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Evolving diagnostic criteria in primary lateral sclerosis: The clinical and radiological basis of "probable PLS"

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ABSTRACT

Introduction: Primary lateral sclerosis is a rare neurodegenerative disorder of the upper motor neurons. Diagnostic criteria have changed considerably over the years, and the recent consensus criteria introduced 'probable PLS' for patients with a symptom duration of 2–4 years. The objective of this study is the systematic evaluation of clinical and neuroimaging characteristics in early PLS by studying a group of 'probable PLS patients' in comparison to a cohort of established PLS patients.

Methods: In a prospective neuroimaging study, thirty-nine patients were stratified by the new consensus criteria into 'probable' (symptom duration 2–4 years) or 'definite' PLS (symptom duration > 4 years). Patients were evaluated with a standardised battery of clinical instruments (ALSFRS-r, Penn upper motor neuron score, the modified Ashworth spasticity scale), whole genome sequencing, and underwent structural and diffusion MRI. The imaging profile of the two PLS cohorts were contrasted to a dataset of 100 healthy controls. All 'probable PLS' patients subsequently fulfilled criteria for 'definite' PLS on longitudinal follow-up and none transitioned to develop ALS.

Results: PLS patients tested negative for known ALS- or HSP-associated mutations on whole genome sequencing. Despite their shorter symptom duration, 'probable PLS' patients already exhibited considerable functional disability, upper motor neuron disease burden and the majority of them required walking aids for safe ambulation. Their ALSFRS-r, UMN and modified Ashworth score means were 83%, 98% and 85% of the 'definite' group respectively. Motor cortex thickness was significantly reduced in both PLS groups in comparison to controls, but cortical changes were less widespread in 'probable' PLS on morphometric analyses. Corticospinal tract and corpus callosum metrics were relatively well preserved in the 'probable' group in contrast to the widespread white matter degeneration observed in the 'definite' group.

Conclusions: Our clinical and radiological analyses support the recent introduction of the 'probable' PLS category, as this cohort already exhibits considerable disability and cerebral changes consistent with established PLS. Before the publication of the new consensus criteria, these patients would have not been diagnosed with PLS on the basis of their symptom duration despite their significant functional impairment and motor cortex atrophy.

Abbreviations: **ALS**, amyotrophic lateral sclerosis; **ALSFRS-r**, revised amyotrophic lateral sclerosis functional rating scale; **ANCOVA**, analysis of covariance; **C9orf72**, chromosome 9 open reading frame 72; **CC**, corpus callosum; **CST**, corticospinal tract(s); **DTI**, Diffusion Tensor Imaging; **EMG**, electromyogram; **EMM**, estimated marginal mean; **FLAIR**, Fluid-attenuated inversion recovery; **FOV**, field of view; **FWE**, familywise error; **GM**, grey matter; **HC**, healthy control; **HSP**, hereditary spastic paraplegia; **IR-SPGR**, inversion recovery prepared spoiled gradient recalled echo; **IR-TSE**, inversion recovery turbo spin echo sequence; **L**, left; **LMN**, lower motor neuron; **MNI152**, Montreal Neurological Institute 152 standard space; **PLS**, primary lateral sclerosis; **PLSFRS**, primary lateral sclerosis functional rating scale; **PUMNS**, Penn upper motor neuron score; **R**, right; **ROI**, region of interest; **SD**, standard deviation; **SE-EPI**, spin-echo echo planar imaging; **SENSE**, Sensitivity Encoding; **SPIR**, spectral presaturation with inversion recovery; **T1W**, T1-weighted imaging; **TE**, Echo time; **TFCE**, threshold-free cluster enhancement; **TI**, Inversion time; **TIV**, total intracranial volume; **TR**, repetition time; **UMN**, Upper motor neuron

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The introduction of this new category will facilitate earlier recruitment into clinical trials, and shorten the protracted diagnostic uncertainty the majority of PLS patients face.

1. Introduction

Primary lateral sclerosis is a rare, adult-onset, sporadic UMN disorder, typically presenting with spasticity and hyperreflexia, most commonly in the lower limbs. It has been estimated to account for between 3 and 5% of incident motor neuron disease cases [1–4]. PLS carries a markedly better prognosis with considerably longer survival than amyotrophic lateral sclerosis, therefore the distinction between the two conditions, especially soon after symptom manifestation requires care and meticulous evaluation. Due to its insidious onset, low incidence, and clinical manifestations reminiscent of other neurodegenerative conditions, its diagnosis is particularly challenging and patients often face a long and circuitous diagnostic journey. A number of diagnostic criteria have been developed over the years based on clinical features and symptom duration, but most of them were optimised to reduce the risk of labelling patients with PLS who later develop LMN signs. The Pringle criteria (1992) proposed a symptom duration of 3 years for reliable diagnosis, based on previous accounts of transition to ALS [5]. This was a downward revision from 1945 criteria which had advocated for a minimum of 5 year symptom duration [6]. Gordon's 2006 paper examined a case series of patients with UMN signs and found that the majority of patients who subsequently developed LMN signs did so by year four [7]. Since then, a symptom duration of 4 years became the most commonly implemented diagnostic threshold. The recent (2020) consensus diagnostic criteria [8] recognised the practical implications of diagnostic delay and the urgency of including suspected PLS patients into research and pharmacological studies. Accordingly, the new criteria introduced the category of 'probable PLS' with a symptom duration of 2–4 years.

