Quantitative Spinal Cord Imaging: Early ALS Diagnosis and Monitoring of Disease Progression

M. Khamaysa, M. El Mendili, V. Marchand, G. Querin, P.-F. Pradat

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Khamaysa M (1), El Mendili M (1), Marchand V (1), Querin G (2), Pradat P-F* (1, 2)

1. Sorbonne Université, CNRS, INSERM, Laboratoire d'Imagerie Biomédicale, Paris, France
2. APHP, Département de Neurologie, Hôpital Pitié-Salpêtrière, Centre référent SLA, Paris, France

Potential conflicts of interest: None declared

Corresponding author:

Pierre-François Pradat,

Email address: pierre-francois.pradat@aphp.fr

Abstract

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder characterized by the progressive degeneration of motor neurons in the cortex, brainstem, and spinal cord. This degeneration leads to muscular weakness, progressively impairing motor functions and ultimately resulting in respiratory failure. The clinical, genetic, and pathological heterogeneity of ALS, combined with the absence of reliable biomarkers, significantly challenge the efficacy of therapeutic trials. Despite these hurdles, neuroimaging, and particularly spinal cord imaging, has emerged as a promising tool. It provides insights into the involvement of both upper and lower motor neurons. Quantitative spinal imaging has the potential to facilitate early diagnosis, enable accurate monitoring of disease progression, and refine the design of clinical trials.

In this review, we explore the utility of spinal cord imaging within broader context of developing spinal imaging biomarkers in ALS. We focus on both diagnostic and prognostic biomarker in ALS, highlighting its pivotal role in elucidating the disease's underlying pathology. We also discuss the existing limitations and future avenues for research, aiming to bridge the translational gap between academic research and its application in clinical practice and therapeutic trials.

Keywords: Amyotrophic Lateral Sclerosis (ALS); Quantitative spinal Imaging; Biomarkers; MRI; Neuroimaging

Amyotrophic lateral sclerosis (ALS) is an adult-onset relentlessly progressive neurodegenerative disease with an estimated incidence between 1 and 2.6 per 100,000 worldwide [1,2]. Motor neurons degeneration in the cortex, brainstem, and spinal cord, extends to other cerebral regions such as the frontal and prefrontal areas, basal ganglia, cerebellum, and sensory pathways [3-8]. This widespread neurodegeneration underlies the diverse manifestations of ALS, extending beyond the motor system to significantly impact cognitive functions. Notably, cognitive impairment is observed in more than 50% of ALS patients, and around 15% meet the criteria for frontotemporal dementia [9,10]. Consequently, patients with ALS experience progressive muscle weakness in the limbs, dysarthria, swallowing difficulties and diaphragmatic weakness, eventually leading to death primarily due to respiratory failure [11], with a median survival of 2-5 years [12]. Currently approved treatments only mildly slow down the progression of the disease, highlighting the critical unmet need for clinically meaningful neuroprotective drugs.

The necessity for a validated biomarker that could facilitate disease characterization and serve as an indicator of disease progression is of paramount importance in enhancing the design of clinical trials [13]. Therefore, in this synthetic narrative review, we aim to explore the progress in spinal cord imaging and the current findings that underscore its significance in the development of a diagnostic biomarker, monitoring disease progression, prognostication, and its contribution to comprehending the disease process in ALS. We discuss existing limitations and future avenues for bridging the translational gap to use spinal cord imaging in the clinical setting and therapeutic trials.

