroop) and a test of mental flexibility (Trails). These results demonstrate that impaired cognition is associated with increased fronto-temporal grey and white matter degeneration in ALS-FTD compared to ALS patients.

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P158 HIPPOCAMPAL ATROPHY RELATED TO MEMORY PERFORMANCE IN NON-DEMENTED ALS PATIENTS: A VOXEL BASED MORPHOMETRY AND NEUROPSYCHOLOGICAL STUDY

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Keywords: hippocampus, memory, VBM

Background: In patients with amyotrophic lateral sclerosis and frontotemporal lobar degeneration (ALS-FTLD), neuropathological studies have shown abnormalities in the medial temporal lobe. In 20–30% of ALS patients (without FTLD) cognitive dysfunction, including memory impairments, have been found. The nature of structural brain abnormalities, if

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present, underlying memory dysfunction in non-demented ALS patients *in vivo* is unknown.

Objectives: To investigate global and regional brain atrophy in relation to memory function in non-demented ALS patients, using voxel based morphometry (VBM) of MRI images.

Methods: T1-weighted 3T MRI images were obtained from 27 non-demented ALS patients (6 bulbar onset, 21 limb onset) and 23 age, education and gender matched healthy controls. Global and regional brain atrophy was quantified using voxel based morphometry, implemented in SPM5. After automated segmentation, the Diffeomorphic Anatomical Registration Through Exponentiated Lie (DARTEL) algebra was used for registration and normalisation of the grey matter images. Patients and controls underwent a neuropsychological assessment adapted to motor impairment, including tests of memory, executive functions, language and attention. Patients had a vital capacity above 70% of the predicted value. Normative data were used to calculate frequencies of cognitive dysfunction (defined as performance lower than 2 SD below the mean, corrected for age and level of education). Global brain atrophy was quantified and the presence of regional brain atrophy was investigated in relation to cognitive measures.

Results: Neuropsychological investigation: Comparison with normative data revealed that both Immediate and Delayed Recall subtests of the Logical Memory subtest of the Rivermead Behavioural Memory Test (RBMT) were most frequently (19%) abnormal in ALS patients ($n=26$), compared to other cognitive tests. As a group, ALS patients performed worse on an attention/working memory test (letter-number sequencing) and naming. VBM: Overall, ALS-patients showed reduced volume of the right dorsolateral prefrontal cortex and the superior frontal gyrus compared to controls. In ALS patients, performance on the RBMT was correlated to bilateral hippocampal volume (L: $Z=4.40$, $p<0.001$, uncorrected; R: $Z=3.72$, $p<0.001$, uncorrected) and left insula volume ($Z=4.06$, $p<0.001$). These correlations were not found in controls. No differences were found in white matter volumes, or in grey matter volume of the precentral gyrus.

Conclusions: Non-demented ALS patients with mild cognitive dysfunction show bilateral grey matter hippocampal atrophy related to verbal memory function. In addition, two regions in the prefrontal cortex, reported earlier to be involved in non demented ALS patients, show grey matter atrophy. These results provide further support for extramotor involvement, including the medial temporal lobes, in non-demented ALS patients.

P159 7 TESLA MRI SHOWS NO SIGNS OF BLOOD-BRAIN BARRIER DYSFUNCTION IN ALS

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Keywords: 7 Tesla MRI, blood-brain barrier dysfunction, microbleeds

Background: Recently there have been several reports about disruption of the blood-spinal cord barrier (BSCB) in SOD1 transgenic mice (1–3). A reduction of tight junction proteins results in microhemorrhages and the release of neurotoxic haemoglobin-derived products as well as reductions in microcirculation and hypoperfusion. In a later phase inflammatory changes occur (1). We investigated blood brain barrier (BBB) disruptions in early stage ALS with susceptibility weighed imaging (SWI) on 7 Tesla MRI.

Objectives: To investigate the integrity of the BBB in ALS by quantification of microbleeds or hemosiderin depositions.

Methods: Twelve patients with ALS (probable lab-supported, probable or definite ALS according to the El Escorial criteria) and 12 age and sex matched healthy controls were studied. The patients were recently diagnosed with ALS and subjects with vascular comorbidity were excluded. Clinical status was evaluated with the ALS Functional Rating Scale-Revised (ALSFRS-R). We performed SWI on a Philips 7 Tesla MRI scanner. With this type of imaging hemosiderine deposits of less than 0.5mm can be detected. Two blinded physicians counted the microbleeds.

Results: Sporadic microbleeds were detected in the patient group and the control subjects. No differences were found between the two groups.

Discussion and Conclusions: There is no evidence for BBB disruption in early stage ALS based on SWI with high field MRI. Further research is needed to reveal the role of BBB disruptions in the pathogenesis of ALS.

References:

1. Garbuzova-Davis S, Saporta S, Haller E *et al.* PLoS ONE 2007; 2(11):e1205
2. Garbuzova-Davis S, Haller E, Saporta S *et al.* Brain Res 2007; 1157:126–37
3. Zhong Z, Deane R, Ali Z *et al.* Nat Neurosci 2008; 11(4):420–2

P160 CHANGES IN CORTICAL AND SUBCORTICAL MOTOR ACTIVITY IN AMYOTROPHIC LATERAL SCLEROSIS: A FMRI STUDY AT EARLY DISEASE STAGE AND DURING ITS PROGRESSION

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Keywords: fMRI, motor activity

Background: A suitable method to study the dynamics of neurodegeneration and possible compensatory processes in ALS is functional imaging. The neurodegenerative process is already advanced before muscle weakness and wasting occurs. In this study we investigated the motor activations using fMRI with a focus on unaffected limbs. We also compared these data with those from more progressed disease status.

Methods: Two groups were investigated using BOLD-fMRI, while they performed a blocked motor task (finger flexion and extension of fingers in the right hand against rest). Imaging was performed in a 3T system. fMRI data were analyzed with Brain Voyager QX. The first group comprised of 22 healthy volunteers (12 women) aged from 42 to 67 years (mean age 61 years). The second group consisted of 22 patients (9 women) who fulfilled the diagnostic criteria for probable or definite ALS according to the revised El Escorial criteria of the World Federation of Neurology. The mean ALSFRS-R score was 39.5 (range 34 to 45). The interval between the diagnosis and

the study was 6 months (range 1 to 15). Medical Research Council (MRC) scale was used for evaluation of muscle strength. Grip strength of the right hand was evaluated using Martin-Type Squeeze Dynamometer (Vigorimeter, Martin, Tübingen, Germany).

Results: During finger movement we found significant activations in primary motor and premotor cortex (precentral gyrus; BA 4, 6); somatosensory cortex (postcentral gyrus; BA 2, 3); supplementary motor area (SMA, BA 6) and subcortical areas in all groups. The contralateral cortical and subcortical activity in ALS patients was significantly increased compared to the control group even for clinically unaffected limbs. Cluster volumes of contralateral activity were highly similar for ALS patients in different stages of disease and showed no correlation to muscle atrophy or weakness in the performing right hand. However the Beta weight of these clusters had a negative correlation with disease progression and a positive correlation with ALSFRS-score. The rate of ipsilateral sensorimotor activation was increased in ALS and the volume was negatively correlated to weakness in the right hand.

Conclusions: The increased cortical activity in ALS patients for movements with clinically unaffected limbs demonstrates adaptive changes in cortical activity before the manifestation of clinical signs. Decrease of Beta weight in the contralateral sensorimotor area and increase of cluster volume of the ipsilateral sensorimotor area are additional functional changes during disease progression.

P161 CORTICAL FINGERPRINT AND MOTOR NETWORK DEGENERATION IN ALS

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Keywords: diffusion tensor imaging, cortical thickness, dying forward

Background: Motor neuron and corticospinal tract (CST) degeneration are key pathological features of amyotrophic lateral sclerosis (ALS). Automated whole brain cortical thickness measurements can provide objective information on the pattern of neurodegeneration and the involvement of motor areas. The specific pattern of neurodegeneration could be a surrogate marker for ALS (1). We evaluated the involvement of the motor network by measuring the cortical thickness in the precentral gyrus together with diffusion tensor imaging (DTI) on the CST and the corpus callosum.

Objectives: To explore the pattern of neurodegeneration in ALS. To estimate the degree of motor network degeneration based on cortical thinning in the motor areas and DTI of the CST and the corpus callosum.

Methods: Twelve patients with ALS (probable lab-supported, probable or definite ALS according to the El Escorial criteria) and age and sex matched healthy controls were studied. Clinical status was evaluated with the ALS Functional Rating Scale-Revised (ALSFRS-R). Cortical thickness and DTI data was acquired on a 3 Tesla Philips Achieva Medical Scanner. We used a validated and automated method to measure whole brain cortical thickness. We measured fractional anisotropy (FA) values along the CST and the corpus callosum, using tract-basted statistics (2).

Results: We found two regions of significant cortical thinning in the primary motor areas in ALS. These regions correspond to the arm and leg region respectively. Slightly reduced FA values were found along the corpus callosum and the CST in ALS compared to controls. Interestingly, exploratory analysis suggest that this reduction gradually decreases as the CST descends from the cortex to the brainstem. We found the average cortical thickness in the precentral gyrus to be associated with the FA values in the rostral part, but not in the caudal part of the CST. There was also an association between cortical thickness and FA values in the corpus callosum.

Discussion and Conclusions: This study gives an impression of the pattern of neurodegeneration and an indication for motor network degeneration in ALS. This degeneration seems to occur in an anterograde manner. This finding gives new insights into the mechanism of neurodegeneration. The advanced analysis technique permits detailed quantification of FA values along a neural tract. The association of FA values with cortical thickness supports the proposed mechanism.

References:

1. Seeley WW, Crawford RK, Zhou J *et al*. Neuron 2009; 62(1):42–52
2. Mandl RC, Schnack HG, Luigjes J *et al*. Schizophr Bull. 2008 Nov 27

P162 CHANGES OF FUNCTIONAL CONNECTIVITY IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: fMRI, resting-state

Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease involving primarily the upper and lower motoneurons with a rapid progression. Even early descriptions of ALS pointed out that some patients develop dementia and, more recently, neuropsychological, electrophysiological, and neuroimaging results suggest that the disease process involves other parts of the nervous system. The demonstration of functional involvement of brain networks outside of the motor system proper is of great importance for our understanding of ALS. The present contribution seeks to provide evidence for such extra-motor involvement using independent component analysis (ICA), applied to blood oxygen level-dependent (BOLD) time-series obtained during rest.

Methods: A functional imaging approach was used, involving the analysis of resting state activity, followed by the definition of functionally connected brain networks by ICA to assess differences between ALS patients (n = 20) and healthy controls (n = 20). Analysis and visualization of the data were performed using Brain Voyager QX (Brain Innovation BV, Maastricht, The Netherlands) software.

Results: ICA analysis revealed 5 typical brain networks among which two, the so-called default mode network and the sensori-motor network showed distinct differences between patients and controls. The default mode network showed less activation in patients in several regions including the ventral anterior cingulate cortex, posterior cingulate cortex and the left and right inferior

parietal cortex, regions that have been linked previously to executive functions. The sensori-motor network showed group differences in the premotor cortex. As in the present approach no task is imposed on the subject, these data favour a primary functional involvement of the premotor cortex in ALS.