PLS is primarily associated with upper motor neuron dysfunction and pseudobulbar affect [9,10], but extra-pyramidal [11] and cognitive [12–15] manifestations have also been reported. Neuroimaging studies in PLS have consistently captured motor cortex [16–18], corpus callosum [19,20] and corticospinal tract degeneration [21–23], but subcortical grey matter pathology [24,25], brainstem [26,27], extra-motor [18,28,29], and cerebellar [18] changes have also been described. Existing imaging studies in PLS however suffer from considerable sample size limitations and depending on the date of publication, use different diagnostic criteria.

Despite its clinical relevance and implications for therapeutic intervention, the early symptomatic phase of primary lateral sclerosis (PLS) is poorly characterised [30,31]. Suspected PLS patients typically face protracted diagnostic uncertainty and while the risk of conversion to ALS is unclear, it is a dreaded possibility after symptom manifestation. Previous diagnostic criteria in PLS were carefully optimised for diagnostic certainty at the expense of diagnostic delay. The implications of the 4-year symptom duration requirement meant that suspected PLS patients were generally excluded from research studies. This, coupled with the low incidence of the condition [4,32] hampered research efforts compared to the advances seen in the field of ALS [33,34]. The rationale behind previous diagnostic criteria is that a proportion of patients with progressive UMN involvement develop clinical or electromyographic (EMG) evidence of lower motor neuron (LMN) involvement [5,35–38]. In the absence of validated predictive markers of transition to ALS [32], patients with a clinical phenotype compatible with PLS typically remained unclassified for up to four years, irrespective of the level of functional impairment. This is despite fulfilling all other clinical diagnostic criteria and their laboratory profile (imaging, EMG and genetic testing) reliably excluding alternative structural, inflammatory, or neoplastic diagnoses [39]. In the seminal study by Gordon et al., 10 of 13 (77%) patients who subsequently developed LMN involvement, did so within 4 years of symptom onset [35]. In a later study, just 3 of 22 (14%) patients with a symptom duration of less than 4 years transitioned to ALS [40]. Finally, a large study of patients with early UMN disease found that minor EMG changes were of doubtful prognostic significance in terms of function or survival [38].

The cost-benefit ratio of delaying diagnosis to improve diagnostic accuracy is uncertain and the ramifications of accruing pathological burden and functional impairment are seldom discussed. With few exceptions [41,42], studies of PLS have focused on patients with long-established disease and described considerable functional impairment associated with muscle spasticity, electrophysiological (TMS) and radiological abnormalities within central motor pathways [18,43–47]. The pioneering study of Clark et al. described considerable clinical and imaging abnormalities in patients with a symptom duration of less than 5 years, reflecting a substantial disease burden early in the course of the disease [41]. 'Early' PLS patients have also been included in other imaging studies although they have been considered together with more established patients [19,48] instead of appraising disease burden in this cohort separately.

The international research community has recognised the above challenges and number of important steps were made to address these issues. The recent consensus diagnostic criteria facilitates an earlier diagnosis of PLS, thus enabling timely inclusion in pharmaceutical trials [49]. A validated PLS-specific functional rating has also been recently introduced which demonstrates improved sensitivity to the functional changes associated with the condition and is thought to be superior for tracking longitudinal changes than ALSFRS-r [42]. Significantly, the new diagnostic criteria introduces the category of 'probable PLS' for patients with a symptom duration interval of 2–4 years and 'definite PLS' for those with a duration of at least 4 years. This is an important step to refine diagnostic categorisation in PLS which is likely to benefit individualised patient care, therapy development and biomarker studies. Since the publication of the new diagnostic criteria however, the clinical and neuropathological profile of patients with 'probable PLS' have not been systematically evaluated. The nuanced characterisation of disease burden in 'probable' PLS is crucially important as it enables the evaluation of pathological patterns with reference to 'established' PLS and permits the quantitative assessment of disease burden in specific clinical domains and anatomical regions. Imaging data generated from 'probable' and 'established' PLS patients allows the assessment of what percentage of degenerative change may already be present in the 'probable' phase of the disease, which, if considerable, would support the rationale for the new diagnostic criteria. Accordingly, the objective of this study is the evaluation of the clinical disability profile and neuroimaging features of patients with 'probable' PLS with reference to 'definite PLS' patients and healthy controls.

2. Methods

2.1. Participants

Patients were recruited from a population-based register and stratified based on the new consensus diagnostic criteria [49] into 'probable' (symptom duration of 2–4 years) or 'definite' (symptom duration > 4 years) PLS categories. Patients underwent standardised clinical, genetic and imaging evaluation. Imaging data from one hundred age-matched healthy controls were utilised to generate normative values. The study was approved by the Ethics (Medical Research) Committee—Beaumont Hospital, Dublin, Ireland, and all participants

provided informed consent prior to inclusion. Heathy controls were unrelated to participating patients, had no neurological and psychiatric diagnoses and lacked vascular risk factors such as smoking, diabetes, hypertension or atrial fibrillation.

2.2. Clinical evaluation

Demographic data, including handedness, education, gender and age were recorded for each participant. Clinical data were collected at the time of MR imaging and included the total ALSFRS-r, ALSFRS-r sub-scores, estimated date of symptom onset and region of first symptom onset, Penn Upper Motor Neuron Score (PUMNS), modified Ashworth spasticity scale scores [50]. The rate of functional decline for each study participant was calculated as average monthly decline in ALSFRS-r from symptom onset to clinical assessment [51]. The ALSFRS-r consists of 12 items relating to motor function across four regions (bulbar, upper-limb/fine motor, lower-limb/gross motor and respiratory) [50]. Each item is scored from zero (severe) to four (normal function). The total ALSFRS-r and ALSFRS-r subscales can be therefore aid interpretation of functional progression and regional spread at individual or group level. To avoid inter-rater bias, all PLS patients underwent a standardised neurological examination by the same, experienced examiner. Evaluation of UMN signs was performed according to the Penn Upper Motor Neuron Score (PUMNS) which includes an evaluation of spasticity in each limb and records pathologically increased reflexes in the limbs and in the bulbar region [52,53]. The Penn UMN score is usually presented as a composite score of UMN signs. The scale ranges from 0 (normal) to maximum of 32 (for widespread/severe UMN involvement) and evaluates the bulbar region (scores 0–4), upper limbs (scores 0–14) and lower limb (scores 0–14). In contrast to the ALSFRS-r, higher scores indicate greater disease burden. Due to the gradual spread of functional impairment between body regions over years [18,31], regional UMN sub-scale scores were also recorded.