The need for neuroimaging biomarker development

One of the primary hurdles in therapeutic development for ALS is the absence of validated and reliable biomarker. Consequently, contemporary therapeutic clinical trials in ALS rely either on survival and/or functional rating scales as primary end-points and do not use objective biomarkers to monitor response to therapy [14]. The lack of a fully validated biomarker is mainly attributed to the significant clinical heterogeneity of the condition [15]. Disease heterogeneity in ALS has multiple dimensions, including genetic origin, site of onset, rate of decline, the presence of cognitive impairment and the relative degree of upper and lower motor neuron involvement (UMN and LMN, respectively) [16]. Therefore, the

establishment of reliable biomarkers would not only address the challenge of disease heterogeneity but also offer multifaceted benefits. This includes shortening the disease diagnosis period, capturing the dynamic pathophysiological substrate of the disease, facilitating patient stratification, and ultimately enhancing statistical power in therapeutic trials. By targeting groups with homogeneous disease progression rates, such biomarkers could reduce the required sample size and expedite the timeline.

Most imaging studies in ALS focus on comparing groups of ALS patients with either healthy or disease control groups. While this is academically valuable, revealing disease-specific imaging characteristics and cohort-specific degeneration patterns, clinical practice requires the ability to interpret individual patient scans [17]. Recently, there has been a significant shift from group-level descriptive research to creating analysis pipelines designed to interpret single scans from individuals [18,19]. These new methods depend on classifying individuals based on normative data or utilizing advanced machine learning techniques [17-19].

The most extensively studied biomarker in ALS is Neurofilament light chain (NfL) [20]. particularly due to its prognostic relevance. Studies have demonstrated a significant inverse correlation between NfL levels in both serum and CSF and survival time, indicating disease severity and progression [21,22]. Interestingly, the significant reduction in NfL levels during the recent Tofersen antisense oligonucleotide trial in SOD1-mutated ALS played a crucial role in the drug's approval by the FDA and EMA [23]. The role of NfL as an early treatment response marker in ALS was further confirmed by a real-world study [24]. Regarding its diagnostic value, while elevated serum NfL levels have been reported in presymptomatic carriers [25,26] and in various ALS subtypes compared to healthy controls and ALS mimics [27]. Due to its lack of specificity, its diagnostic value is yet to be determined.

In the recent years, neuroimaging has emerged as a promising biomarker in ALS [28]. Notably, the majority of existing imaging studies in ALS only evaluate the post-symptomatic phase of ALS predominantly centering on brain imaging [29]. Through different imaging techniques, neuroimaging enables the visualization and quantification of structural, functional, and metabolic alterations within the central nervous system of ALS patients. Quantitative structural MRI methods, detect subtle changes in grey matter density and

cortical thickness, found grey matter thinning in ALS patients compared to healthy controls in different brain regions [30-33], characterized of anatomical patterns of propagation [18,34], mapped of resilient brain regions [35], unraveled progressive subcortical degeneration [36,37], explored longitudinal connectivity changes [38], and associated distinct pattern of atrophy with *C9orf72* mutation [39-41]. Another imaging modality, diffusion weighted imaging (DWI), highlights white matter fiber alterations indicating fiber damage, characterized by reduced fractional anisotropy (FA) or/ and increased mean diffusivity (MD), radial diffusivity (RD) of the corticospinal tract [42-45]. Nevertheless, other imaging modalities, such as proton spectroscopy that address neuronal metabolite changes in ALS, found reduction in N-acetyl aspartate level (and its ratio to choline or creatine) and decrease in GABA level in the motor cortex, frontal lobe, brainstem and hippocampus [46-48]. Interestingly, changes in metabolite ratios appear early in the course of the disease process in asymptomatic *SOD1+* people in the cervical spinal cord [49]. Furthermore, ^{18}F-fluorodeoxyglucose positron emission tomography (PET) studies in ALS have reported significant reduction of glucose metabolism in the motor, frontal, and anterior temporal cortices, as well as in the basal ganglia. These reductions were strongly correlated with the duration of the disease, motor function capacity, and cognitive performance [50-53]. Functional MRI (fMRI) has also emerged as a valuable tool in ALS research, providing critical insights into the disease's impact on neural connectivity and brain network function, using a variety of resting-state and paradigm-based approaches [54-62]. These functional activation changes in ALS reflect the clinical manifestations, including motor [59-62], cognitive and behavioral function [56-58], postural deficits [63,64].