Conclusions: Analysis of resting state network activity in ALS allowed the demonstration of significant changes in two out of five studied networks, the default mode and sensori-motor networks. The former has been linked to cognitive processes, whereas the latter has been demonstrated to be involved in motor control. The present results once again demonstrate extra-motor involvement in ALS. Among the decisive advantages of the resting state approach is the fact that no task is imposed on the subjects and thus no compensatory processes (e.g. increased effort) have to be considered. Further advantages include the brief examination time and the ability to investigate several networks at the same time.

P163 DIFFUSION TENSOR IMAGING OF THE CORTICOSPINAL TRACT IN RECENT ONSET MOTOR NEURON DISEASE: A LONGITUDINAL STUDY

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Keywords: MRI, diffusion tensor imaging, subtypes

Background: Amyotrophic lateral sclerosis (ALS) is a disease of upper (UMN) and lower (LMN) motor neurons. Whether the initial target of disease is the UMN, LMN, or both simultaneously, has not been settled. ALS and other motor neuron diseases such as primary lateral sclerosis (PLS) and progressive muscular atrophy (PMA) may have different etiologies or different disease modifying factors. Therefore, several scenarios of disease onset and progression may be studied in these phenotypes. Diffusion tensor imaging (DTI) is a surrogate marker for UMN degeneration. Decreased anisotropy suggests diminished integrity of a tract and thus is a surrogate marker for axonal loss. Diffusion tensor tractography allows for the *in vivo* investigation of white matter tracts.

Objectives: To focus on UMN involvement as measured with diffusion tensor tractography of the corticospinal tract (CST) in recent onset ALS and PMA.

Methods: In a prospective cohort study we included patients with bulbar onset ALS (ALS-B), limb onset ALS (ALS-L), PMA, PLS and healthy controls, 12 individuals per group. Patients with ALS and PMA had weakness for less than one year. All patients were seen at baseline and 6 months later, except for the PLS patients who were only seen at baseline. Main clinical outcome measures were the revised ALS functional rating scale (ALS-FRS-R) and finger tapping speed. Diffusion tensor MRI data were acquired by means of a spin-echo EPI sequence. We computed fractional anisotropy (FA) values from the eigenvalues. We reconstructed the corticospinal tract bilaterally in DTI-Studio. FA profiles were derived along the caudo-cranial course of the CST. Volumetric changes in grey and white matter were studied by means of Voxel Based Morphometry (VBM) of three dimensional T1- weighted MR images using DARTEL. Statistical significance for all analyses was set at $p < 0.01$).

Results: Disease rapidity did not differ significantly in PMA from that in ALS-L and ALS-B. In PLS we found a significantly decreased FA along the entire course of the CST. In ALS-L we found a significantly decreased FA only in the centrum semiovale at baseline, whereas at follow-up there was a trend towards a further decrease more caudally. In ALS-B FA was significantly decreased in the caudal CST (pons/mesencephalon) and in the posterior limb of the internal capsule. At follow-up there was a trend towards a further decrease more cranially. In PMA FA showed neither a decrease along the CST compared to controls nor a decrease over time. In PLS only we demonstrated volumetric changes in PLS in the subcortical white matter of the primary motor cortex. Finger tapping speed correlated strongly with FA values. FA change over time was not correlated with a change in any of the clinical variables.

Discussion: We demonstrated CST degeneration at baseline in PLS and both ALS groups but not in PMA, with a trend towards further degeneration at follow-up only in both ALS groups. FA values correlated strongly with finger tapping speed.

P164 REGIONAL WHITE MATTER ALTERATIONS IN RARE MOTOR NEURON DISEASES: A WHOLE BRAIN-BASED ANALYSIS BY USE OF DIFFUSION TENSOR IMAGING METHODS

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Keywords: diffusion tensor imaging, Primary Lateral Sclerosis, Hereditary Spastic Paraparesis

Background: Different motor neuron disorders (MNDs) are mainly defined by the clinical presentation based on the predominance of upper or lower motor neuron involvement and the course of the disease. To date, magnetic resonance imaging (MRI) has mostly served as a tool to exclude other pathologies, but step by step novel approaches based on volumetric/morphometric techniques, magnetic resonance spectroscopy and, most recently, diffusion tensor imaging (DTI) have started to add information on the underlying pathophysiological processes of these disorders *in vivo*.

Methods: The present study was designed to investigate three different MND, i.e. primary lateral sclerosis (PLS, N = 25), pure hereditary spastic paraparesis (HSP, N = 24), and X-linked spinobulbar muscular atrophy (X-SBMA, N = 20), by application of whole-brain based DTI analysis methods in comparison with matched controls. All data analyses were performed by use of the DTI software TIFT (Tensor Imaging and Fiber Tracking).

Results: The analysis of white matter (WM) involvement revealed widespread and characteristic patterns of alterations within the motor system with varying predominance

according to the clinical focus. There were also WM changes in projection to the limbic system and within the corpus callosum, the latter both for HSP and, unexpectedly, for PLS. In detail, multiple areas of significantly reduced fractional anisotropy within the supratentorial motor system were delineated in the PLS group and in the HSP group, and to a lesser degree in patients with X-SBMA. In addition, the X-SBMA group showed large involvement of the limbic projectional system which was less widespread in HSP and PLS.

Discussion: In summary, DTI was able to delineate a characteristic WM pathoanatomy in motor and extra-motor brain areas for different MND via whole brain-based fractional anisotropy assessment. Future advanced MRI-based investigations might help to provide a fingerprint-identification of MND.

P165 AN ALGORITHM TO DETERMINE UPPER MOTOR NEURON INVOLVEMENT IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: corticospinal tract, transcranial magnetic stimulation, diffusion magnetic resonance imaging

Background: The diagnosis of Amyotrophic Lateral Sclerosis (ALS) relies on concomitant upper and lower motor neuron dysfunction in one or more body regions. In some patients, upper motor neuron (UMN) features may be subclinical, potentially resulting in misdiagnosis.

Objectives: The present study combined novel threshold tracking transcranial magnetic stimulation (TMS) with diffusion tensor imaging (DTI) techniques to develop an objective marker of UMN dysfunction.

Methods: Studies were undertaken in 10 ALS patients and results were compared to 10 normal controls. TMS studies were performed using a 90 mm circular coil connected to a BiStim device, with magnetic evoked potentials recorded from abductor pollicis brevis. Fifteen directional DTI scans were obtained using an isotropic voxel resolution of 2.5 mm.

Results: Short interval intracortical inhibition (SICI) was significantly reduced in ALS patients (averaged SICI 1–7 ms ALS $-2.1 \pm 0.8\%$; controls $7.1 \pm 1.2\%$, $P < 0.0001$). This reduction in SICI was associated with reductions in intracortical facilitation and cortical silent period duration, thereby re-affirming the presence of UMN dysfunction in ALS. DTI demonstrated significant reduction in fractional anisotropy (FA) along the corticospinal tract (CST) (left CST ALS 0.58, control 0.62, $P < 0.01$ and right CST ALS 0.57, control 0.60, $P < 0.01$). There was also an increase in trace apparent diffusion coefficient (trace ADC) and perpendicular diffusivity (Perp D), consistent with fibre tract degeneration. There was significant correlation between SICI and DTI parameters, suggesting that SICI was associated with fibre tract degeneration. Based on these correlation studies, an UMN index was developed, which was more sensitive than conventional DTI findings at detecting UMN abnormalities long the CST.

Discussion and Conclusions: TMS and DTI studies have established simultaneous functional and structural abnormalities of the UMN system in ALS. The UMN index may be refined as a diagnostic and prognostic marker in ALS.

P166 EVIDENCE FOR WIDESPREAD GREY AND WHITE MATTER VOLUME DECREASES IN PRIMARY LATERAL SCLEROSIS: A VOXEL-BASED MORPHOMETRY STUDY

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Keywords: voxel-based morphometry, Primary Lateral Sclerosis, GM/WM alterations

Background and Objectives: Motor neuron disorders reflect a heterogeneous group of neurodegenerative diseases, which tend to follow a more or less slow course. Due to differences in prognosis and therapeutic aspects, biomarkers are needed to discriminate different disease entities. In primary lateral sclerosis (PLS), clinical diagnostic criteria were defined and supportive laboratory tests help to exclude other diseases. Magnetic resonance imaging (MRI) and further advanced imaging techniques (e.g. voxel-based morphometry, VBM) are of increasing interest due to their potential to elucidate neuropathological alterations *in vivo*.

Methods: We evaluated 25 patients with PLS (57.0 ± 12.5 years), recruited from our outpatient clinic for MND at the Department of Neurology of the University of Ulm, Germany. All patients underwent standardized clinical, neurological and routine laboratory examinations and met the diagnostic criteria for PLS. 3-D T1-weighted data sets (MP-RAGE) were acquired on a 1.5 Tesla clinical MRI scanner and complete postprocessing for grey and white matter separately was performed according to the optimized VBM protocol by use of SPM2 (Statistical Parametric Mapping).

Results: Voxel clusters of significantly decreased grey matter volumes ($p < 0.05$, corrected) were localized in the superior frontal gyrus in close proximity to the parasagittal cortical representation area of the lower limbs, within the nucleus ventralis posterolateralis of the right thalamus and in projection to the middle frontal gyrus of the right hemisphere, respectively. The WM analysis revealed widespread clusters of significantly decreased WM volume in subcortical brain areas adjacent to the motor system at $p < 0.05$, corrected, with a slight preponderance of the right hemisphere.

Discussion: Neuroimaging evidence was obtained for structural GM and WM alterations in patients with PLS. These results are in accordance with the proposition that the most prominent pathologic hallmark in PLS is reflected by the loss of pyramidal neurons in motor and premotor cortical areas and associated axonal degeneration. Particularly with regard to the widespread WM alterations adjacent to the cortical representation areas of the motor system of the lower limbs, these results may serve as a fingerprint-characterization for PLS in comparison to other MND.

P167 UTILIZING DIFFUSION TENSOR IMAGING (DTI) FOR THE ASSESSMENT OF UPPER MOTOR NEURON INVOLVEMENT IN FLAIL LEG VARIANT ALS

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Keywords: flail leg syndrome, diffusion tensor imaging, UMN involvement

Background: The flail leg ALS variant is a lower motor neuron syndrome characterized by progressive distal onset weakness and wasting restricted to the lower limbs for at least 12 months. It can be identified in 3–6% of all ALS cases. Patients survive, on average, twice as long in comparison to classical ALS patients. The integrity of upper motor neuron projections in the flail leg variant of ALS has yet to be studied. Fractional anisotropy (FA) measures, derived from diffusion tensor weighted imaging (DTI), serve as sensitive *in vivo* markers of the integrity of cerebral fibre tracts.

Objectives: To assess the degree of degeneration of the intracranial portion of the corticospinal tract in flail leg variant ALS in comparison to classical ALS using DTI.

Methods: We studied two patients with flail leg syndrome (aged 55 and 60 years; ALSFRS-R 36-, 38/48, disease duration 40 and 26 months, respectively). For comparison, we studied 5 patients with the clinical diagnosis of definitive ALS (aged 47–61 years; ALSFRS-R 12–42/48, median 27/48; disease duration 10–54 month, median 14), using DTI at 1.5 Tesla. After selection of a seed region of interest, we tracked the nerve fibres along the dorsal pyramidal tract which represents the projection site for the legs. FA along these fibre tracts was averaged and individually compared between the flail leg patients and the classical ALS controls using z-score transformation.

Results: Among the 2 patients with flail leg ALS relative to the classical ALS controls, the z-scores of FA values were (right/left) 0.9 and 1.6 for the one, and 1.5 and 3.3 for the other patient. This suggests that the fibre tracts are relatively preserved in the dorsal part of the pyramidal tract.