2.3. Magnetic resonance imaging

T1-weighted images were acquired on a 3 Tesla Philips Achieva system with a 3D Inversion Recovery prepared Spoiled Gradient Recalled echo (IR-SPGR) pulse sequence using an 8-channel receive-only head coil. The IR-SPGR pulse sequence parameters were as follows; TR/TE = 8.5/3.9 ms, TI =1060 ms, field-of-view (FOV): 256 × 256 × 160 mm, spatial resolution: 1 mm3, flip angle = 8°, SENSE factor = 1.5, acquisition time: 7 min 30 s. DTI images were acquired using a spin-echo echo planar imaging (SE-EPI) sequence with a 32-direction Stejskal-Tanner diffusion encoding scheme. FOV = 245 × 245 × 150 mm, spatial resolution = 2.5 mm3, 60 slices were acquired with no interslice gap, TR/TE = 7639 / 59 ms, SENSE factor = 2.5, b-values = 0, 1100 s/mm2, with SPIR fat suppression, dynamic stabilisation and a total acquisition time of 5 min 41 s. A dual approach was implemented to characterise anatomical patterns of pathology in ‘probable’ and ‘definite’ PLS. First, standard ‘whole-brain’ analyses were carried out to appraise grey and white matter alterations. Subsequently, additional region-of-interest (ROI) analyses were undertaken to assess integrity imaging measures in clinically relevant anatomical regions.

2.4. Grey matter analyses

Morphometric grey matter changes in the PLS cohorts were evaluated using the FMRIB's FSL suite v6.0. [54,55] Pre-processing steps included skull-removal (BET), motion-corrections and tissue-type segmentation [56]. Grey-matter partial volume data were aligned to the MNI152 standard space using affine registration [57]. A study-specific GM template was subsequently created to which the grey matter images from each subject were non-linearly co-registered. Permutation based non-parametric inference was used for group comparisons with the threshold-free cluster enhancement (TFCE) method.

2.5. White matter analyses

Following eddy current corrections a tensor model was fitted to the raw diffusion data to generate maps of fractional anisotropy (FA), axial diffusivity (AD), mean diffusivity (MD) and radial diffusivity (RD). The tract-based statistics (TBSS) pipeline of the FSL image analysis suite was utilised for non-linear registration and skeletonisation of each subject's diffusion image. FA, AD, MD and RD images were merged into a single 4D image file and a mean FA mask was created. The voxelwise diffusivity profile of the study groups was evaluated by permutation-based non-parametric inference using matrix-defined contrasts.

2.6. Region of interest analyses

Version 7.1.0 of the FreeSurfer image analysis suite was used for cortical thickness measurements. [58] The standard pre-processing stream was implemented including the removal of non-brain tissue, segmentation of the subcortical white matter and deep grey matter structures, intensity normalization, tessellation of the grey matter-white matter boundary, and automated topology correction. [59] Average cortical thickness values were retrieved from the pre- and paracentral gyrus using the labels of the Desikan-Killiany atlas [60]. White matter integrity metrics were retrieved from the corpus callosum and corticospinal tracts using FMRIB's FSL. The study specific white matter skeleton was masked by labels of the Jülich histological atlas [61] for the left corticospinal tract, right corticospinal tract and the corpus callosum to generate study specific white matter ROIs [62] and retrieve average axial diffusivity, fractional anisotropy, mean diffusivity and radial diffusivity values form the above white matter regions [63].

2.7. Genetic testing

Twenty-eight of the 39 (72%) PLS patients underwent whole genome sequencing and were screened for ALS and HSP-associated mutations. Thirty-three PLS patients (85%) were screened for *C9orf72* repeat expansions. Genome sequence data first underwent quality control, aligned to the GRCh37 genome template, annotated and analysed using cutadapt V.1.9.1 [64], SAMtools V1.7 [65], Picard V.2.15.0 (http://picard.sourceforge.net/), Plink V.1.9 [66], R V.3.2.3 (http://www.r-project.org/), SnpEff V.4.3 [67] and Gemini V.0.20.1 [68]. Samples were screened for mutations in 33 genes implicated in ALS [69] and 70 genes linked to HSP [70]. The presence of the *C9orf72* hexanucleotide repeat expansion was determined using repeat-primed polymerase chain reaction (PCR) as described previously [71].

