Cortico-efferent tract involvement in primary lateral sclerosis and amyotrophic lateral sclerosis: A two-centre tract of interest-based DTI analysis

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ABSTRACT

Background: After the demonstration of a cortico-efferent propagation pattern in amyotrophic lateral sclerosis (ALS) by neuropathological studies, this concept has been used for in vivo staging of individual patients by diffusion tensor imaging (DTI) techniques, both in ‘classical’ ALS and in restricted phenotypes such as primary lateral sclerosis (PLS).

Objective: The study was designed to investigate that microstructural changes according to the neuropathologically defined ALS alteration pattern in PLS patients could be confirmed to be identical to ‘classical’ ALS patients. The novelty in this approach is that the results were independent of the subject samples and the data acquisition parameters (as was validated in two samples from two different centres). That way, reproducibility across (international) centres in addition to harmonisation/standardisation of data analysis has been addressed, for the possible use of MRI-based staging to stratify patients in clinical trials.

Methods: Tractwise analysis of fractional anisotropy (FA) maps according to the ALS-staging pattern was applied to DTI data (pooled from two ALS centres) of 88 PLS patients and 88 ALS patients with a ‘classical’ phenotype in comparison to 88 matched controls in order to identify white matter integrity alterations.

Results: In the tract-specific analysis, alterations were identical for PLS and ALS in the tract systems corresponding to the ALS staging pattern, independent of the subject samples and the data acquisition parameters. The individual categorisation into ALS stages did not differ between PLS and ALS patients.

Conclusions: This DTI study in a two-centre setting demonstrated that the neuropathological stages can be mapped in vivo in PLS with high reproducibility and that PLS-associated cerebral propagation, although showing the same corticofugal patterns as ALS, might have a different time course of neuropathology, in analogy to its much slower clinical progression rates.

1. Introduction

Primary lateral sclerosis (PLS), as a motor neuron disease (MND) which almost exclusively affects upper motor neurons (Wais et al., 2017), has been recognised as one of the restricted phenotypes of amyotrophic lateral sclerosis (ALS) in the revision of the El Escorial diagnostic criteria, including the notion that PLS develops into ALS in the vast majority of patients (Agosta et al., 2015a; Ludolph et al., 2015).

Systematic ex vivo data in patients with a PLS phenotype for the diagnostic proof as definite ALS by the neuropathological demonstration of cerebral TDP43 pathology according to the neuropathological staging concept of ALS (Braak et al., 2013; Braak et al., 2017) are lacking yet. In order to assess this neuropathological pattern in MND patients in vivo by magnetic resonance imaging, a group of PLS patients has been subjected to the established hypothesis-guided tract-of-interest (TOI)-based diffusion tensor imaging technique of the brain (Kassubek et al., 2015a; Dodel et al., 2016; Dodel et al., 2017).

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2014; Kassubek et al., 2018), and the results showed the same microstructural pathological patterns in PLS patients as in ALS, in support of the hypothesis that PLS is a phenotypical variant of ALS (Müller et al., 2018).

Standardisation of advanced neuroimaging including postprocessing is a major challenge in the establishment of these techniques as biological markers e.g. in neurodegenerative diseases. The application of the TOI technique has previously been applied to show that the PLS brain alteration pattern is like in ALS (Müller et al., 2018). In addition, it has been demonstrated that different DTI protocols can be pooled even in a large multi-centre study (Müller et al., 2016). In the current study, the hypothesis should be tested that the finding that the PLS brain alteration pattern is like in ALS is independent of the subject samples, the scanner (including field strength), and the DTI scanning protocol. Therefore, the application of the TOI technique was applied to data of PLS patients from two different MND centres (Ulm, Germany and Milan, Italy). In addition, the two-centre approach allowed for the analysis of a large sample size, that way aiming at the categorisation of these DTI data according to ALS stages in the individual patients. Such multi-centre approaches with up-to-date, ultimately harmonised neuroimaging protocols will further improve the understanding of ALS pathogenesis and deliver objective biomarkers for use in multi-centre therapeutic trials (Filippi et al., 2015).

2. Methods

2.1. Subjects and patient characteristics

Data were pooled from two different tertiary referral centres for MND (Department of Neurology, University of Ulm, Germany and San Raffaele Scientific Institute, Vita-Salute San Raffaele University, Milan, Italy). All patients underwent standardised clinical, neurological, and routine laboratory examinations. All subjects gave written informed consent for the study protocol according to institutional guidelines which had been approved by the Ethics Committee of Ulm University, Germany (No. 19/12) and the local ethical committee on human studies of Milan, Italy (No. RF-2010-2313220).