Conclusions: The fibre tracts representing projections from the upper motor neuron of the legs were relatively preserved in patients with flail leg syndrome relative to classical ALS patients. These findings support the notion that DTI is a significant tool to measure the integrity of cerebral fibre tracts in neurodegenerative disorders.

P168 ELECTRICAL IMPEDANCE MYOGRAPHY AS A PRE-SYMPTOMATIC BIOMARKER OF ALS

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Keywords: electrical impedance myography, biomarker, pre-symptomatic

Background: Amyotrophic lateral sclerosis is characterized by a pre-symptomatic phase during which neuronal degeneration occurs prior to the appearance of clinical symptoms. The unknown duration of this preclinical phase as well as the possibility that motor neuron loss is extensive prior to the appearance of symptoms represent serious challenges to a better understanding of the cause and risk factors for ALS, and to the development of effective therapies. These observations provide a rationale for the identification of biomarkers of the preclinical phase of the disease that might be used to permit early diagnosis and initiation of therapy earlier in the course of the disease.

Objectives: To evaluate the utility of electrical impedance myography (EIM), a novel electrophysiological technique that is sensitive to changes in the biophysical properties of muscle, as a biomarker of pre-symptomatic disease.

Methods: The Pre-Familial ALS (Pre-fALS) study is an ongoing prospective observational study of people at risk for developing ALS. Asymptomatic individuals from SOD1 positive (SOD+) fALS pedigrees are recruited and evaluated annually. Both SOD1+ and SOD1− family members are included. Multi-frequency EIM was performed by an evaluator blinded to SOD1 mutation status. EIM involves the application to a limb of a low-intensity, alternating current at a spectrum of frequencies ranging from 10 kHz to 1 MHz with measurement of the resulting voltages across a pair of electrodes placed over a muscle of interest. EIM provides both a measure of the offset in timing of current flow produced by the cell membranes (the reactance) and a measure of the obstruction to current flow offered by intracellular and extracellular fluid (the resistance). EIM data for the spectrum of frequencies were collapsed into single values by calculating the slope of the reactance values across a range of frequencies. Subjects were categorized as likely SOD1+ or likely SOD1− based on EIM results by an independent blinded evaluator.

Results: Twenty-five participants were studied, including 17 SOD1+ and 8 SOD1− subjects. Mean age (range) in the SOD1+ group was 48 (27–63) compared to 49 (31–64) in the SOD1− group. There were more females in the SOD1+ group (76% vs 50%). Height and weight were comparable between the two groups. Fourteen of the 17 SOD1+ subjects were correctly classified as such based on the results of EIM alone (sensitivity 82%). Five of the eight SOD1− subjects were correctly classified as such based on EIM alone (specificity 62.5%). The kappa statistic is 0.44, supporting moderate agreement between the two tests.

Discussion and Conclusions: These cross-sectional data suggest that the EIM reactance slope may be a useful biomarker of pre-symptomatic disease in SOD1+ individuals. Confirmation and validation of these findings will await the results of our ongoing longitudinal evaluation of the Pre-fALS study cohort.

P169 ASSESSING ALS PROGRESSION OVER SHORT TIME PERIODS WITH FREQUENT MEASUREMENTS: A COMPARISON OF ELECTRICAL IMPEDANCE MYOGRAPHY, HANDHELD DYNAMOMETRY AND ALSFRS-R

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Keywords: biomarker, electrical impedance myography, handheld dynamometry

Background: Electrical impedance myography (EIM) is a technique for the assessment of neuromuscular disease that relies upon the application of high-frequency, low-intensity electrical current to localized areas of muscle and the measurement of the resulting surface voltages. To date, EIM

has shown good reproducibility and a high sensitivity to progressive neurogenic change in muscle. For this reason, it was reasoned that EIM may be capable of detecting muscle deterioration in ALS over short periods of time. Ultimately, since the technique is easy to apply, it could serve as a useful outcome measure in ALS clinical trials.

Objectives: To assess EIM's potential to detect ALS disease progression over short time periods in comparison to hand-held dynamometry (HHD) and the ALSFRS-R.

Methods: Two ALS patients were recruited as part of a multi-center study evaluating EIM in ALS. The first was a 64-year-old man with sporadic disease and slow progression and the second was a 44-year-old woman with the SOD1 A4V mutation and clinically more rapid progression. EIM, handheld dynamometry, and ALSFRS-R data were obtained as frequently as every 2 weeks for a period of several months. EIM measurements were made over several upper and lower extremity muscles on one side. HHD was performed bilaterally.

Results: In the slowly progressing patient, at the end of 3 months, EIM phase remained stable, but for the most rapidly progressing muscle, a reduction of 18% was observed. ALSFRS-R had increased by 2.5% and mean HHD showed a 5% reduction. For the more rapidly progressing patient, at the end of 6 weeks, mean EIM phase declined 17.1%, but for the most rapidly progressing muscle, a 42% decline was observed. In comparison, mean HHD demonstrated a 21.2% decline with an 8% decline in ALSFRS-R. In the patient with slow progression, EIM phase appeared to drift up and down over a several week period at different times in several muscles.

Discussion: These findings support that reductions in EIM as well as in HHD can be detected over relatively short periods of time in even slowly progressing patients. Moreover, frequent monitoring of ALS patients may reveal previously unappreciated variations in outcome measures over a period of just several weeks. Specifically, the fluctuations observed in the EIM data in the slowly progressing patient may represent evidence of ongoing denervation and subsequent reinnervation.

Conclusions: Intensive monitoring of ALS with EIM and HHD may provide novel insights into disease progression. These techniques could be used either independently or together to help assess disease progression over just 2–3 month time periods, offering an approach to shortening the length of Phase II clinical trials whilst also providing a new tool to study disease biology.

P170 DOES LMN LOSS IN ALS DERIVE FROM PRIMARY UMN DEGENERATION? THE STERNOCLEIDOMASTOID-TRAPEZIUS MUSCLE MODEL

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Keywords: upper motor neuron, lower motor neuron, trans-synaptic degeneration

Background: The concept that ALS commences in the UMN, and that LMN loss results from a trans-synaptic degenerative process, has been supported by the finding of early cortical hyper-excitability, although there is no classical pathological support for this suggestion. One approach to this concept is to investigate ipsilateral trapezius (TM) and sternocleidomastoid (SCM) muscles in the same patients. Since the TM receives UMN input from the contralateral motor cortex and SCM from both hemispheres, as shown by transcranial magnetic stimulation, asymmetry of LMN changes in these two muscle would support the concept of primary UMN degeneration.

Objectives: To study the role of UMN in LMN degeneration.

Methods: Forty-two normal control subjects and 62 age and sex-matched ALS patients with definite or probable disease (mean age 62 years, SD 14: mean disease duration 12.9 months, SD 7.2; 24 bulbar-onset) were investigated. The diagnosis of ALS was established at the time of the first EMG evaluation and clinical follow-up confirmed the diagnosis. MUP analysis (mean amplitude, duration and% of polyphasic potentials) was studied in TM and SCM in the more severely affected upper limb in each patient (right side when both were equally affected). In 11 patients in whom clear asymmetrical upper limb involvement was observed both sides were fully investigated to test symmetry. Non-parametric tests were applied and $p < 0.01$ was considered significant.

Results: In controls, SCM mean amplitude (0.53 mV vs 0.61 mV) and duration (9..88 ms vs 10.7) were significantly lower than in TM, but % polyphasic potentials was greater in SCM (16.61 vs 9.88). No spontaneous activity was observed in controls. In ALS patients amplitude, duration and % polyphasic potentials were significantly greater as compared with controls for both muscles. In SCM 6.5% and 55% showed fibs-sw and fasciculation potentials (FPs) respectively. In TM in these patients these values were 14.5% and 69% respectively, a non-significant difference. Regarding MUP analysis mean amplitude (0.91 mV vs 1.08) and duration (11.87 ms vs 12.72) were higher for TM and % polyphasic potentials (25.9 vs 15.5) higher for SCM, compared to controls; in addition the percent differences in MUP measurements between these muscles were similar in controls and patients with ALS. In the group of 11 patients in whom both sides were investigated there was a non-significant asymmetry in the tested measurements.

Discussion: TM and SCM are innervated by the same nerve and are anatomically adjacent. Both are recruited in voluntary movement and posture, and also as accessory respiratory muscles. However, their corticospinal input is different. These results do not support a primary role for UMN pathophysiology at the onset of ALS, although we have confirmed that these muscles are affected early in ALS and show frequent FPs, which are useful to confirm diagnosis.

P171 THE NEUROPHYSIOLOGICAL INDEX – PERFORMANCE IN PHASE II CLINICAL TRIALS

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Keywords: neurophysiological index, clinical trial, biomarker

Background: The neurophysiological index (NI) was devised as a measure of peripheral disease burden in amyotrophic lateral sclerosis (ALS). It undergoes more rapid decline than other clinical measures of disease progression, including

forced vital capacity and functional rating scales. As such, the NI has been proposed as an electrophysiological endpoint for clinical trials in ALS.

Objectives: To evaluate the performance of the NI in two Phase II clinical trials in ALS.

Methods: Standard nerve conduction studies were undertaken on the ulnar nerve-abductor digiti minimi system at the wrist in 34 patients with ALS (22 males and 12 females, aged 54 ± 1.5 years). Testing was undertaken on four occasions over 12 weeks. The NI was calculated according to the formula: compound muscle action potential (CMAP; mV) F-wave frequency (%) / distal motor latency (DML; ms). Patients were also scored with the Amyotrophic Lateral Sclerosis Functional Rating Scale-revised (ALS FRS-r). Normative electrophysiological data was also collected from 22 healthy control subjects (5 males and 17 females; aged 41.8 ± 14.5 years).

Results: Assessment of the NI was brief (completed within three minutes) and was successfully undertaken in all ALS patients, confirming feasibility in an ALS cohort. The CMAP (7.5 ± 0.4 mV), DML (2.8 ± 0.1 ms), F-wave frequency ($81 \pm 4\%$) and NI (2.4 ± 0.2) were reduced at baseline, in comparison to healthy control subjects (CMAP, 11.5 ± 0.6, $P < 0.00001$; median F-wave frequency, 100%, $P < 0.00001$; DML, 2.3 ± 0.1 ms, $P = 0.0001$; NI, 4.9 ± 0.3, $P < 0.001$). After 12 weeks follow-up, CMAP (7.0 ± 0.44; $P = 0.002$), F-wave frequency (0.7 ± 0.05; $P = 0.002$) and NI (1.89 ± 0.22; $P < 0.0001$) were significantly reduced. By contrast, DML increased significantly (2.9 ± 0.07; $P = 0.03$) at the end of the 12-week observation period. Over this period, the ALS FRS-r underwent a marginal reduction of 5% (baseline, 40.6 ± 0.7; week 12, 38.6 ± 0.83; $P < 0.00001$).

Discussion and Conclusions: Changes in the NI suggested that it may serve as an accurate biomarker of disease progression in ALS. A reduction in NI reflected the loss of peripheral motor units, an expected feature of ALS. The reduction in NI was more substantial than clinical measures of disease progression, including the ALS FRS-r. The NI may be considered for future ALS clinical trials given its high level of responsiveness to disease progression and ease of use.