Moreover, quantitative spinal cord magnetic resonance imaging MRI emerged as a promising diagnostic and prognostic biomarker avenue in ALS [65]. Especially because spinal cord MRI has the advantage to investigate the two motor system components, UMNs and LMNs, that are involved in ALS [66]. However, at the clinical setting, spinal cord imaging is performed only in the diagnosis phase to visually inspect the structural spinal changes to exclude ALS mimics. At the research setting, technological as well as methodological constraints have precluded reliable quantitative spinal imaging in ALS.

Overcoming challenges in spinal cord imaging

Spinal cord imaging poses significant challenges, primarily due to technical difficulties impacting image quality and quantification of MRI metrics. Moderate MRI resolution causes spinal cord and its immediate surrounding tissue types to contribute to the same voxel, leading to a blurring of tissue boundaries (e.g., between CSF and white matter (WM), white matter and grey matter (GM), CSF and vessels, and WM), resulting in partial volume effects. These effects are particularly concerning when quantifying spinal MRI measures in neurodegenerative diseases due to its thinning [67,68]. Various techniques have been developed to enhance spatial resolution and reduce partial volume effects. These include using higher magnetic field strengths, supported by a growing body of evidence advocating the benefits of ultrahigh field spinal cord MRI [69-71], an increased number of phased array coils with parallel imaging [72,73], and an acquisition protocol employing axial slices with high in-plane resolution and substantial slice thickness [66]. Additionally, the spinal canal's surrounding tissues have varying magnetic susceptibility profiles, creating an inhomogeneous magnetic field that can cause geometric distortions and signal intensity loss. Strategies to improve field homogeneity, such as shimming with shim coils to counteract field inhomogeneities [74]. Parallel imaging, and precise slice positioning, can minimize the effects of magnetic field inhomogeneity [72,75,76].

An important aspect of spinal imaging in ALS is the interpretation of qualitative radiological cues often detected during clinical MRI reviews. For example, high signal intensity along the corticospinal tract on axial views in the lateral columns is frequently observed [77-80]. However, this finding is not specific to ALS and should be interpreted with caution, as it can also occur in conditions such as primary lateral sclerosis and hereditary spastic paraplegia [81-83]. Similarly, the 'owl-eyes' sign—also known as the 'snake-eyes' or 'fried-eggs' sign—characterized by hyperintensity of the anterior horns, must be carefully assessed, as it may also be seen in conditions such as chronic spondylotic myelopathy, cord ischemia, spinal-bulbar muscular atrophy, post-poliomyelitis syndrome, and spinal muscular atrophy [84-86]. Additionally, in younger patients with upper limb-predominant amyotrophy, Hirayama disease should be considered as a differential diagnosis. In these cases, cervical MRI should be performed in both neutral and flexion positions to identify characteristic

findings, such as enlargement of the posterior epidural space in flexion [87]. This underscores the importance of carefully interpret qualitative MRI findings in ALS and considering a broad differential diagnosis.

Motion artifacts, caused by repetitive displacement due to respiration, cerebrospinal fluid flow, cardiac pulsation, and patient movement during MRI, present another significant challenge [66,88,89]. Techniques to mitigate these artifacts include synchronizing acquisition with respiratory or cardiac cycles ('cardiac gating') [73,75], employing saturation bands and cervical collars to restrict signals from moving structures [90], using velocity-compensating gradient sequences, and averaging signals over multiple motion phases [65]. Rapid MRI sequences, such as fast spin echo, parallel imaging, partial Fourier imaging, and reducing the k-space matrix size, have proven effective in diminishing both physiological and subjective motion artifacts [91-93].