Eighty-eight PLS patients (50 from Ulm and 38 from Milan) were included who met the proposed diagnostic criteria for PLS (Pringle et al., 1999; Singer et al., 2007; Wais et al., 2017). The group of patients from Ulm has been reported previously (Müller et al., 2018), the patient sample from Milan was partially reported (Agosta et al., 2014). To be eligible, subjects had to meet the following criteria: no family history of MND, no clinical diagnosis of frontotemporal dementia (FTD), age at onset > 40 years, no mutations of major genes related to hereditary spastic paraparesis if investigated, no other major systemic, psychiatric or neurological illnesses, no history of substance abuse. It was mandatory for inclusion that routine MRI scans excluded any brain or cervical cord abnormalities suggesting a different etiology of the clinical symptoms. Disease duration in the PLS group was 6 ± 4 years (5 ± 3 years for the PLS patients from Ulm and 7 ± 5 years for the PLS patients from Milan), all data are given as arithmetic mean ± standard deviation (SD).

PLS patients presented a revised ALS functional rating scale (ALS-FRS-R) (Cedarbaum et al., 1999) of 36 ± 7 in average (36 ± 8 (range 16 to 47) for the PLS patients from Ulm and 37 ± 6 (range 11 to 46) for the PLS patients from Milan).

A group of 88 ALS patients (50 from Ulm and 38 from Milan) were selected to match for age and gender to the PLS group. The diagnosis of all cases was made according to the El Escorial diagnostic criteria (Ludolph et al., 2015), all these patients showed clinical involvement of the first and the second motor neuron. ALS patients presented with an ALS-FRS-R of 37 ± 9 in average (38 ± 9 (range 16 to 45) for the ALS patients from Ulm and 36 ± 9 (range 17 to 44) for the ALS patients from Milan); no differences in ALS-FRS-R between the PLS group and the ALS group were found. None of the patients with ALS or PLS had any history of other neurological or psychiatric disorders.

PLS and ALS patients were compared to a group of 88 age- and gender-matched controls (50 from Ulm and 38 from Milan). Gross brain pathology including vascular brain alterations was excluded by conventional MRI. All healthy control subjects had no family history of neuromuscular disease and had no history of neurologic, psychiatric, or other major medical illnesses and were recruited from among spouses of patients and by word of mouth.

A summary of all subjects' characteristics is given in Table 1. The group comparisons for age and gender indicated no significant differences for the different subject groups.

2.2. MRI acquisition

DTI scanning in Ulm was performed on a 1.5 Tesla Magnetom Symphony (Siemens Medical, Erlangen, Germany); DTI scanning in Milan was performed on a 3.0 Tesla Intera (Philips Medical Systems, Best, The Netherlands). At 1.5 T, two DTI study protocols were used. DTI study protocol A consisted of 13 volumes (45 slices, 128 × 128 pixels, slice thickness 2.8 mm, pixel size 2.0 mm × 2.0 mm) (TE=93 ms and TR=8000 ms) representing 12 gradient directions (b=800 s/mm²) and one scan with gradient 0 (b = 0). The echo time (TE) and repetition time (TR) were 93 ms and 8000 ms, respectively. The number of averaged acquisitions was five. DTI study protocol B consisted of 52 volumes (64 slices, 128 × 128 pixels, slice thickness 2.8 mm, pixel size 2.0 mm × 2.0 mm) representing 48 gradient directions (b=1000 s/mm²) and four scans with b=0. TE and TR were 95 ms and 8000 ms, respectively.

At 3.0 T, the DTI study protocol consisted of 34 volumes (55 slices, 96 × 96 pixels, slice thickness 2.5 mm, pixel size 0.94 mm × 0.94 mm) representing 32 gradient directions (b=1000 s/mm²) and two scans with b=0. TE and TR were 80 ms and 8986 ms, respectively. The number of averaged acquisitions was two.