P172 THE IMPACT OF THE AWAJI-SHIMA CRITERIA ON YIELD FROM CONCENTRIC NEEDLE EMG STUDIES IN PATIENTS WITH SUSPECTED ALS

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Keywords: Awaji-shima, Airlie-House, EMG

Backgound: Amyotrophic lateral sclerosis (ALS) is one of the most debilitating and devastating of the neurological diseases. An early diagnosis is desirable for optimal management and is mandatory for patient enrolment into clinical trials. Clinical neurophysiology can be helpful in establishing a diagnosis in clinically suspected cases. However the Airlie-House criteria have been repeatedly reported to lack the sensitivity required to facilitate early diagnosis. The Awaji-Shima recommendations were formulated in 2006 with the aim of increasing the sensitivity of these diagnostic criteria allowing earlier recognition of the disease. This audit aims to examine how clinical neurophysiology could be employed more effectively to facilitate early diagnosis.

Objectives: To describe the proportion of patients in which the needle EMG studies provided the referring physician with clinically significant additional information. To investigate whether application of the Awaji-Shima recommendations retrospectively to a cohort of patients with suspected ALS improves the yield from the electrophysiological examination and leads to change in the final diagnostic categorisation of the cohort.

Methods: A review was undertaken of the medical records and neurophysiological data of all patients referred to the neurophysiology department at Beaumont Hospital in Dublin with suspected ALS over a 3 year period.

Results: Twenty-nine patients with ALS were identified with a mean age 61.5 (range 37–79 years) and a male to female ratio of 2.2:1. Nineteen patients had spinal onset ALS (64%), 8 had bulbar-onset ALS (28%) and 2 had generalized-onset ALS (8%). Average follow up period was 13.8 months. Nerve conduction studies showed significant motor only abnormalities in 7 patients. EMG studies provided the referring clinician with new clinically significant information in 72% of cases.
The diagnostic category of the patient changed after the EMG study in 42.3% of patients using the Airlie Hose criteria and 69.2% using the Awaji-shima recommendations. The proportion of patients in the probable/definite group was 34.4% prior to the EMG study. This proportion increased after the EMG study to 37.9% when the Airlie House criteria were used and 72.5% using the Awaji-shima criteria.

Discussion and Conclusion: Utilisation of the Awaji-shima recommendations resulted in a significant increase in the proportion of patients in the probable/definite ALS category. The two most common reasons for failure of the EMG study to change the patients diagnostic category were: 1) limited EMG study due to patient intolerance; and 2) presence of widespread chronic innervation in the absence of evidence of active denervation.
This study suggests that the Awaji -shima recommendations increase the yield from concentric needle examination in patients with suspected ALS.

P173 EXPERIENCE WITH THE AWAJI ISLAND MODIFICATIONS TO THE DIAGNOSTIC CRITERIA FOR ALS

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Background: Most cases of amyotrophic lateral sclerosis (ALS) are sporadic, there is no single confirmatory laboratory test and the diagnosis is based on a set of clinical and electrophysiological criteria upon exclusion of other definable diseases. An early and correct diagnosis is important, especially for drug trials aimed at slowing disease progression. To increase sensitivity, an expert group recommended modified electrodiagnostic criteria.

Objectives: To evaluate the performance of the Awaji modified criteria in the electromyography (EMG) laboratory of an ALS referral center.

Methods: We reviewed charts for electrodiagnostic and clinical findings in 70 consecutive patients referred to the EMG laboratory for suspected motor neuron disease over a six-month period to see if they met current or modified ALS electrodiagnostic criteria.

Results: Of the 70 EMG studies included for analysis, 19 did not have neurogenic changes on EMG; 11 studies showed neurogenic changes in 1 region. 40 met the current Revised El-Escorial EMG criteria (EEC-R) (having 2 or more regions with fibrillation/positive sharp waves and neurogenic motor unit potentials), and an additional 3 met the modified Awaji criteria for probable ALS (EEC-A) (having 2 or more regions with fasciculations and chronic motor unit potentials).

Interpretation: In this sample, the modified Awaji criteria showed increased sensitivity. In our center this resulted in 3 additional diagnoses of probable ALS (7%) over a 6-month period. More experience is needed to understand if this modest increase in sensitivity meaningfully improves diagnosis and clinical trial recruitment.

P174 AN EVALUATION OF THE AWAJI SHIMA CRITERIA IN THE DIAGNOSIS OF MOTOR NEURONE DISEASE

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Keywords: El-Escorial Criteria, Awaji-Shima criteria, EMG

Background: New criteria for the diagnosis of Amyotrophic Lateral Sclerosis/Motor Neurone Disease (ALS/MND) were recently proposed at an international symposium in Awaji-Shima, Japan. They differ from the accepted revised El-Escorial criteria by considering fasciculation potentials to be evidence of acute denervation. In addition when assessing diagnostic certainty the Awaji-Shima criteria equate electrodiagnostic evidence of lower motor involvement with clinical examination findings.

Objectives: To establish if the Awaji-Shima criteria enable an earlier diagnosis of MND to be made without increasing the number of false positives.

Methods: A retrospective review was performed of 205 consecutive sets of notes, from patients who underwent neurophysiological assessment for suspected MND during the period 2000–2003. The clinical signs and EMG findings were assessed according to the two sets of criteria. The diagnoses reached using the criteria were compared to the final interval diagnosis.

Results: 107 patients of 205 had a final interval diagnosis of MND. The Awaji Shima criteria had a sensitivity of 60.7% and specificity of 95.9%, compared to the revised El-Escorial criteria with a sensitivity and specificity of 28.0% and 95.9% respectively.

Discussion: The Awaji-Shima criteria increased the sensitivity of diagnosis without affecting the specificity or the false positive rate. Accepting fasciculations as evidence of acute denervation increased the diagnostic certainty of MND. These findings suggest that the Awaji-Shima criteria will enable an earlier diagnosis and potentially earlier recruitment into clinical therapeutic trials.

P175 MUNIX PERFORMED ON BRACHIAL BICEPS MUSCLE WITH QUANTITATIVE AND QUALITATIVE ASSESSMENT OF FORCE

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Keywords: MUNE, MUNIX, voluntary muscle force

Background: Motor unit number estimation (MUNE) is an electrophysiological method designed to quantify motor unit loss in target muscles of interest. This study is a part of multidisciplinary work studying diagnostic features of ALS. MUNE has the potential role of quantifying progression rate in ALS and measuring the response in future treatment trials. This study focuses on a new MUNE method applied on control subjects.

Objectives: To evaluate the ability of a novel MUNE technique, Motor Unit Number Index (MUNIX) (1), to quantitatively assess the number of MUs in a proximal muscle. Thus the purpose of this study was to examine if surface interference pattern (SIP) in MUNIX changed with specified levels of voluntary force. MUSIX is a parameter derived from the test to express motor unit potential (MUP) size.

Methods: Fifteen untrained healthy subjects (7 males, 8 females) – age 27–53 years – with no history of pathology in the upper limb volunteered to participate in the study. In all tests the brachial biceps muscle (BB) of the dominant upper limb was tested. MUNIX was performed with subjective assessment of force. The examiner was blinded for the result in the following test which was planned on a different day. Here the subjects were positioned with 90° flexion at the elbow in dynamometer LIDO Active Multijoint IIa. The computer software package LIDOACT 5.3D was used for data collection. SIP was obtained in 9 recordings (KeyPoint Classic) with gradually increasing voluntary isometric force from slight to maximum activity with force increments of 10% maximal voluntary contraction (MVC). Data analysis with MUNIX. exe was performed offline.

Results: There was a linear relationship between SIP area and force level throughout the test. The relation between number index and force level was similar to that between number index and SIP area. Correlation between number index in BB and CMAP amplitude was high ($p < 0.01$). No relation was found between MUNIX/MUSIX and maximal voluntary contraction.

Discussion and Conclusions: The demonstrated linear relationship between SIP area and force performance supports the hypothesis proposed for MUNIX in which force is substituted by SIP area. In ALS the superimposition among MUPs decreases as the number of MU decreases, thereby theoretically making MUNIX more sensitive for detecting the number of actual MUs.
There is a linear relationship between SIP area and the level of MVC, which is expected according to the mathematical model of MUNIX. MUNIX has the potential to follow the pathophysiological changes in the lower motor system in ALS

patients and a prospective study is ongoing to define more clearly the sensitivity and specificity of MUNE in patients with ALS.

Reference:

1. Nandedkar SD, Nandedkar DS, Barkhaus PE *et al.* IEEE Trans Biomed Eng. 2004; 51(12):2209–11

P176 MOTOR UNIT NUMBER ESTIMATION (MUNE) USING SURFACE EMG INTERFERENCE PATTERN (S-EMG IP) AND COMPOUND MUSCLE ACTION POTENTIAL (CMAP)

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Keywords: Motor Unit Number Estimation, electromyography, compound muscle action potential

Background: Different Motor Unit Number Estimation (MUNE) methods have been described to estimate motor unit (MU) loss in ALS.

Objectives: To develop a MUNE technique (MUNIX) that would be easy and rapid to perform. Using surface EMG recordings, MUNIX could give information on both the estimated number (i.e., "index") of MUs, and also on their size (MUSIX).

Methods: In an 8 month serial study of 10 probable or definite ALS patients and 2 healthy controls, we recorded bilateral hypothenar and thenar CMAPs as obtained in conventional motor nerve conductions. In each instance, the active recording electrode was placed over the muscle to obtain the maximal evoked response. We then made surface EMG interference pattern (S-EMG IP) recordings at different voluntary levels of activation using the same montage to record the CMAP. These signals were analyzed to calculate the Motor Unit Number Index (MUNIX) and Motor Unit Size Index (MUSIX).

Results: Thenar CMAP amplitude decreased by 16% and 24% at 4 and 8 months, respectively. In contrast, MUNIX decreased by 29% and 42% at these two intervals. MUSIX increased by 21% compared to baseline over the 8 month period. Compared to normals however, this represented an almost 78% increase. The hypothenar CMAP declined by 10% and 17% at 4 and 8 months, respectively. MUNIX decreased 21% and 37% at these two interval measures. Hypothenar MUSIX also increased by 21% compared to baseline. But compared to normals, this represented a 91% increase. Each muscle took about 5 minutes to study and could be performed on a standard electromyograph using a special software program.

Discussion and Conclusions: MUNIX and MUSIX provide complimentary information that cannot be derived from the CMAP alone. MUNIX decreased more than the CMAP. MUSIX increased, but not enough to compensate for MU loss. This suggested inadequate compensatory reinnervation. Hypothenar muscle shows greater increase in MUSIX than thenar muscle. This could result in normal CMAP amplitude despite reduced MUNIX. This technique should be of use in studying ALS progression (changes in MUNIX) and reinnervation (changes in MUSIX), where it demonstrates the expected pattern of motor unit (MUNIX) decline as the disease progresses. CMAP amplitude must be controlled in serial investigations.

P177 COMPARISON OF MUNES BY MULTIPLE POINT STIMULATION AND INCREMENTAL STIMULATION IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: motor unit number estimates, multiple point stimulation, incremental stimulation

Objectives: To compare two common techniques for motor unit number estimation (MUNE), multiple point stimulation and incremental stimulation, in patients with amyotrophic lateral sclerosis (ALS).