2.8. Statistical analyses

Demographic variables for all three groups and the clinical variables for the two patient groups were compared using analysis of variance and independent samples *t*-tests, respectively, for continuous variables. Chi-square and Fisher's exact tests were used to compare group proportions for categorical variables. Statistical analysis of demographic and clinical data was performed using IBM SPSS Statistics Version 26. Voxel-wise imaging data was analysed using non-parametric permutation-based statistics. Design matrices included group membership and demeaned covariates. Age, gender and education were included as covariates in tract-based spatial statistics [72]. Morphometric grey matter analyses were adjusted for age, gender, education and total intracranial volumes (TIV). Voxel-wise statistics were performed with the threshold-free cluster enhancement (TFCE) approach, resulting statistical maps were thresholded and presented following FWE corrections. Region-of-interest imaging statics were performed on raw data retrieved from individual scans. Average cortical thickness was retrieved from the pre- and para-central gyri and diffusivity metrics retrieved

from spatially-aligned skeletonised white matter data. Retrieved imaging metrics were also interpreted using IBM SPSS v. 26. Assumptions of normality were examined using the Kolmogorov-Smirnov test. Skewness and kurtosis were assessed separately for each study group. Since all variables followed a normal distribution, parametric statistics were applied. Group differences in imaging metrics retrieved from ROIs were examined using multivariate analysis of covariance (MANCOVA) with age, gender and education as covariates. A 'p' value of less than 0.05 was considered significant in post-hoc comparisons following Bonferroni corrections for multiple comparisons to reduce Type I error.

3. Results

3.1. Clinical characteristics

Thirty-nine PLS patients met the inclusion criteria, 32 who had clinically 'definite' PLS and 7 with 'probable' PLS. All 'probable' PLS patients were clinically followed and subsequently met criteria for 'definite' PLS. The demographic details of all participants are presented in Table 1. The groups were matched for age, gender and handedness. No ALS or HSP-associated mutations were identified in either PLS cohort. The clinical profiles of the 'probable' and 'definite' PLS groups are presented in Table 1. All 'probable' PLS patients and all but one 'definite' PLS patient had experienced their first symptoms in the lower limbs. Although the 'definite' PLS group exhibited more severe functional disability, 'probable' PLS already also had substantial impairments. Early functional impairment was most striking on the gross motor scale, reflecting lower limb involvement where the group mean was just 6.6 in the probable group compared to 5.5 in the definite group ($p = .137$). Six of the seven 'probable' PLS patients were already relying on a walking aid for safe ambulation. However, none of the patients in the 'probable' group were dependent on a wheelchair compared with 3 (9%) patients in the definite group. Relative to the early severity of lower limb dysfunction, upper limb impairment was less severe in both groups. In comparison to the 'definite' PLS group, bulbar dysfunction was less severe in the 'probable' PLS group ($p = .016$). The rate of functional decline (ALSFRS-r slope) prior to study entry was almost twice as high in the 'probable' PLS group ($p = .0001$). Mirroring the ALSFRS-r sub-scale findings, the PUMNS was remarkably similar in the two groups with no significant difference in group means ($p = .544$).

3.2. Grey matter profiles

The voxel-wise analysis confirmed significant motor cortex atrophy in the 'definite' PLS group relative to healthy controls. Fig. 1. By contrast, the probable PLS group had less severe and more focal structural alterations within the same anatomical region. Region-of-interest thickness analyses also demonstrated cortical thinning in bilateral precentral and paracentral gyri in both 'probable' and 'definite' PLS patients. The direct comparison of 'probable' and 'definite' groups did not reach statistical significance on either whole-brain or ROI analyses. Regional cortical thickness profiles are presented in Table 2 and Fig. 2. The percentage change in cortical thickness in each patient group relative to healthy controls is shown on a radar plot in Fig. 3.

3.3. White matter profiles

Significant, symmetrical white matter alterations were identified in the 'definite' PLS group, primarily involving the corticospinal tracts throughout their intracranial course as well as the corpus callosum. Fig. 1. No statistically significant white matter alterations were detected in the 'probable' PLS group on voxel-wise analyses. ROI analysis also demonstrated widespread diffusion abnormalities in the 'definite' PLS group. In the 'definite' PLS group the most significant changes were observed in MD and RD in the corpus callosum with FA just above the significance threshold. The 'probable' PLS group exhibited limited white matter pathology; increased MD was detected in the right corticospinal tract, but differences in other diffusion parameters did not reach statistical significance.

** indicates a statistical significance of $p < .01$ Abbreviations: CC – Corpus callosum, CST – Corticospinal tract, L – Left, R – Right.

4. Discussion

This study reveals considerable clinical disability and structural disease burden in patients with 'probable' PLS, comparable to that observed in a cohort of established, 'definite' PLS patients. The functional and neurological profile of 'probable' PLS patients confirm severe disability relatively soon after symptom manifestation. Motor cortex changes are well established in 'definite' PLS patients based on *post mortem* and imaging studies [5,73–75]. In our study, we demonstrate considerable motor cortex atrophy in early PLS patients with less than 4-year symptom duration comparable to that observed in 'definite' PLS patients. Cortical changes are more focal in 'probable' PLS patients compared to the widespread degeneration identified in the 'definite' cohort. Some of the focal changes identified in the 'probable' group are consistent with the bulbar representation of the motor homunculus even though that group exhibited only limited bulbar impairment. This could represent presymptomatic structural degeneration or merely the limitations of ALSFRS-r in PLS [76–78].

Voxelwise white matter analyses confirmed extensive symmetrical

Table 1
(a) The demographic details of 'probable' PLS (symptom duration of 2–4 years), 'definite' PLS (symptom duration > 4 years) and healthy control groups (HC). **(b)** The functional and neurological profiles of the 'probable' and 'definite' PLS patients. ALSFRS-r - revised ALS functional rating scale, UL – Upper limb, LL – Lower limb, UMN – Upper motor neuron, LMN – Lower motor neuron, SD – Standard deviation.