Another methodological challenge is the practice of conducting cervical-spine analyses using brain MRI acquisitions [94,95]. While some research groups employ this approach, it is suboptimal as only the very superior segments (C1-C4) can be evaluated, and the clinically relevant spinal segments are typically out of the field-of-view. This limitation underscores the necessity for dedicated spinal imaging protocols in ALS research to ensure comprehensive assessment of the spinal cord, particularly in the clinically relevant regions.

Alongside the challenges inherent to spinal cord MRI acquisition, intrinsic features of ALS complicate the development of reliable biomarkers. These features manifest variably, encompassing age and site of onset, symptom progression, motor neuron involvement, cognitive and behavioral changes, genetic background determinants, underlying pathological mechanisms, and patients' survival. In addition, from a clinical perspective, especially in the interpretation of individual scans, one should also note the potential impact of sex on normative spinal metrics. Sexual dimorphism is well recognized in cerebral imaging and is likely to be an important factor in spinal imaging as well [96,97]. In addition to these factors, recent evidence suggests that body size interacts with the structure of the central nervous system, where body height correlated strongly or moderately with brain GM volume, cortical GM volume, total cerebellar volume, brainstem volume, and cross-sectional

area (CSA) of cervical SC white matter , and should be considered as a biological variable in the design of clinical neuroimaging studies[98].

To address this heterogeneity, larger, homogenized longitudinal MRI datasets are crucial, enabling machine learning and artificial intelligence modelization. However, challenges related to patients' conditions, such as their inability to lie down for prolonged periods and difficulties in traveling, must be addressed. Therefore, enriching study designs to include patients early in their disease course is essential. (**Figure 1**)