Methods: Surface recorded motor unit action potentials of the median nerve/thenar muscle were measured on 60 healthy controls and 60 patients with ALS. The compound muscle action potential (CMAP) amplitude of the maximal baseline to negative peak was recorded. For multiple point stimulation, the stimulus sites included the skin of the wrist, 6 cm above the wrist, elbow, and 6 cm above the elbow. Individual motor unit responses were obtained by moving the stimulating electrode and isolating threshold responses with distinct morphologies. Then, with finely graded stimulus intensity at one point, 3 steps in a CMAP were investigated. 10–12 different single motor unit action potentials (SMUPs) were recorded. For incremental stimulation, stimulus intensity was slowly increased from subthreshold levels until a small, all-or-none response was evoked. The intensity was slowly increased until the response increased in a quantal fashion. This process was repeated for a total of 10 increments. Individual motor unit amplitudes were obtained by subtracting amplitudes of each response from that of the prior response. Both techniques were performed twice, electrodes changed and results averaged.

Results: For healthy controls, MUNE was 227.8 ± 30.2 for multiple point stimulation, 197.7 ± 26.4 for incremental stimulation. Test-retest correlation coefficients and coefficients of variation for the mean of two MUNEs were $(0.88 \sim 0.91)$ and $(13.20 \sim 15.24)\%$ for multiple point stimulation, (0.86) and $(13.30 \sim 15.65)\%$ for incremental stimulation. For ALS patients, MUNE was (63.5 ± 5.7) and (58.6 ± 7.0), respectively.

Conclusions: Both MUNE methods are similar reproducible and are equally effective at documenting progression of a lower motor neuron disorder in patients with ALS.

P178 LOW CMAP AMPLITUDE DECREASES REPRODUCIBILITY OF STATISTICAL MUNE

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Keywords: motor unit number estimation, reproducibility, compound muscle action potential

Background: Reproducibility of motor unit number estimation (MUNE) is of great importance for follow-up studies. Many factors can affect the reproducibility of Statistical MUNE.

Objectives: To evaluate the reproducibility of Statistical MUNE in healthy subjects and amyotrophic lateral sclerosis (ALS).

Methods: Twenty-seven healthy subjects and 29 patients with ALS were recruited. Statistical MUNE was performed in the median nerve/thenar muscle twice in the same day. Number-weighted MUNE (n-MUNE) was calculated. Coefficient of variation (CV) from trial 1 to trial 2 was determined by subtracting the n-MUNE of trial 1 from the n-MUNE of trial 2 and dividing that value by the n-MUNE of trial 1.

Results: In healthy subjects, the n-MUNE from trial 1 and trial 2 were 119.5 ± 9.8 and 120.6 ± 10.6 respectively ($P > 0.05$). In ALS subjects, the n-MUNE from trial 1 and trial 2 were 47.2 ± 23.5 and 44.8 ± 23.0 respectively ($P > 0.05$). $P_{50}(P_{25}, P_{75})$ of CV for n-MUNE were 9.5% (5.6%, 25.0%) in ALS and 5.0% (2.3%, 9.4%) in healthy controls ($Z = 2.387$, $P = 0.017$). In 25% of cases with ALS, CV was more than 25%, and the amplitude of compound muscle action potential in those nerves was from 1.9 mV to 4.3 mV, while in the other 75% patients, CV was less than 25% and the amplitude was from 3.8 mV to 12.4 mV.

Discussion and Conclusions: n-MUNE in healthy subjects and ALS has good test-retest reproducibility. The reproducibility of n-MUNE in ALS is not as good as in healthy subjects. It is recommended that statistical MUNE should be performed in nerves with higher CMAP amplitude for follow-up studies.

P179 MONITORING DISEASE PROGRESSION USING HIGH-DENSITY MOTOR UNIT NUMBER ESTIMATION IN ALS PATIENTS

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Keywords: MUNE, EMG, motor unit number

Background: In ALS the loss of α-motor neurons is masked by collateral reinnervation, so that muscle strength, force measurements and many electrophysiological techniques are unreliable measures of disease progression. Motor Unit Number Estimation (MUNE) is a technique that provides a more direct measure for the amount of motor neuron loss. The number of motor units can be estimated by dividing the compound muscle action potential (CMAP) by a representative motor unit potential (MUP). The representative MUP is usually taken as the mean of single MUPs. In this study we compare the high-density MUNE technique with other measures to determine its usability as a marker of disease progression.

Objectives: To determine disease progression using high-density motor unit number estimation and compare these results with other measures such as the ALS functional rating scale and force.

Methods: High-density surface EMG uses a large number of small densely spaced electrodes over one muscle. Using high-density surface EMG, MUPs can be recognised by their spatial and temporal profiles. The extra information helps to obtain a larger sample of single MUPs. This increases the accuracy and reduces the variability. In a longitudinal study, MUNE measurements were preformed in 18 ALS patients over a period of 8 months. Measurements took place at baseline and at four month intervals. To establish reproducibility, MUNE values were measured twice, at baseline and within two weeks. MUNE values were compared with muscle strength (MRC), functional hand grip strength and thenar pinch strength and the ALS functional rating scale (ALSFRS). Baseline MUNE values were compared with a group of 26 healthy controls.

Results: Mean MUNE for the 26 healthy controls was 280 (range: 131–493). Reproducibility was performed in a subset of 14 subjects (ICC = 0.88). Mean MUNE value at baseline in ALS patients was 152 (range: 13–480). Reproducibility values showed good agreement similar to the healthy group (ICC = 0.87). Longitudinal data showed that MUNE decreased more than CMAP and ALSFRS. In percentage to baseline and over 8 months ALSFRS decreased 14%, MRC 17%, CMAP 36%, functional hand grip 27%, thenar pinch 48%, and MUNE 51%.

Discussion and Conclusions: MUNE decreased most as compared to other measures indicating that it is a sensitive measure to motor neuron loss. Furthermore, high-density MUNE showed good reproducibility in healthy subjects and ALS patients. Pinch force seemed to be sensitive as well but requires a MRC of 4 or higher. We conclude that MUNE should be considered to be used in addition to other markers of disease progression.

P180 MOTOR RESPONSES ELICITED BY DIRECT MECHANICAL STIMULATION OF PERIPHERAL NERVES IN PATIENTS WITH EARLY STAGE ALS

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Keywords: electrophysiology, axonal excitability

Background: Previous studies have demonstrated that ALS patients have widespread disturbances in axonal excitability, probably related to potassium and sodium channel disorders. The evaluation of motor responses elicited by mechanical stimulation (brisk compression or percussion) of peripheral nerves (MREC) could be used as an alternative bedside method to quantify peripheral motor axon excitability. There are no previous studies of MREC in ALS patients.

Objectives: To describe the electrophysiological and clinical features of MREC in early-stage ALS patients.

Methods: Fifteen patients with a diagnosis of definitive ALS (<1 year of evolution) according to the El Escorial criteria and 17 age-matched healthy controls were studied. MREC were elicited using two types of mechanical stimulation to the peroneal nerve at the fibular head: 1) Mechanical compression: briskly sliding the index and middle fingers, transversally, while making pressure over the nerve trunk (like pulling a guitar string); 2) Tapping the nerve trunk with a modified neurologist's reflex hammer that also triggered the electromyographic recordings. Motor responses were recorded with surface electrodes placed over the EDB muscle. The nerve was stimulated 10 times, at intervals of 30 seconds. Peak to peak amplitude and the percentage of occurrence of MREC were measured in all subjects.

Results: The percentage of occurrence was significantly higher in ALS patients than in normal subjects for both types of stimulation: mechanical stimulation ($75.5 \pm 21.3\%$ vs. $25.5 \pm 12.2\%$, $p < 0.009$); percussion: ($35.5 \pm 11.3\%$ vs. $12.5 \pm 5.2\%$, $p < 0.04$). ALS patients showed significantly increased MREC amplitudes, compared to healthy controls, to both variants of stimulation: mechanical compression (1200 ± 250 uv. vs. 900 ± 236 uv., $p < 0.001$) and percussion (650 ± 145 uv. vs. 450 ± 175 uv., $p < 0.001$). No significant correlations were found between the incidence of MREC and the severity of fasciculation potentials, upper motor neuron signs or EMG signs of denervation.

Discussion and Conclusion: These results demonstrate that ALS patients have increased responses to mechanical stimulation of nerve fibers. Direct mechanical compression of the nerve trunk is more effective than percussion for eliciting MREC. The assessment of axonal excitability could be important in the study of the wide spectrum of pathologies involved in motor neuron disease and its differential diagnosis. These findings demonstrate that patients with early stage ALS have increased peripheral motor axon excitability, which can be identified with MREC. The clinical evaluation of MREC, just compressing or percussing the nerve and observing the elicited muscle twitch, is a simple, replicable and widely available tool to test axonal excitability in ALS patients.

P181 NEUROPHYSIOLOGICAL STUDY IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS IN A TOTALLY LOCKED-IN STATE

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Keywords: totally locked-in state, somatosensory evoked potential, electroencephalogram

Background: Fifteen to eighteen percent of amyotrophic lateral sclerosis (ALS) patients present with ophthalmoplegia and fall into a totally locked-in state (TLS) after initiation of tracheostomy positive-pressure ventilation (TPPV).

Objectives: To assess the neurophysiological function in TLS-ALS patients using the techniques of somatosensory evoked potentials (SEP) and electroencephalogram (EEG).

Methods: SEPs were recorded by electrical stimulation of the median nerve at the wrist in seven TLS patients (3 men and 4 women, age: 53 to 81 years). All patients showed complete ophthalmoplegia and tetraplegia with TPPV all day long. Communication with them was entirely impossible. The brain MR images showed severe frontotemporal atrophy in all of the patients. The disease duration ranged from 5 to 13 years and the duration of ventilator use was 3 to 10 years. One patient (66 year-old man) had a superoxide dismutase 1 (SOD1) gene mutation (Exon 5, Cys146Arg). Erb's potential, cervical/brainstem N13 potential (C5S-Fz), parietal N20 (CP3/4-Fz) and frontal N30 (F3/4-A), and central conduction time (latency differences between N13 peak and N20 peak) were analyzed. EEG was recorded from the scalp monopolar electrodes placed on Fp1/2, F3/4, C3/4, P3/4, O3/4, F7/8, T3/4 regions, for about 15 to 30 minutes in the afternoon. The frequency analysis of theEEG was performed for a relatively stable 5 seconds of the total recording time using the technique of Fast Fourier Transform (FFT).

Results: Six out of the seven patients showed decreased or abolished parietal N20 and frontal N30. The latencies of N13 and central conduction time were also delayed. The patient with SOD1 gene mutation showed poor Erb's potentials and no responses of the brainstem and cortical components. In EEG analysis, only two patients showed occipital alpha waves with slow frequency range (8 to 10 Hz), and the other three showed theta to delta waves predominantly on the central-frontal regions. In one patient with involuntary jaw movements, the FFT analysis could not be examined due to EMG artifact. The patients with occipital slow alpha waves showed preserved parietal N20 components, and the patients with no N20 components showed theta to delta range EEG without alpha waves.

Discussion and Conclusions: Along with disease progression of ALS, the sensory system could be involved. The lesions might be in the spinal cord, brainstem and primary sensory cortex. SOD1-associated ALS might involve the peripheral sensory neurons. EEG is desynchronized and slowed in the advanced stages, suggesting impaired consciousness of the patients. There might be a correlation between the presence of N20 in SEP and the result of EEG-FFT analysis. In TLS-ALS patients, communication aids should be applied during the stages with preserved SEPs and alpha rhythms of EEG.