(a)	**Probable PLS** $n = 7$	**Definite PLS** $n = 32$	**HC** $n = 100$	***P* value**
Age – years (SD)	61.2(11.1)	62.2 (9.8)	58.8 (11.5)	0.304
Sex- Male (%)	4 (57%)	19 (59%)	48 (48%)	0.506
Handedness-Right (%)	7 (100%)	28 (88%)	94 (94%)	0.349
Education-years	10.7 (3.0)	12.5 (3.3)	14.3 (3.3)	**0.002**

(b)	**Probable PLS** n = 7	**Definite PLS** n = 32	**Probable *vs* Definite (%)**	**P value**
Symptom Duration months (Range)	36.1 (26–46)	126.0 (56–307)	29%	**0.001**
Lower Limb Onset- n (%)	7 (100%)	31 (97%)		0.821
Functional Impairment				
Mobility				
Unaided	14%	9%		
ambulation	86%	82%		
Walking aid	0%	9%		
Wheelchair				
ALSFRS-r Total (SD)	39.6 (4.1)	34.5 (5.3)	63%	**0.024**
Bulbar	11.1 (0.9)	9.1 (2.1)	31%	**0.016**
Fine Motor	10.1(1.6)	8.8 (2.0)	59%	0.112
Gross Motor	6.6 (1.5)	5.5 (1.6)	83%	0.137
Respiratory	11.7 (0.8)	11.0 (1.4)	30%	0.215
ALSFRS-r decline (point per month)	−0.25 (0.08)	−0.13 (0.08)	192%	**0.001**
UMN burden				
UMN Total-mean (SD) Max = 32	18.5 (7.6)	20.2 (6.1)	92%	0.544
Bulbar UMN (max = 4)	1.4 (1.4)	1.8 (1.4)	78%	0.571
UL UMN sum (max = 14)	8.1 (3.8)	8.3 (3.4)	89%	0.544
LL UMN sum (max = 14)	10.0 (2.8)	9.8 (2.4)	98%	0.705
Spasticity- UL Mean	2.1 (0.8)	2.7 (0.9)	78%	0.199
Spasticity- LL Mean	2.9 (0.7)	3.4 (0.8)	85%	0.140

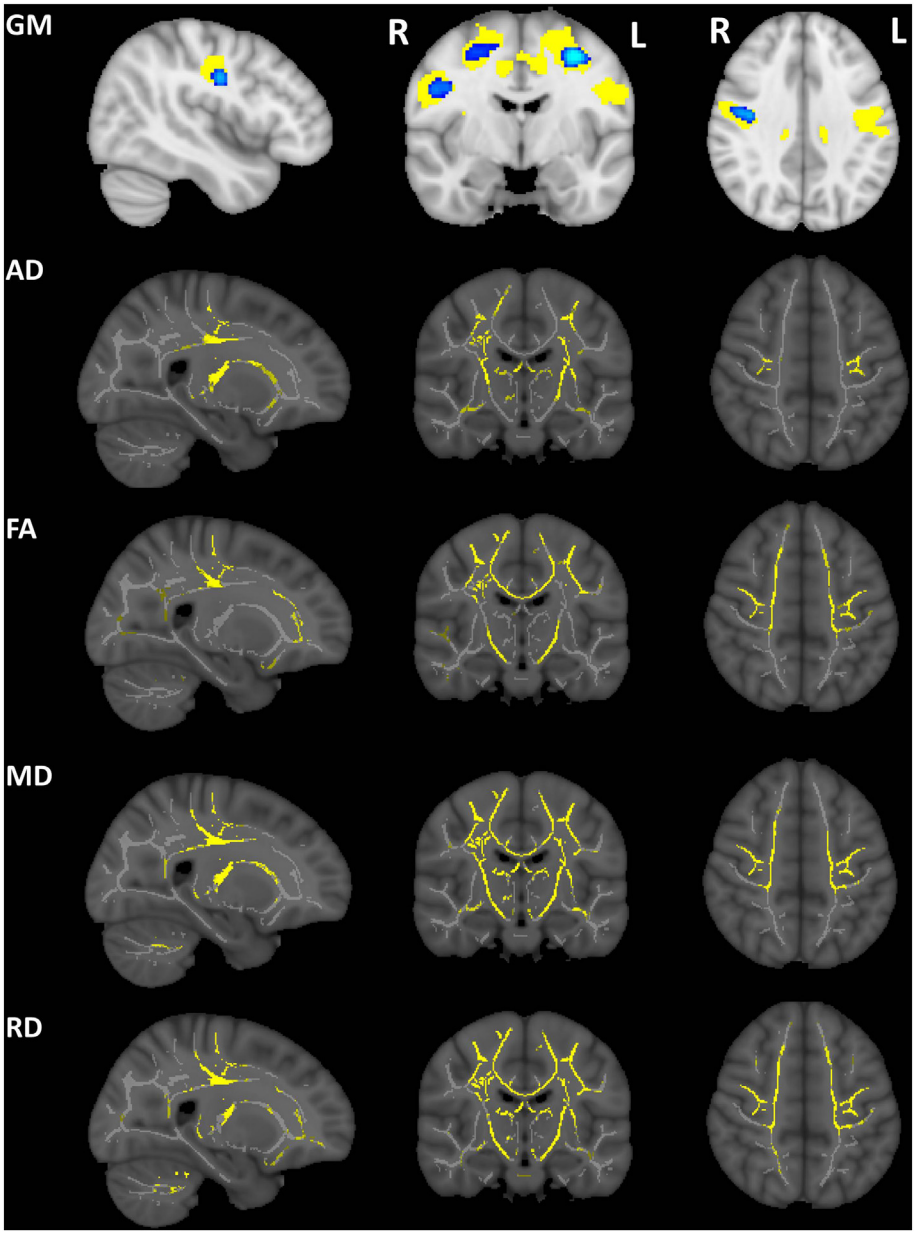

Fig. 1. GM – morphometric changes in the 'definite PLS' cohort are represented in yellow colour ($p < .01$ TFCE FWE) with reference to healthy controls and blue clusters represent morphometric changes at $p < .01$ TFCE FWE in 'probable PLS' patients. AD, FA, MD, and RD alterations are shown in 'definite' PLS patients at $p < .01$ TFCE FWE in contrast to healthy controls. Diffusivity metrics did not show statistically significant changes in 'probable PLS' patients with reference to controls. The direct grey and white matter contrasts between 'definite' and 'probable' PLS patients did not reach statistical significance. GM changes are shown with the following MNI coordinates $x = 44$, $y = -7$, $z = 34$, white matter changes represented at $x = 24$, $y = -14$, $z = 48$. Radiological convention was used to depict focal changes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