2.3. Data analysis

The postprocessing and statistical analysis was performed by use of the analysis software Tensor Imaging and Fiber Tracking (TIFT – Müller et al., 2007a). Fractional anisotropy (FA) maps were calculated for quantitative mapping of microstructure (Le Bihan et al., 2001). The normalisation of the data to the Montreal Neurological Institute (MNI) stereotaxic space was performed iteratively (Müller and Kassubek, 2013). In the stereotaxic normalisation procedure, study specific templates (b0 and FA) have been generated across all subjects. For this

| Table 1 | Subjects characteristics. *disease progression rate = (48-ALSFRS-R score at clinical examination)/disease duration (years). |
|---|---|---|---|---|---|---|
| PTOS | | | | | | |
| Male/female | 50/38 | 42/46 | 47/41 | Chi squared: 0.1 |
| 1.5 T | 29/21 | 22/28 | 25/25 |
| 3.0 T | 21/17 | 20/18 | 22/16 |
| Age/years (mean ± std. dev.) | 63 ± 9 | 61 ± 10 | 60 ± 13 |
| 1.5 T | 62 ± 10 | 58 ± 10 | 58 ± 16 |
| 3.0 T | 64 ± 8 | 65 ± 10 | 63 ± 8 |
| 3.0 T | 36 ± 7 | 37 ± 9 | n.a. |
| Disease duration/years (mean ± std. dev.) | 37 ± 6 | 36 ± 9 | n.a. |
| 1.5 T | 5.5 ± 4.2 | 2.0 ± 1.9 | n.a. |
| 1.5 T | 4.5 ± 3.2 | 2.3 ± 2.2 | n.a. |
| 3.0 T | 6.7 ± 5.0 | 1.6 ± 1.3 | n.a. |
| Disease progression rate* (mean ± std. dev.) | 3.4 ± 4.4 | 9.1 ± 9.1 | n.a. |
| 1.5 T | 3.4 ± 2.6 | 8.2 ± 8.8 | n.a. |
| 3.0 T | 3.5 ± 6.1 | 10.3 ± 9.5 | n.a. |
analysis step, the absolute b0 intensity and the FA value (which both could differ due to the various scanner acquisition protocols) are not of importance as the optimisation algorithms are based on correlation optimisation (as the cost function) where relative spatial differences are of higher importance than absolute FA and b0 intensity values. For the harmonisation of FA differences resulting from different acquisition protocols, FA maps of controls recorded with the different protocols were used for calculation of 3-D correction matrices according to a previously reported protocol (Rosskopf et al., 2015; Müller et al., 2016). Then, FA maps of PLS and ALS patients and controls were harmonised by application of the respective 3-D correction matrix (linear first order correction). In a consecutive step, an 8 mm (FWHM) Gaussian filter was applied for smoothing of FA maps in order to achieve a good balance between sensitivity and specificity (Unrath et al., 2010). In general, these procedures are not limited to FA. However, FA was focussed on in this study as a sensitive DTI metric to microstructural changes (Song et al., 2002) which also is the parameter used for the first application of the TOI approach (Kassubek et al., 2014).

FA maps of controls recorded with the different protocols were used for calculation of 3-D correction matrices according to a previously reported protocol (Rosskopf et al., 2015; Müller et al., 2016). Then, FA maps of PLS and ALS patients and controls were harmonised by application of the respective 3-D correction matrix (linear first order correction). In the final step, FA maps of all subjects were corrected for the covariate age.

Statistical comparison by Student’s t-test was performed voxelwise for FA values to detect changes between the subject groups by whole brain-based spatial statistics (WBSS). Voxels with FA values below 0.2 were not considered for calculation as cortical grey matter shows FA values up to 0.2. Statistical results were corrected for multiple comparisons using the false-discovery-rate (FDR) algorithm at p < 0.05 (Genovese et al., 2002). Further reduction of the alpha error was performed by a spatial correlation algorithm that eliminated isolated voxels or small isolated groups of voxels in the size range of the smoothing kernel leading to a threshold cluster size of 256 voxels.