P182 ELECTROPHYSIOLOGICAL PROPERTIES OF LUMBAR MOTONEURONS OF ADULT ANAESTHETIZED MICE: IMPLICATION FOR THE STUDY OF ALS

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Keywords: intracellular recordings, intrinsic properties, adult mouse model

Background: Our understanding of the pathophysiological mechanisms of ALS requires the study of the electrophysiological properties of motoneurons and the mechanical properties of their motor unit in animal models. However, data available so far have been obtained on isolated immature preparations, long before the apparition of any symptoms, or have been focused on the sole contractile properties of muscles. Intracellular motoneuron recordings in mice older than 2 weeks can only be achieved *in vivo*. However, the electrophysiological properties of wild-type (WT) mouse spinal motoneurons have been examined in only a few studies.

Objectives: To present a systematic investigation of the subthreshold and the discharge properties of motoneurons in adult mice.

Methods: We have developed an anaesthetized adult mouse preparation in which we obtained stable intracellular recordings of lumbar motoneurons, and the force of its motor unit. We recorded, in 20 WT mice, 37 motoneurons with a resting membrane potential more hyperpolarized than -50 mV and an overshooting action potential larger than 65 mV.

Results: Three salient features were observed: 1) The membrane time constant was brief (0.5 to 4.0 ms, $2.5 +/ -1.0$ ms, $N = 35$) despite input resistances from 1.2 to

7.1 MOhm (3.6+/−1.7 MΩ, N=37). One consequence is the high frequency of the subthreshold resonance at the resting potential (7–30 Hz, 17+/−6 Hz, N=23). This resonance was likely due to the Ih current. 2) The spikes repolarized quickly (spike width at half height: 0.25–0.50 ms, 0.30+/−0.05 ms, N=25) and the duration (26–67 ms, 45+/−11 ms, N=19) and time constant of the AHP (5–13 ms, 10+/−2 ms, N=19) were short. 3) During depolarizing ramps of current, the motoneurons displayed high frequency oscillations (100–150 Hz) preceding each spike. The fast membrane kinetics favored the appearance of ample oscillations. The interval between two successive spikes was variable depending on the number of oscillations. We called this regime of highly variable discharge the "sub-primary zone". It was observed in all motoneurons that discharged repetitively (N=20). In 15 of them, this zone was followed by a more "classical" primary zone at higher current intensities, on which the gain can be measured meaningfully (5 to 50 Hz/nA, 16+/−10 Hz/nA, N=15). In the primary zone, the AHP dominated the interspike interval and the discharge frequency was much more regular.

Conclusions: This work will serve as a foundation for subsequent works, focused on studying the evolution of the motoneuron's electrical properties, as well as the contractile properties of its motor unit during the development of MND.

P183 ALTERATION IN THE INTRINSIC EXCITABILITY AND UPREGULATION OF THE IONIC CURRENTS OF MUTANT SOD1 MOTONEURONS: *IN-VITRO* RECORDINGS AND COMPUTER SIMULATIONS

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Keywords: electrophysiology, modeling, excitotoxicity

Background: Excitotoxicity is one of the hypotheses for mechanisms contributing to motoneuron degeneration in ALS. Surprisingly, studies of excitotoxicity have largely ignored investigating the changes in voltage-sensitive ion channels, the primary determinant of the motoneuronal excitability.

Objectives: To assess the intrinsic excitability and measure the ionic currents in motoneurons of wild-type (WT) and transgenic mice during the presymptomatic stage, long before disease onset.

Methods: We used two approaches to investigate the excitability of motoneurons: 1) whole cell patch clamp recordings of ionic currents from lumbosacral motoneurons of neonatal (P_0–P_{12}) WT and transgenic mice (G93A model) in the slice preparation; 2) development of realistic computer models of WT and mSOD1 lumbar motoneurons in which ionic currents could be estimated. Models were based on the reconstructed morphologies of neonatal WT and mSOD1 motoneurons (P_8–P_{10}), and model parameters were optimized to match the electrical properties recorded experimentally from the same motoneurons.

Results: The results from the *in vitro* recordings and computer simulations confirmed each other and showed that the intrinsic excitability of mSOD1 motoneurons is altered long before symptom onset. More specifically, the magnitudes of the Na^+ and Ca^{2+} PICs were significantly increased in mSOD1 motoneurons relative to WT. The *in vitro* recordings from the slice preparation indicated that the motoneuronal PICs increased with age and mSOD1 motoneurons P_6 and older had significantly larger PICs than WT littermates (mean±SD: WT PIC 87±99 pA, n=33; mSOD1 PIC 152±147 pA, n=40). Tetrodotoxin (Na^+ channel blocker) and isradipine (L-type Ca^{2+} channel blocker) were applied to quantify the Na^+ and Ca^{2+} contributions to the PIC. Na^+ currents mediated a larger portion of the PIC than Ca^{2+} and were significantly larger in mSOD1 motoneurons over P_6 (Na^+ PIC: WT 199±100 pA, n=14; mSOD1 336±262 pA, n=23). In accordance with the *in vitro* recordings, computer models of mSOD1 motoneurons exhibited an increase in the magnitudes of the Na^+ and Ca^{2+} PICs relative to WT models. For instance, mSOD1 motoneuron models predicted an increase in the total, Na^+, and Ca^{2+} PICs by 45%, 55%, and 40% relative to WT models, respectively. Experimentally, these percent increases in PIC amplitudes were comparable and 80%, 60% and 50% were measured in mSOD1 motoneurons, respectively. Computer simulations also predicted alteration in the membrane biophysical properties of mSOD1 motoneurons.

Discussion and Conclusions: Spinal motoneurons that are vulnerable in ALS have low Ca^{2+} buffering capacity. Increases in Ca^{2+} entry to these cells, through Ca^{2+} channels or the prolonged depolarization induced by Na^+ channel activation, could contribute to their degeneration. These results indicate that upregulated ionic mechanisms in mSOD1 motoneurons may contribute to their degeneration in ALS.

P184 PERIPHERAL NERVE PATHOLOGY IN CANINE DEGENERATIVE MYELOPATHY WITH MUTATION IN SUPEROXIDE DISMUTASE 1 GENE

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Keywords: peripheral neuropathy, dog, lower motor neuron

Background: Canine degenerative myelopathy (DM) is a progressive neurodegenerative disease which has recently been shown in several canine breeds to be a result of a missense mutation in the superoxide dismutase 1 (*SOD1*) protein (1). Based on this mutation and similarities in disease phenotype, canine DM appears to be a spontaneously occurring model for human amyotrophic lateral sclerosis (ALS). In canine DM, upper motor neuron spasticity and general proprioceptive pelvic limb ataxia occurs in dogs older than 8 years of age and if euthanasia is delayed, clinical signs will ascend causing flaccid tetraparesis and other lower motor neuron signs. A similar course of clinical progression has been described in upper motor neuron onset human ALS.

Objectives: To determine if pathologic changes typical of denervation are present in muscle (biceps femoris and gastrocnemius), and nerve (peroneal) of dogs with advanced DM.

Methods: Specimens were collected either as biopsies or following euthanasia from Pembroke Welsh Corgi (8), Boxer (7), German Shepherd Dog (2), Kerry Blue Terrier (1) and Chesapeake Bay Retriever (1) dogs homozygous for the *SOD1* mutation and confirmed as DM with microscopic demonstration of DM-specific lesions in spinal cords. Muscle specimens were evaluated in both frozen and paraffin sections and peripheral nerve specimens were evaluated in resin sections. Abnormalities were generally classified as denervation atrophy, nerve fiber loss and axonal degeneration or demyelination, and were scored 0 to +++ (normal to marked, respectively). Similar specimens were collected and processed from age-

matched control dogs including Boxer (3), German Shepherd Dog (1), Rhodesian Ridgeback (3) and English Cocker Spaniel (1) shown to be clear or heterozygous for the mutation.

Results: The most dramatic and consistent abnormalities were found in the Pembroke Welsh Corgi in which denervation atrophy (+++) was present in all muscle specimens and nerve fiber loss (++ to +++), myelin ovoids (++), myelin splitting and ballooning (++ to +++) and inappropriately thinly myelinated fibers (++ to +++) consistent with mixed axonal degeneration and demyelination were found in peroneal nerves. Similar but milder changes (+ to ++) were present in peripheral nerves in the Boxer, German Shepherd Dog, Kerry Blue Terrier and Chesapeake Bay Retriever breeds with variable muscle atrophy (0 to ++).

Discussion and Conclusions: Longer disease duration in the Pembroke Welsh Corgi (24 to 48 months) versus other affected breeds (7 to 12 months) may explain the more extensive disease severity. In conclusion, this study provides pathologic evidence for peripheral nerve involvement in canine DM consistent with the clinical signs of lower motor neuron disease.

Reference:

1. Awano T, Johnson GS, Wade C *et al.* Proc Natl Acad Sci USA 2009; 106:2794–2799

P185 DETERMINANTS OF DOUBLE DISCHARGES IN AMYOTROPHIC LATERAL SCLEROSIS AND KENNEDY DISEASE

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Keywords: double discharge, Kennedy disease, transcranial magnetic stimulation (TMS)

Background: Motor unit double discharges (DDs) occur frequently in motor neuron disorders such as Amyotrophic Lateral Sclerosis (ALS) and Kennedy disease (KD). While this is likely to be because of changes in the intrinsic properties of motor neurons in such disorders, changes in corticomotoneuronal inputs may also contribute.

Objectives: To assess the contribution of corticomotoneuronal inputs to DDs.

Methods: The prevalence and intra-doublet interval (IDI) of motor unit DDs as well as their timing with respect to transcranial magnetic stimulation (TMS)-induced primary peaks (PPs) in the peristimulus time histogram (PSTH) were studied in 23 ALS patients (96 motor units), 11 patients with KD (45 motor units) and 13 control subjects (60 motor units). Three DD patterns with respect to the PP were defined: pre-peak DD, peak-DD and post-suppression DD.

Results: In patients with KD more motor units (82%) fired DDs than in ALS patients (51%) and control subjects (63%) ($p = 0.013$). The motor units in patients with KD exhibited the largest motor unit action potential (MUAP) amplitude and shortest interspike interval (ISI). The prevalence of pre-peak DD in Kennedy patients was 4.06 fold higher (95% CI 0.53–2.81; $p = 0.0014$) than in controls whereas in ALS patients the prevalence of peak DD was 4.79 fold higher (95% CI 1.09–21.10; $p = 0.041$) than in controls. Only in ALS the pre-peak IDI (17.1 ± 5.4 ms) and peak IDI (14.4 ± 5.3 ms) were significantly prolonged ($p < 0.003$) compared with controls (12.3 ± 5.3, 6.2 ± 4.1 respectively). MUAP amplitude ($r = 0.344$; $p < 0.005$), estimated amplitude of the excitatory postsynaptic potential (EPSP), ($r = 0.483$; $p < 0.001$) and ISI ($r = 0.456$; $p < 0.001$) were significantly correlated with pre-peak DD prevalence but not with the prevalence of peak DD.

Discussion and Conclusions: The high peak DD prevalence and prolonged IDIs in ALS are consistent with complex and multiple corticomotoneuronal volleys indicating that in ALS upper motor neuron abnormalities also contribute to the generation of double discharges. In ALS and KD MUAP amplitude, ISI and EPSP size reflecting motor unit remodelling and associated changes of intrinsic anterior horn cell membrane properties determine the prevalence of DD.

P186 TRIGEMINO-CERVICAL RESPONSE IN PATIENTS WITH X-LINKED SPINAL AND BULBAR MUSCULAR ATROPHY

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Keywords: trigemino-cervical response, X-linked spinal and bulbar muscular atrophy, lower brainstem

Objectives: The trigemino-cervical response (TCR) was investigated in patients with X-linked spinal and bulbar muscular atrophy (SBMA) to evaluate its effect for disclosing the bulbar involvement in this disorder.