corticospinal tract and corpus callosum alterations in the 'definite' PLS group in keeping with previous studies [18,79–83]. The white matter profile of 'probable' patients however revealed limited pathology. Voxelwise analyses did not capture white matter degeneration on TBSS in 'probable' patients in any of the diffusivity metrics. Even on ROI analyses, just one diffusivity metric (MD) captured right corticospinal tract changes compared to healthy controls. The limited white matter involvement in 'probable' PLS patients is in sharp contrast to the significant early-stage grey-matter degeneration. These findings suggest that grey matter pathology may be precede widespread CST degeneration which only becomes evident after longer symptom duration. An alternative interpretation is that grey matter metrics are more sensitive to capture ALS-associated pathological changes [84]. This is different from ALS where longitudinal studies suggest that CST and CC degeneration is an early feature of the disease and progressive grey matter degeneration dominates the later stages [85–87]. The corpus callosum was the least affected in the 'probable group' compared to the 'definite' group, which suggests that that corpus callosum degeneration may be a later feature of the condition. The relevance of these observations is twofold; from a diagnostic perspective metrics which detect early changes are particularly useful, in the case, grey matter metrics in the motor cortex. From a monitoring standpoint, imaging indices which exhibit progressive changes are advantageous, in this case, measures of white matter integrity.

The clinical profile of the 'probable' PLS patient's is also remarkable. 'Probable' PLS patients had already accrued considerable functional disability, equivalent to almost two-thirds of the total loss of function of the 'definite' group despite their much shorter symptom duration. In previous studies, the ALSFRS-r and the PUMNS are usually reported as a composite score of all regions. However, this approach fails to account for the characteristic regional spread that is typically observed in PLS. In this study, all but one patient experienced what has been described as the ascending pattern of symptom spread, progressing from lower limb onset, to upper limb involvement and ultimately to the bulbar region (21). The benefits of sub-scale analysis have been demonstrated in ALS which is thought to provide important biological insights [88]. The sub-scale analysis of ALSFRS-r and PUMNS confirms the lower limb predominance of disease burden in early-stage PLS [2,38,39]. Even at this relatively early phase in the disease, mean lower limb ALSFRS-r subscale, lower limb spasticity (Mod. Ashworth) and lower limb PUMN

Table 2
Regional grey and white matter values of probable PLS (Prob PLS), definite PLS (Def PLS) and healthy control (HC) groups . Estimated marginal means (EMM) and standard error (SE) are adjusted for age, gender and education. Significant ($p < .05$) intergroup differences are in bold. Bonferroni correction used for multiple comparisons used for all post-hoc analysis. L –left, R – right, CST – Corticospinal tract.

Region	*Study group*	*EMM*	*SE*	MANCOVA	*Prob PLS vs HC*	*Def PLS vs HC*	*Prob PLS vs Def PLS*
Cortical Thickness							
Precentral (L)	HC	2.553	0.015	< **0.001**	< **0.001**	< **0.001**	1
	Prob PLS	2.295	0.058				
	Def PLS	2.304	0.027				
Precentral (R)	HC	2.527	0.015	< **0.001**	< **0.001**	< **0.001**	1
	Prob PLS	2.282	0.058				
	Def PLS	2.308	0.027				
Paracentral (L)	HC	2.410	0.018	< **0.001**	**0.012**	< **0.001**	1
	Prob PLS	2.205	0.067				
	Def PLS	2.244	0.032				
Paracentral (R)	HC	2.395	0.017	< **0.001**	**0.029**	. < **0.001**	0.588
	Prob PLS	2.222	0.064				
	Def PLS	2.260	0.030				
Fractional Anisotropy							
CST (L)	HC	0.514	0.002	< **0.001**	0.321	< **0.001**	0.486
	Prob PLS	0.500	0.008				
	Def PLS	0.487	0.003				
CST (R)	HC	0.524	0.002	< **0.001**	0.480	< **0.001**	1
	Prob PLS	0.510	0.009				
	Def PLS	0.504	0.004				
Corpus Callosum	HC	0.642	0.003	< **0.001**	0.831	< **0.001**	0.057
	Prob PLS	0.625	0.014				
	Def PLS	0.586	0.007				
Mean Diffusivity							
CST (L)	HC	0.000695	0.000002	< **0.001**	0.261	< **0.001**	0.204
	Prob PLS	0.000712	0.000009				
	Def PLS	0.000731	0.000004				
CST (R)	HC	0.000672	0.000003	< **0.001**	**0.048**	< **0.001**	1
	Prob PLS	0.000697	0.000010				
	Def PLS	0.000705	0.000005				
Corpus Callosum	HC	0.000836	0.000006	< **0.001**	1	< **0.001**	**0.048**
	Prob PLS	0.000834	0.000022				
	Def PLS	0.000893	0.000010				
Axial Diffusivity							
CST (L)	HC	0.001135	0.000003	< **0.001**	1	< **0.001**	0.546
	Prob PLS	0.001145	0.000012				
	Def PLS	0.001163	0.000005				
CST (R)	HC	0.001110	0.000004	< **0.001**	0.246	< **0.001**	**1**
	Prob PLS	0.001136	0.000014				
	Def PLS	0.001141	0.000007				
Corpus Callosum	HC	0.001564	0.000006	0.426	0.741	1	0.579
	Prob PLS	0.001535	0.000024				
	Def PLS	0.001570	0.000011				
Radial Diffusivity							
CST (L)	HC	0.000475	0.000003	< **0.001**	0.213	< **0.001**	0.252
	Prob PLS	0.000495	0.000010				
	Def PLS	0.000515	0.000005				
CST (R)	HC	0.000453	0.000003	< **0.001**	0.081	< **0.001**	1
	Prob PLS	0.000478	0.000011				
	Def PLS	0.000486	0.000005				
Corpus Callosum	HC	0.000471	0.000006	< **0.001**	1	< **0.001**	**0.027**
	Prob PLS	0.000484	0.000024				
	Def PLS	0.000554	0.000011				