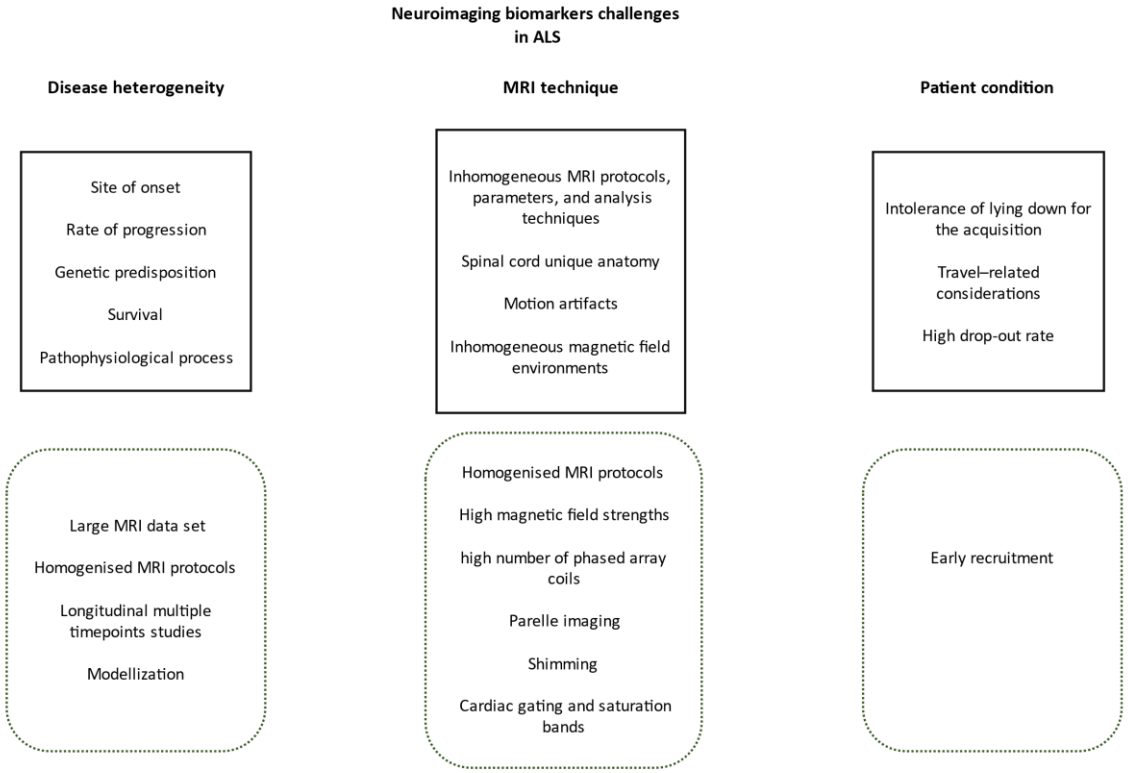

Figure 1. The challenges of spinal cord imaging in ALS biomarker development and Corresponding proposed solutions for overcoming them.

The role of spinal imaging in the development of a biomarker and characterizing the disease pathology

The role of spinal imaging in the development of diagnostic biomarkers in ALS

The spinal cord imaging measure in ALS present a promising avenue in diagnostic biomarker development. Several spinal cord imaging studies have employed semiautomated segmentation techniques to calculate the cord cross-sectional area (CSA). Notably,

structural spinal imaging studies have found that ALS patients had significantly lower CSA at the cervical cord level compared to healthy controls matched for age and sex [99] and have had significant progressive cervical cord atrophy over 6-12 months follow up period [100].

Advances in MRI acquisition techniques and in the processing pipelines combined with development of spinal cord templates [101-104] have enabled an improved contrast and a specific characterization of spinal cord GM and WM impairment. That enabled not only quantifying the global CSA of the spinal cord, but also the CSA of both WM and GM separately. A recent multiparametric spinal imaging study has investigated the GM and WM atrophy using high- resolution anatomical imaging in 10 ALS patients (with predominance spinal onset ALS) compared to 20 matched healthy controls. It has found a reduction in the WM/GM and global CSA along the entire cervical spinal cord, with the highest decrease in the lower cervical spinal levels (C4-C6) [76]. Another longitudinal spinal imaging study, which employed a 3D T2-weighted fast spin-echo sequence investigated the GM CSA at the vertebral level C4-C6 in 25 ALS patients over a one-year follow-up period, in comparison to 22 healthy controls. The study found that baseline GM CSA served as a more sensitive marker of atrophy, compared to the global spinal cord CSA.

Diffusion tensor imaging (DTI) is also a promising imaging technique that measures the diffusion of water molecules in tissues and allows the assessment of microstructural changes in white matter. DTI metrics, such as FA, MD, axial diffusivity (AD) and RD, (**Figure 2**) provide valuable insight into pathological changes in the spinal cord, such as degeneration, loss of structural integrity, axonal damage and demyelination [105].