Pathways for defined brain structures according to the ALS-staging system (Braak et al., 2013; Brettschneider et al., 2013) were identified with a seed-to-target approach (Kassubek et al., 2014; Kassubek and Müller, 2016; Rosenbohm et al., 2016). TOIs for the definition of the four ALS stages were used as previously defined, i.e. the corticospinal tract (CST, representative for stage 1), the corticorubral and corticopontine tracts (representative for stage 2), the corticostriatal pathway (representative for stage 3), and the proximal perforant path (representative for stage 4) (Kassubek et al., 2014; Kassubek et al., 2018). As a reference path, the tract was used originating from the corpus callosum (CC) area V where no involvement in ALS-associated neurodegeneration could be anticipated. Tractwise fractional anisotropy statistics (TFAS – Müller et al., 2007b) was performed by comparing the FA values in a respective tract system between the groups (Student’s t-test). Acquisition protocol A has a smaller FOV compared to the other protocols. Thus, a minor part of the lower corticorubral tract (corresponding to ALS stage 2) is not covered by the FOV. However, as FA values were thresholded at 0.2, this (minor) part of the corticorubral tract did not contribute to the analysis and did not influence the results.

Comparisons between subject groups were performed separately for centre 1 and 2 as well as for the pooled data set from all subjects of both centres that contributed to this study. Fig. 1 shows the data analysis workflow for the different subject groups.

2.4. ALS staging at the individual level

Staging categorisation was performed according to the published protocol (Kassubek et al., 2014; Kassubek et al., 2018). The results for staging categorisation were obtained for each patient at the individual level.

3. Results

3.1. Whole brain-based spatial statistics of FA maps

The comparison at the group level by WBSS for PLS patients vs. controls demonstrated multiple clusters of regional alterations at p < 0.05 (corrected for multiple comparisons), these widespread FA reductions were observed along the CST and also in frontal and prefrontal brain areas in the FA maps of both centres independently (Fig. 2A, Table 2). Both PLS and ALS patients showed a widespread FA reduction pattern compared to controls, while ALS patients vs. PLS patients showed no significant differences. For the pooling of the data from both sites, i.e. the results of 176 ALS/PLS patients vs. 88 controls, projectional views for the regional FA reduction maps are depicted in Fig. 3A (group comparisons). A summary of all significant clusters at the group level is provided in Table 4.

3.2. Differences of FA in the tract systems

The hypothesis-guided analysis of the FA differences in the ALS-related tract systems by use of TFAS showed differences of the averaged FA values between the PLS and the control groups, showing most prominent FA alterations in the CST, followed by FA reductions in the other ALS-staging-related tracts (Fig. 1B). Here, significant FA reductions could be observed independently for both centres for the CST (related to ALS stage 1) as well as for the corticopontine and the corticorubral tract (related to ALS stage 2), and for the corticostriatal pathway (related to ALS stage 3). For the proximal portion of the perforant path (ALS stage 4), FA reductions in PLS patients compared to controls were observed only in centre 1. For the grand average of the stage-related tract systems, FA reductions were significant for both centres. No significant FA alterations were observed in the reference path. A summary of all alterations in the tract systems at the group level is provided in Table 3.

The results in the two-centre setting were consistent with the single centre results; both, ALS and PLS group vs. the control group showed most prominent FA alterations in the CST, followed by FA reductions in the other ALS-staging-related tracts (Fig. 3B). Here, significant FA reductions could be observed independently for the CST, for the corticopontine and the corticorubral tract, for the corticostriatal pathway, and for the proximal portion of the perforant path (ALS stages 1–4) in PLS patients and ALS patients each compared to controls. For the grand
average of the staging-related tract systems, significant FA reductions were observed for PLS patients and ALS patients compared to controls. No significant FA alterations were found for any comparison in the reference path. A summary of all alterations in the tract systems at the group level is provided in Table 5.

3.3. ALS staging at the individual level

ALS staging categorisation was performed for 88 PLS and 88 ALS patients; here, 76% of the PLS patients were stageable and 80% of the ALS patients were categorised into ALS stages (Fig. 4A). Out of the ALS patients, 33% were in ALS stage 1, 9% in ALS stage 2, 7% in ALS stage 3, and 31% in ALS stage 4, the distribution in ALS-stages for PLS patients was similar, i.e. 27% were in ALS stage 1, 10% in ALS stage 2, 12% in ALS stage 3, and 26% in ALS stage 4. Examples of ALS staging categorisation for single PLS patients recorded at 1.5T or 3.0T are shown in Fig. 4B.