score had reached 83%, 85% and 98% of the respective measures in the 'definite' PLS group. By contrast, the ALSFRS-r bulbar sub-score and UMN bulbar score were 31% and 78% respectively of the mean values of the 'definite' PLS group.

The findings of this study have potential implications for the design future clinical trials in PLS and support the rationale for more permissive diagnostic criteria [49]. The key finding of our study is that considerable structural changes have already occurred in the first 4 years after symptom manifestation. The considerable motor cortex atrophy identified in the 'probable' cohort at $p < .01$ FWE suggests irreversible degenerative changes and indicates that the therapeutic window is not in the 'definite' phase of the disease. Probable PLS patients endure considerable disability less than 4 years after symptom manifestation, which coupled with the significant radiological changes, provides a strong argument for early intervention, inclusion in clinical trials and the introduction of disease-modifying therapies. Our observations make a compelling argument for the more lenient diagnostic criteria and support the recent introduction of the 'probable' category. The fact that none of the 'probable' PLS patient subsequently transition to ALS provides further justification to diagnose suspected patients after a symptom duration of two years. The disability profile of the 'probable' group also suggests the potential therapeutic benefit of disease-modifying therapies after 4 years of symptom duration may be relatively limited as this cohort is already dependent on mobility aids, albeit progression to upper limb or bulbar impairment could theoretically be delayed or halted.

The rate of functional decline in the 'probable' PLS group was almost twice that of the 'definite' PLS group ($p = .001$), which suggest a