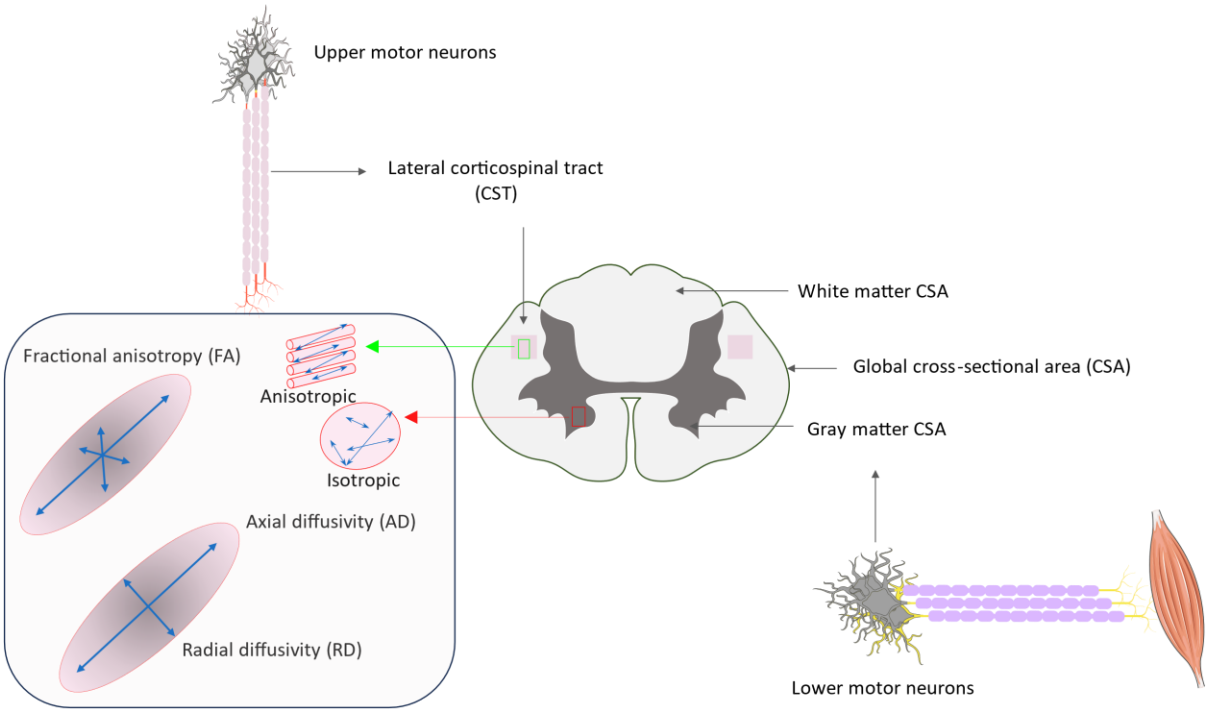

Figure 2. An illustration of the quantitative spinal structural and diffusional metrics

A spinal DTI study have investigated the cervical spinal cord has shown significantly lower FA in the cervical cord compared to healthy controls [99]. A further DTI spinal imaging study have investigated the longitudinal diffusional alteration of the cervical spinal cord over a nine-month follow-up have shown significant decrease FA and augmentation of MD in ALS patients [100]. More precisely, with dedicated template-based analysis, a recent multimodal spinal MRI study including a DTI protocol investigated microstructural diffusional changes at the C2 and C5 vertebral levels in the whole WM, bilateral corticospinal tract (CST), dorsal columns, and GM. At the C2 level, significantly lower FA in the CST and whole WM, lower AD in the CST, dorsal columns, and whole WM, and higher RD in the whole WM in ALS patients compared to healthy controls were found. At the C5 level, significantly lower FA in the CST and whole WM and higher RD in the whole WM were found [76]. Moreover, other DTI spinal studies have reported similar diffusional changes in DTI metrics characterized by a decrease in FA and an increase in MD in CST [106,107]. Interestingly, RD and FA have held the highest discriminatory effect between ALS patients and healthy controls [108]. Notably, few imaging studies found that spinal changes in ALS patients can manifest independent of cerebral changes, reinforcing the importance of spinal imaging in recording ALS pathological changes [100,109] .

Interestingly, spinal quantitative MRI measures at the cervical level were able to detect WM atrophy at each cervical vertebral level from C2-C7 in asymptomatic ALS patients who were carriers of the G4C2 pathogenic expansion compared to non-carriers. At the 18-month follow-up from baseline, the same study reported progressive degeneration evidenced by the progressive reduction in FA [110].

The quantitative structural and diffusional changes in the spinal cord linked to ALS could be crucial in establishing early diagnostic biomarkers. This is especially salient given the unique opportunity to track clinical indicators of ALS, such as investigation of both components of the neuroaxis, the upper motor neurons (UMN) and the lower motor neurons (LMN). Further advancements, with the introduction of 7 Tesla MRI systems that allow for higher cross-sectional resolution of 0.37 × 0.37 mm^2, compared to ranges from 0.5 to 1 millimeter (mm) in-plane.with 3 Tesla MRI, are believed to better quantify MRI measures. This will enable the development of more robust biomarkers [70,111].