4. Discussion

ALS is one of the neurodegenerative diseases with a characteristic pattern of neuronal and regional vulnerability in the sense of sequential involvement of different central nervous system regions as proposed by human autopsy studies (Braak et al., 2013, Jucker & Walker 2013). With regard to neuropathology, PLS has been reported as being characterised by severe demyelination near the corpus callosum, whereas the demyelination that occurs in ALS is seen in the superior frontal gyrus (Kolind et al., 2013). In PLS, pathological changes have been found in the cortex and brainstem, and some of these changes resemble those seen in sporadic ALS, including (at least in some cases) TDP-43 pathology (Dickson et al., 2007; Kosaka et al., 2012). Nonetheless, additional neuropathological and morphological studies involving a
larger number of PLS cases will be required to fully characterise TDP-43 lesions in PLS, although this may prove difficult, inasmuch as PLS is ca. 0.5% as prevalent as ALS.

The approach of TOI-based white matter studies can help elucidating in vivo what structures have been involved in the spread across the brain for individual patient classification. In the current study, it could be demonstrated in a large dataset of 88 patients each that PLS and ALS shared the same lesion pattern of specific cortico-efferent white matter tracts corresponding with the ALS neuropathological spreading (Kassubek et al., 2018), independent of the subject samples and the data acquisition parameters. No alterations between groups were observed in the reference tract. *p < 0.05, **p < 0.001.

It could be demonstrated that the percentages of PLS patients which belonged to certain stages were rather identical to the ALS patients in the study, although their disease durations were much higher, in accordance with the different clinical phenotype and disease course.

A further analysis of these differences in the development of the cortico-efferent pathology might be of high importance for our mechanistic understanding of what factors might influence the evolvement of the different (and more benign with respect to survival) disease course in PLS.

In addition, disease duration of the 3.0 T PLS sample was significantly higher than the 1.5 T PLS sample. However, although the ALS-associated slope of FA alterations over time has been reported at the individual level (Baldaranov et al., 2017), only a weak correlation could be shown at the group level (Kassubek et al., 2014). Still, the higher disease duration in the 3.0 T PLS patient sample may be responsible for the lower rate of not stageable PLS patients in the 3.0 T PLS patient sample compared to the 1.5 T PLS patient sample (11% for 3.0 T vs 34% for 1.5 T). From the results of this cross-sectional study, it could be assumed that the cortico-efferent pathology in the brains of PLS patients does not proceed in the same time like in ALS, since then more patients in higher staging categories would have been expected. However, this aspect needs to be addressed in longitudinal MRI studies in PLS.

In this study, we showed neuropathologically defined ALS alteration patterns for PLS patients at a single centre level as well as at a two-centre level. For this task, three different DTI protocols were used at two different field strengths, three different gradient direction schemes on platforms from two different manufacturers had to be merged into a common analysis scheme. The alteration patterns for ALS/PLS patients could be validated in the bicentric approach, that way improving the statistical power in investigation. After calculation of 3-D correction

![Fig. 3.](A) Whole brain-based spatial statistics (WBSS) of FA maps at the group level for ALS patients, PLS patients, and controls. WBSS of FA maps demonstrated multiple clusters of regional FA reductions at p < 0.05 (corrected for multiple comparisons), projectional views. (B) Tractwise fractional anisotropy statistics (TFAS) of FA maps at the group level for ALS patients, PLS patients, and controls. TFAS demonstrated significant regional FA reductions in ALS-related tract systems and in the grand average between ALS patients and controls as well as between PLS patients and controls. No alterations between groups were observed in the reference tract. *p < 0.05, **p < 0.001.

### Table 3

*p*-values for differences between PLS patients and controls groups for different ALS-related tract systems. Significant FA alterations (*p* < 0.05) are coloured.

| FA | PLS vs controls | CST (stage 1) | corticopontine/corticorubral tract (stage 2) | corticostriatal pathway (stage 3) | proximal portion of the perforant path (stage 4) | grand average | reference |
|----|----------------|---------------|---------------------------------|---------------------------------|---------------------------------|---------------|-----------|
|    | center 1 (Ulm) | 4\times10^-4  | 1\times10^-5                    | 0.002                           | 0.01                            | 0.005         | 0.3       |
|    | center 2 (Milan) | 1\times10^-11 | 0.04                           | 0.05                            | 0.9                             | 4\times10^-5  | 0.5       |
matrices according to a previously reported protocol (Rosskopf et al., 2015; Müller et al., 2016), FA maps of PLS and ALS patients and controls were harmonised and could be pooled to a large data sample. After pooling, still, to some extent, residual centre-specific FA-influencing factors might remain. However, as the ratio patients/controls was identical for both centres in this study, linear inter-centre differences do not contribute to the results when calculating differences between patients and controls.