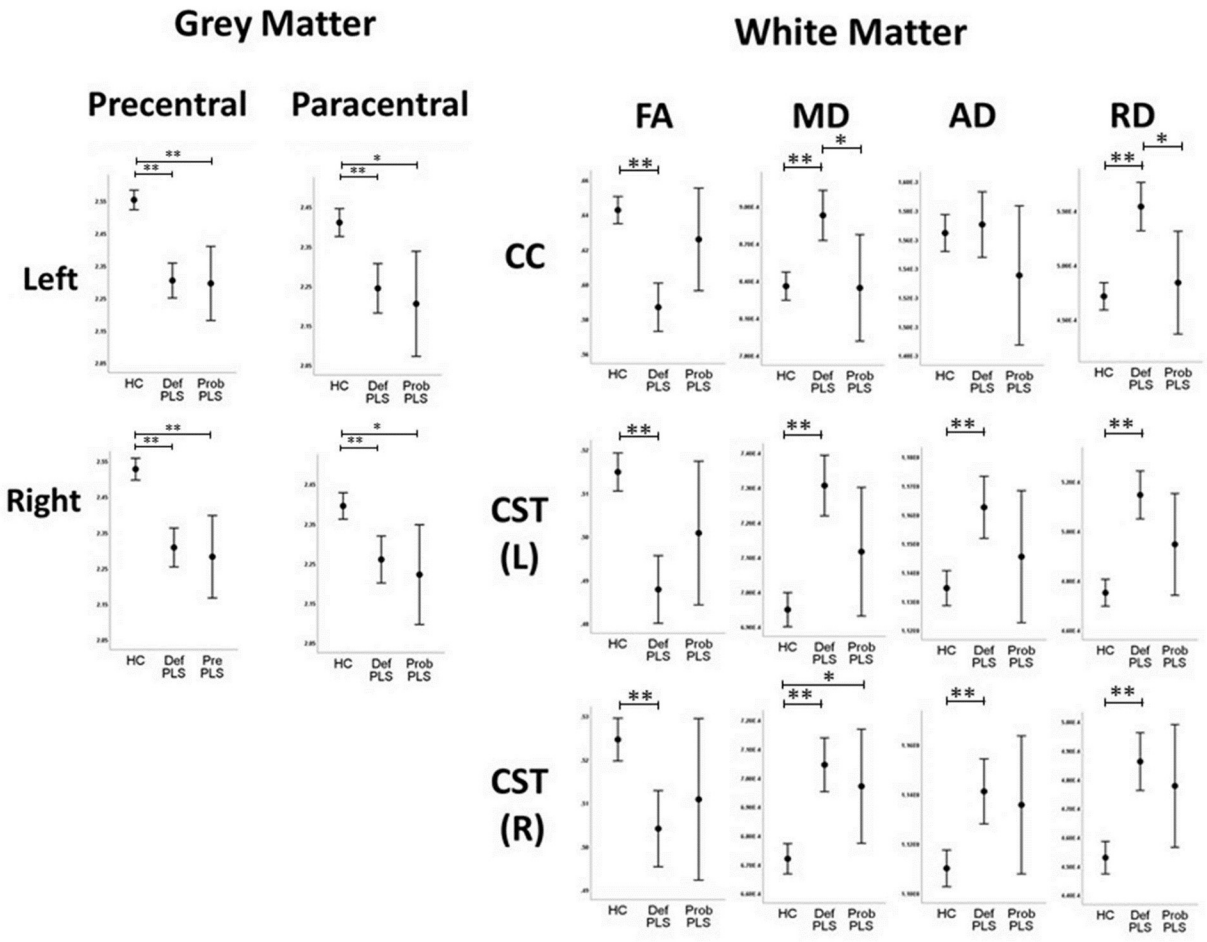

Fig. 2. The imaging profiles 'probable' and 'definite' PLS patients with reference to healthy controls (HC) based on estimated marginal means adjusted for age, gender and education. Error bars represent 95% confidence intervals. * indicates statistically significant intergroup differences at $p < .05$ following corrections for multiple comparisons and adjustments for demographic variables.

potential deceleration after reaching a certain disease burden or symptom duration. While our inference on longitudinal processes based on cross-sectional data is indirect, a recent multi-timepoint natural history study confirmed linear functional decline in the first 8 years of PLS followed by a plateau [89]. The stringent symptom duration requirement of previous diagnostic criteria stem from the fear of transition to ALS, as UMN predominant ALS patients may initially exhibit little evidence of lower motor neuron degeneration [38]. There is a

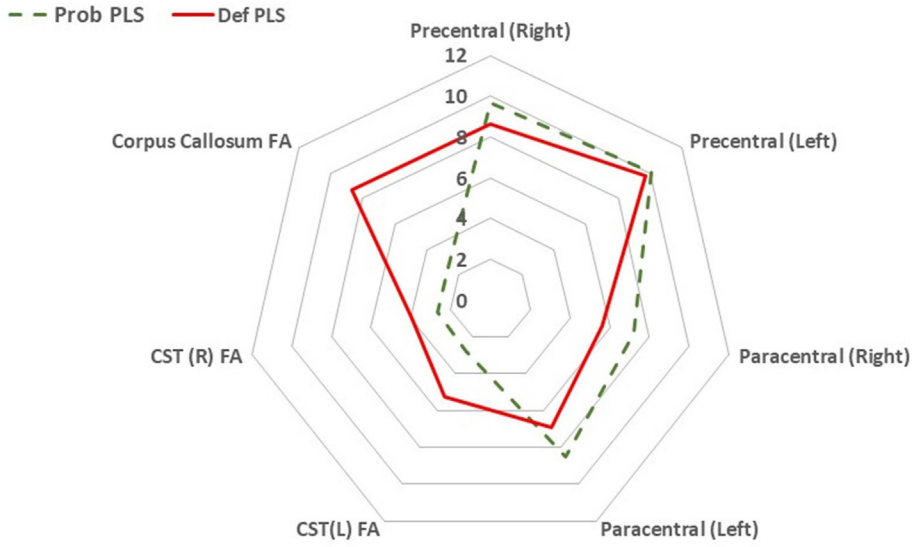

Fig. 3. The imaging profile of 'probable' and 'definite' PLS. Percentage change is presented with reference to the estimated marginal mean of healthy controls for each structure. Estimated marginal means of volumes were adjusted for age, gender, and education.

striking lack of robust epidemiology studies which specifically address this question and appraise the risk of conversion to ALS in UMN syndromes [90–92].

A limitation of this study is that it does not include any individuals with clinically 'suspected' PLS with less than 2 years symptom duration. However, in our own experience, presentation to a specialised clinic for diagnostic clarification with UMN symptoms for less than 2 years is relatively unusual. Our conversations with patients indicate that mobility may be affected early in the course of the disease and walking may already be impaired in the first two years. A category of 'suspected' PLS therefore seems judicious on clinical grounds, and neuroimaging that cohort may provide important insights regarding accruing cerebral disease burden. The drawback of introducing 'suspected-PLS' for patients with s symptom duration of less than 2 years, is the risk of transition to ALS, which needs to be specifically studied. It is also evident that symptom duration and disease duration are disparate entities; just like in other motor neuron diseases pathology is likely to accrue long before symptom manifestation until a critical threshold is reached [93]. The presymptomatic phase of PLS is arcane, as unlike in ALS [94] no presymptomatic imaging studies exist in PLS. In the absence of highly penetrant PLS-associated mutations the natural disease trajectory of the disease may be best evaluated by robust multi-timepoint longitudinal studies which would aim to include patients soon after symptom manifestation irrespective of meeting diagnostic criteria and follow-up with standardised clinical and radiological assessments [93]. Another limitation of this study is that clinical profiling focused on motor dysfunction despite the clinical relevance of extra-motor manifestations [12,95–97].The drawbacks of relying on ALSFRS-r also need to be acknowledged, especially that PLS specific instruments have now been developed [77]. Compared to more detailed instruments [76], ALSFRS-r only provides an indication of motor disability and is thought to be disproportionately representative of lower motor neuron dysfunction [88].

5. Conclusions

We have demonstrated that the Gordon criteria are relatively stringent with regards to symptom duration and exclude patients with considerable UMN disability and cortical disease burden. Our clinical and imaging findings therefore support the recent introduction of the 'probable' PLS category. The limited white matter pathology in patients with a symptom duration between 2 and 4 years justifies the categorisation of these patients under a separate label: 'probable PLS'. This symptom duration interval may represent an invaluable window for therapeutic intervention, where the diagnosis is already beyond reasonable doubt but only limited neurodegenerative change has occurred.

Declaration of Competing Interest

None declared.

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