Furthermore, the use of newer imaging modalities such as Positron Emission Tomography (PET) has emerged. A study by demonstrated a significant increase in ^{18}F Fluoro-Deoxy-Glucose uptake in the spinal cord, particularly in the cervical segment of ALS patients [112]. This evidence of alterations in spinal cord metabolism opens up new avenues for potential biomarkers.

Despite this progress, several gaps and challenges remain. Identifying specific biomarkers for heterogenous ALS clinical phenotypes and different stages of the disease require large homogenized cohort and the inclusion of ALS mimics. For instance, the spinal imaging study revealed that segmented analysis at different cervical vertebral levels can detect early grey matter atrophy, starting 7 to 20 months before symptom onset and suggest that the cervical atrophy spreads from GM to WM across King's stages in ALS [113]. However, due to the technical difficulties, yet thoracic and lumbar region of the spinal cord are to be investigated. Additionally, the cause-effect relationships between these imaging findings and the ALS pathophysiology itself remain to be elucidated.

The role of spinal cord imaging in disease monitoring and prognostication

Quantitative MRI spinal imaging addressed changes in the architecture and integrity of the spinal cord for their promising potentials as prognostic biomarker that could indicate a clinical significance for clinical trials and daily practice. There is a strong consensus in the spinal Imaging research community about reductions in CSA in ALS patients [99,100,114,115]. A spinal cord imaging study, investigated the cervical cord at the vertebral level of C2/C3, found a significant correlation between spinal cord atrophy and functional impairment, as measured by the ALSFRS-R [114]. Another spinal imaging study found a significant correlation between the cord CSA at the level from C2-T2 and the manual muscle testing scores, as well as the ALSFRS-R arm sub-score. In a 12-month longitudinal spinal imaging study, a significant monthly reduction of 0.14 mm^2 in CSA correlated significantly with changes in ALSFRS-R and manual muscle testing arm sub-scores [116]. Interestingly, a multimodal study compared clinical variables and MRI metrics found that MRI measurements, particularly the reduction in CSA at the C3/C4 and C5/C6 levels, were more predictive of survival than the ALSFRS-R, muscle strength, and disease duration [117].

Most morphometric spinal cord imaging studies in ALS have predominantly investigated the global CSA of the spinal cord [100,107,114,116]. Only a few studies have separately quantified GM/WM CSAs, leveraging the enhanced GM/WM contrast and higher resolution offered by MR imaging [76,113,118]. The observed GM atrophy was found to probably reflect the degeneration LMNs [76]. A recent study suggested that the cord atrophy in ALS spread from GM to WM. Specifically, it found that cord atrophy in patients at King's stage 1 were mainly identified in the GM, while cord atrophy in those at King's stage ≥2 started to involve the WM [113]. And GM and WM atrophy at the levels from C2-C6 correlated with ALSFRS-R [113]. Furthermore, using 2D Phase-Sensitive Inversion Recovery imaging, a significant reduction in GM area at the level from C2-T1 was identified in ALS patients compared to healthy controls [118].

The atrophy of the spinal cord, whether in the gray matter (GM), white matter (WM), or overall, was observed in ALS compared to healthy controls [76,118]. This observation holds promising potential for investigation in presymptomatic patients, as increasing evidence of a long presymptomatic phase suggests that the current evidence from symptomatic ALS only

provides a snapshot of decades of progressive neurodegenerative change [29,119-121]. which investigation could help to better identify the cut-off for phenoconversion and facilitate the establishment of early treatment for certain genotypes. Moreover, it could contribute to establishing a reliable biomarker for response to therapy.