It is due to one of the limitations of the study that a differentiation with respect to the temporal course between PLS and ALS was not possible on the basis of the current data, because the study design was cross-sectional, while only longitudinal data from the same timepoints during the course of the disease of PLS and ALS patients would allow to compare the sequential spreading model in both MND phenotypes. Another limitation is the lack of autopsy-based confirmation of TDP43 pathology in the patients.

In summary, the two-centre study demonstrated that the neuropathological stages can be mapped in vivo in PLS with high reproducibility and that PLS-associated cerebral propagation, although occurring in the same corticofugal patterns as ALS, might have a different time course. As a consequence, the used neuroimaging technique can help monitoring how PLS spreads across the brain by a self-perpetuating process (Agosta et al., 2015b), and, like in ALS (Kassubek et al., 2018), TOI-based FA mapping scores may be a candidate read-out for potential disease-modifying strategies in PLS, when future studies with individual longitudinal staging categorisation will have elucidated the time course of the propagation.

Author contributions

Hans-Peter Müller: study concept and design, data analysis and interpretation of data, drafting of manuscript.

Federica Agosta: Data collection, interpretation of data, critical revision of manuscript for intellectual content.

Martin Gorges: Data collection, data analysis, critical revision of manuscript for intellectual content.

Rebecca Kassubek: Data analysis, critical revision of manuscript for intellectual content.

Edoardo Gioele Spinelli: Data collection, critical revision of manuscript for intellectual content.

Albert Ludolph: Interpretation of data, study supervision, critical revision of manuscript for intellectual content.

Massimo Filippi: study concept and design, interpretation of data, study supervision, critical revision of manuscript for intellectual content.

Jan Kassubek: study concept and design, interpretation of data, study supervision, drafting of manuscript.

Author disclosures

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Table 4

Clusters from whole brain-based spatial statistics. WBSS (p < 0.05, FDR-corrected) of FA maps.

| Cluster no. | Cluster size | MNI x/y/z (maximum) | L/R | p | Anatomical localization |
|------------|-------------|---------------------|-----|---|-------------------------|
| ALS vs controls | 1 | 50,309 | −11/-24/-12 | L/R | < 0.000001 | decrease lower/upper CST |
| | 2 | 23,570 | −28/-19/26 | L/R | < 0.000001 | decrease upper CST |
| | 3 | 2758 | −43/26/6 | L | < 0.000001 | decrease temporal lobe |
| | 4 | 1074 | −31/17/17 | L | < 0.000001 | decrease frontal lobe |
| | 5 | 701 | 38/-35/5 | R | < 0.000001 | decrease temporal lobe |
| ALS vs controls | 1 | 60,145 | 12/-28/-12 | L/R | < 0.000001 | decrease lower/upper CST |
| | 2 | 1602 | −26/17/13 | L | < 0.000001 | decrease frontal lobe |
| | 3 | 1309 | −12/25/-12 | L | < 0.000001 | decrease lower CST |

Table 5

p-values for differences between groups for different ALS-related tract systems. Significant FA alterations (p < 0.05) are coloured.

| FA | CST (stage 1) | corticopontine/ corticorubral tract (stage 2) | corticostriatal pathway (stage 3) | proximal portion of the perforant path (stage 4) | grand average | reference |
|----|---------------|---------------------------------------------|---------------------------------|---------------------------------------------|--------------|-----------|
| ALS vs controls | 2*10^-15 | 3*10^-6 | 2*10^-4 | 0.02 | 8*10^-10 | 0.9 |
| PLS vs controls | 1*10^-12 | 1*10^-4 | 5*10^-4 | 0.03 | 4*10^-9 | 0.8 |
| ALS vs PLS | 0.5 | 0.4 | 0.6 | 0.8 | 0.4 | 0.8 |
J. Kassubek is Section Chief Editor of Frontiers in Neurology; Advisory board member for UCB Pharma, BIAL, NeuroDerm, Teva Pharmaceuticals, Medtronic, AbbVie. Honoraria as a speaker from UCB Pharma, Teva Pharmaceuticals, Novartis, Zambon, Medtronic, Desitin, AbbVie, Boehringer Ingelheim, GlaxoSmithKline, Merz Pharmaceuticals.

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Statement

All human studies have been approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

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