Methods: We studied 30 normal subjects, 30 patients with amyotrophic lateral sclerosis (ALS) and 30 patients with SBMA. In all normal subjects, stimulation of the infraorbital nerve on one side produced bilateral short latency waves, which consisted of a positive/negative wave described with the mean peak latency (P19/N31). The mean square root of the ratio between the amplitude of P19/N31 and the mean rectified surface EMG activity preceding the stimulus was described by A value.

Results: The latency of ipsilateral P19 in SBMA patients was 24.34 ± 4.82 ms, N31 was 36.20 ± 4.91 ms, and the A value was 1.24 ± 0.33, respectively. At the same time, the contralateral P19, N31 and A value in SBMA patients were 23.91 ± 4.84 ms, 35.45 ± 4.76 ms and 1.19 ± 0.25, respectively. The parameters of TCR between SBMA patients and the healthy controls were statistically different ($P < 0.01$). Although there was no statistical difference in the latencies of TCR between patients with SBMA and ALS, the A value was statistically different between these two groups.

Conclusions: TCR can be helpful in disclosing lower brainstem lesions in SBMA patients.

P187 SLOW SACCADES IN AMYOTROPHIC LATERAL SCLEROSIS: A PSP VARIANT?

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Keywords: saccades, bulbar-onset, progressive supranuclear palsy

Background: Classical teaching is that eye movements are spared in amyotrophic lateral sclerosis (ALS), except in those patients whose lives are prolonged by artificial ventilation. Over the past twenty years, however, a number of papers have reported that ALS patients may show a range of eye movement disorders similar to those encountered in other degenerative and hereditary neurological diseases. In particular, numerous case reports describe slow saccades and supranuclear vertical gaze palsies similar to that found in patients with Progressive Supranuclear Palsy (PSP) particularly in bulbar-onset patients. In addition, extrapyramidal features are occasionally found in ALS patients.

Objectives: To examine reflexive saccades in PSP and ALS patients, in particular bulbar-onset patients compared to controls. To compare results between ALS and PSP patients.

Methods: Eye movements were recorded in 44 ALS patients (14 bulbar-onset and 30 spinal-onset), 7 PSP patients and 45 age-matched controls using infra-red oculography. Saccadic speed and latency were measured for horizontal saccades.

Results: Saccades were similar between ALS patients and controls. However, bulbar-onset patients had slower saccades compared to controls ($p = 0.3$) as did PSP patients compared to controls ($p = 0.1$). Saccades were similar between bulbar-onset ALS and PSP patients for speed and latency.

Discussion: The authors propose that slow saccades occur in ALS, particularly in those with significant bulbar disease. Saccades may be similar in bulbar-onset ALS and PSP. This study, however, is limited by the examination of only horizontal saccades as vertical saccades tend to be involved earlier and more severely in PSP. In bulbar-onset disease, however, it is likely that more extensive pathological changes in the brainstem contribute to slowed saccades with or without a supranuclear gaze palsy. In these instances ocular motor abnormalities may imitate those of PSP. Clinicians should therefore be aware of the potential for confusion due to the overlap between the ocular motor findings in both conditions.

P188 USEFULNESS OF OROPHARYNGOESOPHAGEAL SCINTIGRAPHY FOR EVALUATING TRACHEO-BRONCHIAL ASPIRATION IN NEUROLOGICAL PATIENTS

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Keywords: aspiration, scintigraphy, dysphagia

Objectives: Dysphagia and bolus aspiration are two of the most frequent and debilitating symptoms of various neurological diseases. Swallowing disturbances are the most important cause of tracheobronchial inhalation, which causes pneumonia ab-ingestis, whose mortality rate ranges from 20% to 62%. Oropharyngoesophageal scintigraphy (OPES) permits functional quantitative assessment of the various stages of swallowing, and also reveals and quantifies the presence of bolus aspiration. In this study, we employed OPES to dynamically evaluate deglutition in neurological patients (Amyotrophic Lateral Sclerosis, Parkinson's disease, stroke, etc.) to diagnose and quantify bolus aspiration.

Methods: We enrolled 29 neurological patients complaining of dysphagia (16 women and 13 men, mean age 68.2 ± 12.1 years). All patients underwent OPES with ^{99m}Tc-nanocolloid using both a liquid and a semi-solid bolus sequentially. The following parameters were analysed: Oral, Pharyngeal and Esophageal Transit Time, Oro-Pharyngeal Retention Index, Esophageal Emptying Rate, and Aspiration Rate (AR). AR was calculated as: $AR = (IA/AT_0 - AT_1)/100$, where IA stands for Inhaled Activity, while AT_0 and AT_1 stand for Oral Activity before and after swallowing the radioactive bolus, respectively.

Results: OPES revealed tracheobronchial inhalation in 22/29 patients. In particular, 5 patients showed laryngeal aspiration with a mean 7% AR fraction, 10 patients showed tracheal aspiration (mean 18% AR) and the remaining 6 patients had bilateral broncho-pulmonary aspiration (mean 45% AR mean). In one patient with massive bilateral broncho-pulmonary aspiration caused by post-radiotherapy damage of cranial nerves, OPES indicated functional laryngectomy as the most appropriate surgical treatment.

Conclusions: OPES allows an objective, quantitative evaluation of bolus inhalation into the tracheobronchial tract. It is easy to perform, well tolerated by patients, repeatable, and inexpensive. Although the resolution of this technique is too low to clearly define anatomic structures, OPES is useful for determining the exact fraction of the inhaled bolus into the tracheobronchial tree in neurological patients with dysphagia and represents a useful and accurate tool to monitor response to medical and/or surgical therapy.

P189 *IN VIVO* MEASUREMENT OF THE ELASTIC PROPERTIES OF THE SKIN AS A BIOMARKER FOR AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: biomarker, skin elasticity, in vivo study

Background: CNS and skin are both derived from the neural crest and many diseases affect both systems. Published clinical studies in patients with ALS report that decubitus ulcers are

uncommon in immobile patients and that skin is supple with reduced elasticity. Various structural and biochemical changes in skin biopsies occur, including: decreased collagen bundles with amorphous material between them (1) and a non-inflammatory vasculopathy and deposits of beta-amyloid protein near blood vessels and epidermal appendages (2). Significant negative correlation between the diameter of collagen fibrils in the skin and the duration of disease is also reported (1). The biomechanical properties of the skin reflect the complex interplay among epidermis, dermal collagen and elastin networks and underlying structures. The infrequency of decubitus ulcers and the reduced skin elasticity in ALS patients could result from multiple biochemical and structural changes mirroring those in the nervous system.

Objectives: To test the hypothesis that longitudinal quantitative measurements of skin elasticity may be a useful non-invasive biomechanical biomarker of disease progression and a diagnostic aid in patients with ALS.

Methods: We quantitatively evaluated multiple components of skin elasticity in patients with ALS and control individuals using the Cutometer®, a device that measures non-invasively elements of skin elasticity including elastic recovery after release of maximum negative pressure and elastic displacement from initial position. Forty-one consecutively available patients with ALS (based on the El Escorial Criteria) and thirty-one family members (controls) were enrolled and evaluated at baseline. 37 subjects and 26 controls were evaluated at 3 months and 32 subjects and 24 controls at 6 months with Cutometer® measurements of the arm and back. Neurological variables including the ALSFRS-R and FVC were also assessed in patients.

Results: Skin elasticity was significantly reduced in the ALS arm readings ($p < 0.001$) at baseline, indicating that patients with ALS have diminished skin elasticity as compared to unaffected control individuals. Back skin elasticity in ALS patients was significantly correlated with the ALSFRS-R ($p < 0.01$) over time. The elasticity of the back skin in ALS was significantly correlated with the FVC ($p < 0.05$) over time (3 months follow up data analysis shown and six month data analysis will be available at the conference).

Discussion and Conclusions: Skin elasticity is readily measured quantitatively and non-invasively and appears to be a promising biomarker for detecting the ALS phenotype and predicting disease progression. Further studies are needed to elucidate the relationship of this biomarker to specific biochemical changes relevant to the pathogenesis of ALS.

References:

1. Ono S, Toyokura Y, Mannen T *et al.* Neurology 1986; 36:948–956
2. Kolde G, Bachus R, Ludolph AC. Lancet 1996; 347: 1226–1227

P190 THE SKIN AS A MIRROR OF ALS PATHOLOGY: MATRIX METALLOPROTEINASES IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: matrix metalloproteinase, skin, cerebrospinal fluid

Background: Amyotrophic lateral sclerosis (ALS) is the most common form of motor neuron disease that mainly affects the cortical and spinal motor neurons but may also include other organs such as the skin. Matrix metalloproteinases (MMP) have been suggested to play an important role in ALS pathology.

Objectives: To determine whether gelatinase MMP-2 and MMP-9 could provide a link between neuronal degeneration and skin alterations observed in ALS and whether they were related to markers of oxidative stress.

Methods: We measured CSF, serum and skin tissue homogenate concentrations of MMP-2 and MMP-9 using ELISA and malondialdehyde (MDA), an established marker of lipid peroxidation, using High Performance Liquid Chromatography (HPLC) in 54 ALS patients and 36 controls.

Results: We found CSF and skin MMP-9 concentrations to be elevated in ALS patients as compared to controls ($p < 0.001$, $p = 0.03$, respectively). We observed the elevation of MMP-9 in CSF to be independent of the stage of disease or clinical subtypes and to be highest in patients with a rapid progressive course of disease ($p = 0.008$). In contrast, we found no significant difference of CSF, serum or skin concentrations of MMP-2 as compared to controls. We observed CSF MMP-2 to decrease with duration of disease ($p = 0.04$, $R = -0.31$). MDA was elevated in serum of ALS ($p < 0.001$), though no correlation with MMP-2 or MMP-9 was observed.
We observed MMP-9 to be significantly elevated in the spinal cord of SOD1 as compared to WT mice at an age of 60 days ($p = 0.03$), 90 days ($p = 0.03$) and 120 days ($p = 0.01$). We furthermore found MMP-9 to be significantly elevated in skin tissue homogenate of SOD1 as compared to WT at an age of 60 days ($p = 0.04$) and 90 days ($p = 0.04$). We observed a significant correlation of skin and spinal cord MMP-9 concentrations in SOD1 at an age of 30 days ($p = 0.04$) and for all time points combined ($p = 0.04$).

Discussion and Conclusions: These results indicate a general upregulation of MMP-9 in ALS. This may mirror ongoing neuronal degeneration and glial activation and could be linked to alterations in collagen metabolism observed in the skin of ALS patients. MMP-2 seems to be less responsive to disease associated stimuli than MMP-9, though it is functionally related to it. This may be due to differences regarding the regulation of MMP gene expression.
MMP-9 could provide a link between neuronal degeneration and skin pathology in ALS. The skin may provide an easily accessible source of *in vivo* biomarkers in ALS.

P191 PERIPHERAL OXIDATIVE STRESS BIOMARKERS IN SPORADIC ALS AT THE ONSET

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Keywords: oxidative stress, biomarkers, redox imbalance

Background: Among the pathogenic hypotheses on motor neuron degeneration leading to amyotrophic lateral sclerosis (ALS), the reactive oxygen species generation and oxidative stress theory has been put forward. Evidence of accumulation of oxidative damage to proteins, lipids, and DNA in ALS patients has been reported. However, the exact role of oxidative stress at the onset or at a very early stage of the disease is still unclear.

Objectives: To assess oxidative stress biomarker levels in sporadic ALS patients at the onset of disease.

Methods: Oxidative stress biomarkers (advanced oxidation protein products (AOPP) and ferric reducing antioxidant power (FRAP)) were examined in blood samples from 32 sporadic ALS (sALS) patients (13/19 M/F, mean age 63.3 ± 10.8 years) at the onset of the disease (mean time between onset of the symptoms and diagnosis with collection of the blood sample 5.7 ± 2.2 months). A group of 54 healthy matched subjects (25/29 M/F, mean age 69.3 ± 9.2 years) were recruited as controls. The determination of AOPP and FRAP levels was based on spectrophotometric detection.

Results: Plasma AOPP levels were found to be increased in sALS patients compared to controls (346.409 ± 40.1, 246.9 ± 40.9 nmol/microliter, $p < 0.01$). After stratification by gender, the association was significant in the female group (393.6 ± 41.9 vs 238.6 ± 42.5, $p < 0.05$), but not for the male group (316.5 ± 38.9 vs 256.5 ± 34.6, $p = 0.21$). Plasma FRAP levels were found to be decreased in sALS patients compared to controls (0,696 ± 0.057 vs 1.314 ± 0.09 nmol/microliter, $p < 0.001$). After stratification by gender, we observed that plasma FRAP levels were significantly decreased in both males (0.706 ± 0.06, $p < 0.001$) and females (0.688 ± 0.09, $p < 0.05$) compared to controls (1.514 ± 0.06 nmol/microliter). No correlation between AOPP or FRAP values and site of onset of the disease was observed.

Conclusions: These findings support a role for oxidative stress in ALS, even in a very early phase of the disease. FRAP and AOPP can represent useful biomarkers to detect redox imbalance in such disease, thus providing a non invasive tool to monitor disease status and response to therapies.

P192 THE INDIRECT INVOLVEMENT OF RADICAL PROCESSES IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: tryptophan derivatives, nitric oxide, fatty acids

Background: Accumulating data indicate that oxidative and radical stress plays a major role in the pathogenesis of Amyotrophic Lateral Sclerosis (ALS). It causes damage to main cellular components such as lipids, proteins and nucleic acids, resulting in cell death by necrosis, apoptosis, or autophagy. In our laboratory, we have indirectly evaluated these radical processes by identifying specific immunoglobulins of G, M and A isotypes, directed against neoantigens resulting either from hidden self antigens or self antigens modified by radical species. Here, we reported the binding of circulating antibodies in ALS patient sera, directed against: tryptophan derivative (TD) conjugates, NO and NO_2-modified amino acid conjugates, fatty acids (FA) and their hydroxylated (OHFA) form conjugates.

Objectives: The first objective of this study was to define the specific circulating antibodies present in ALS patient sera directed against TD conjugates, NO and NO_2-modified amino acid conjugates and FA and OHFA conjugates. The second objective was to determine their interest for a better understanding of the diversity of radical processes.

Methods: The TD antigens kynurenin, 3OH-kynurenin and kynurenic, picolinic, xanthurenic, anthranilic, 3OH-anthranilic, quinolinic, quinaldic acids, were each linked to bovine serum albumin (BSA) using carbodimide coupling reaction. NO- and NO_2-conjugates resulted from the following compounds: nitrotyrosine, tyrosine, phenylalanine, citrulline, tryptophan, asparagine, creatin, cysteine, methionine, histidine, arginine, linked to BSA via glutaraldehyde reaction and then nitrosylated. We have used hydroxylated or non-hydroxylated caproic, caprylic, capric, lauric acids; and myristic, palmitic, oleic and azelaic acids. FA and OHFA were linked to BSA using ethylchloroformiate coupling reaction. Using ELISA tests and these conjugates, the circulating antibodies were identified.

Results: These studies have shown that: 1) specific circulating antibodies directed against TD, NO- and NO_2-modified amino acid conjugates and OHFA components were found with a specific pattern; 2) specific isotype profiles were reported: IgA responses were directed only against kynurenin, kynurenic, quinolinic, quinaldic, and 3OH-anthranilic conjugates. We have found a preferential IgG response to NO-tryptophan, IgM response to NO-phenylalanine, NO-tryptophan and NO-BSA, and IgA to NO-tryptophan and 3 isotypes for nitrotyrosine. Only IgM responses to hydroxylated caproic acid were found.

Discussion and Conclusions: These data indirectly confirm that neurotoxic TD, NO and NO_2-modified amino acid conjugates are implicated in neurodegenerative mechanisms. The presence of TD, NO, NO_2^- and OHFA modified endogenous epitopes confirms the alterations in protein structures that could lead to modification or inhibition of enzyme and mitochondrial activities. Subsequently, the latter are able to induce apoptotic and autophagic processes involved in the pathogenesis of ALS. These data indirectly show the diversity of radical processes in ALS.

P193 INVESTIGATION OF LEVELS OF THE VASCULAR ENDOTHELIAL GROWTH FACTOR IN CEREBROSPINAL FLUID IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: VEGF, CSF, hypoxia

Background: Deletion of the hypoxia-response element in the Vascular Endothelial Growth Factor (VEGF) promotor causes adult-onset progressive motor neuron degeneration in mice. Neuroprotective and neurotrophic properties of the vascular endothelial growth factor were revealed in recent studies. These results suppose that VEGF participates in the pathogenesis of Amyotrophic Lateral Sclerosis (ALS).

Objectives: To measure levels of VEGF in the cerebrospinal fluid (CSF) of ALS patients.

Methods: The study concerned 24 ALS patients and 11 control subjects (traumatic patients without craniocerebral

injuries).VEGF was measured by enzyme-linked immunosorbent assay. Spirometry was performed in all ALS patients. Percentage of predicted lung vital capacity was calculated for every patient.

Results: The results have shown that VEGF levels in CSF are significantly decreased in patients under 40 years old compared with controls and with patients over 40 years old. There were significant negative correlations between VEGF levels and hematocrit and between VEGF levels and ALS Functional Rating Scale. There were significant positive correlations between VEGF levels and percentage of predicted Lung Vital Capacity (only in a subgroup of patients who had less than 85% of predicted Vital Capacity) and between VEGF levels and blood pH (only in a subgroup of ALS patients over 40 years old).

Discussion and Conclusions: VEGF is a hypoxia inducible factor. If the VEGF level increases in hypoxia it should have the following correlations: positive correlation with hematocrit, negative correlation with ALS Functional Rating Scale, negative correlations with predicted percentage of Lung Vital Capacity and blood pH. Inverse correlations suppose a disorder of hypoxia inducible factors in ALS patients.
Distinction of VEGF levels in the CSF of patients less than forty years of age is evidence of some heterogeneity of disease and may be a mark of difference in pathogenesis of ALS in adult and middle aged patients. Revealed correlations of CSF VEGF levels with some laboratorial indexes suggest an inadequate hypoxia reaction in ALS patients. Further investigations could help to assess the relevance of this inadequate hypoxia reaction in the pathogenesis of ALS.

P194 CORRELATION OF CEREBROSPINAL FLUID GLIAL MARKERS WITH SURVIVAL IN AMYOTROPHIC LATERAL SCLEROSIS

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Keywords: biomarker, cerebrospinal fluid, glial activation

Background: Biochemical markers in the cerebrospinal fluid (CSF) are increasingly studied in amyotrophic lateral sclerosis (ALS) to evaluate their relevance for differential diagnosis, disease progression, and for the understanding of pathophysiological processes. We therefore evaluated glial proteins in the CSF of patients with ALS and other motor neuron diseases (MND) in order to assess whether baseline levels of CSF measures in ALS are associated with the course of the disease.

Methods: 108 subjects with MND were included (ALS, n = 90; lower motor neuron diseases such as spinal muscular atrophy, n = 12; upper motor neuron diseases, n = 6). Follow-up data were available in 23 ALS patients. CSF baseline levels of astroglial S100beta (all MND patients) and microglial sCD14 concentrations in CSF and serum (57 ALS patients, 13 patients with other MND and 28 controls) were related to diagnosis, duration of the diseases, clinical scores at time of lumbar puncture and survival.

Results: Compared with lower motor neuron diseases, CSF S100beta levels were higher in ALS ($p = 0.002$) and upper motor neuron diseases ($p = 0.004$). In ALS patients, CSF S100beta concentrations showed an inverse correlation with survival ($r = -0.457$, $p = 0.011$, $n = 23$). CSF/serum ratios of sCD14 were decreased in ALS and lower motor neuron diseases as compared to controls ($p < 0.001$). CSF sCD14 positively correlated with the survival time ($r = 0.643$, $p = 0.009$, $n = 13$). There were no correlations of CSF baseline levels of both glial markers with clinical scores such as the ALSFRS or muscular strength, or the duration of the diseases.

Conclusions: Although CSF S100beta and sCD14 concentrations were not related to clinical scores at time of lumbar puncture, biochemical markers may provide important information about the condition of glial cells and therefore may be useful for prognostic purposes in ALS.

P195 CORRELATIONS BETWEEN PATHOLOGY AND GENE EXPRESSION IN AMYOTROPHIC LATERAL SCLEROSIS

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Background: Amyotrophic lateral sclerosis (ALS) is poorly understood at the molecular level and there are as yet no effective therapeutics to stop the insidious progression of weakness that ultimately leads to respiratory failure and death. The degree of neuronal loss in the spinal cord appears to be related to the degree of clinical severity of ALS (1). Interestingly, motor neuron degeneration generally begins focally in one region of the spinal cord and sequentially works its way to adjacent regions of the nervous system (2) suggesting that a local comparison of different segments of the spinal cord or brain with different degrees of neuronal loss may offer clues to the molecular pathogenesis of disease progression.

Objectives: To compare gene expression in the spinal cords and brains of patients with ALS in regions with differing degrees of neuronal loss and compare this to controls.

Methods: Pathological changes were measured histologically for motor neuron numbers, demyelination, and gliosis; and gene expression was determined by quantitative PCR for a number of genes implicated in the disease process.

Results: Regions of the spinal cords and brains of patients with ALS disease showed differing degrees of neuronal loss and activation/inflammation of non-neuronal cells. While there was no consistent change in SOD1, TDP43, and EAAT2 expression (genes implicated in pathogenesis), there was a significant downregulation of NRG1 gene expression (a neuronally-expressed gliotrophic gene that supports axoglial interactions). On the other hand, the neurotrophin BDNF appeared to have the opposite pattern of expression to NRG1 in the ALS patients. These results suggest that motor neuron loss is correlated with decreased NRG1 but increased BDNF gene expression in ALS patients.

Discussion and Conclusions: The downregulation of NRG1 with upregulation of BDNF could help define altered cell-cell signal patterns in ALS and could serve as a biomarker for measuring neuronal loss in this disease.
Recently, we have postulated a feedback loop between NRG1 and BDNF as a mediator for both axoglial and neuromuscular stability. This raises an intriguing hypothesis that this feedback loop could be defective in ALS and that NRG1

deficiency could be an important biomarker for neuronal loss. This work also sets the stage for higher throughput studies that can identify disruptions in normal regulatory networks that contribute to disease progression and could represent important new biomarkers and therapeutic targets for ALS.

References:

1. Cruz-Sánchez FF, Moral A, Rossi ML *et al*. J Neural Transm. 1996; 103:1317–1229
2. Ravits J, Laurie P, Fan Y *et al*. Neurology 2007; 68: 1576–1582