Basic metrics derived from DTI have been significantly altered in ALS patients [99,100,113,122]. Notably, these metrics demonstrate correlations with disease severity indicators. For instance, baseline FA in the cervical spinal cord exhibits a strong correlation with the ALSFRS-R [99,107]. Similarly, both baseline FA and RD in the cervical spinal cord, from the vertebral level C2 to C6, correlate with finger and foot tapping speeds [122]. Additionally, DTI metrics of the corticospinal tract (CST) at the C2 vertebral level are associated with ALSFRS-R, disease duration, and muscle strength [76].

Building upon the spinal imaging findings, recent findings have further highlighted the prognostic capabilities of MRI parameters in ALS, particularly in terms of predicting patient survival and respiratory function. MRI parameters have shown a notable predictive capability regarding patient survival, surpassing traditional clinical variables such as ALSFRS-R, manual muscle testing, and disease duration [117]. An important observation was made from analyzing changes in cervical spinal cord volume the diagnosis and at a 3-month interval, where a decrease in spinal cord volume correlated with the changes observed in slow vital capacity after a year [123]. Interestingly, a recent study suggested the added prognostic value of incorporating brainstem and spinal cord imaging. it found that a model combining age at onset, sex, spinal cord diameter, and brainstem volumes significantly enhances the accuracy in predicting the need for NIV within six months of diagnosis [124]. Collectively, these developments mark significant progress in understanding how spinal imaging biomarkers may contribute to the prognosis of ALS.

The contribution of spinal cord imaging to the understanding of ALS pathogenesis

The contribution of spinal imaging studies, particularly those focusing on longitudinal changes in quantitative MRI measures of the spinal cord in ALS, underscores the progressive nature of this condition and aligns with the concept of disease propagation [94,100,115]. While ALS has traditionally been viewed as a disease primarily affecting motor neurons, recent

imaging studies at the brain and spinal cord levels suggest that it may also involve significant alterations in somatosensory pathways [107,125,126]. Additionally, cerebellar involvement in ALS has gained increasing attention [127], but only cerebro-cerebellar connectivity has been comprehensively studied [128]. However, spinocerebellar tracts, which could provide critical insights into the neurodegenerative processes in ALS, have not yet been adequately studied. This emerging area of cerebellar imaging presents a new frontier for ALS research. The direction of the process of neurodegeneration in ALS, as suggested by previous experimental studies, neurophysiological findings, and postmortem literature, is hypothesized to occur in one of two directions: top-down ('dying forward') [129-131] or bottom-up ('dying backward') [122,132-134]. It is important to note that these hypotheses cannot be investigated through histopathological studies, as these studies are typically conducted in the later stages of the disease.

Despite the significance of neuroimaging in investigating the disease process distribution, only a limited number of imaging studies have explored quantitative MRI measures at both brain and spinal cord levels to investigate this phenomenon [99,100,115,135]. A cross-sectional study found a moderate correlation between corticospinal tract fiber coherence, characterized by lower FA, in both brain and cord portions [99]. A longitudinal study observed significant baseline GM loss in motor areas, including paracentral, precentral, and temporal regions, as well as spinal cord atrophy, when compared to healthy controls. Over an 8-month follow-up period, there was a significant progressive reduction in both brainstem volume and cervical spinal cord area [115].

Another longitudinal imaging study observed significant alterations in cervical spinal cord measurements over a 9-month follow-up period. These changes were characterized by a reduction in cord area and FA, coupled with an increase in MD. In contrast, intracranial CST diffusivity measures remained unchanged [100]. Conversely, other spinal imaging studies addressing the directionality of neurodegeneration by analyzing multiple vertebral spinal level DTI and magnetization transfer ratio suggested a larger degeneration toward caudal segments, supporting the dying backward hypothesis [107,122].

Neurodegeneration in ALS develops over many years before symptoms appear. Most imaging studies is conducted after symptoms start, when big changes have already occurred

[29,136]. Therefore, studies conducted at the presymptomatic phase are particularly important to develop sensitive quantitative monitoring markers that are required to detect pathological progression and response to therapy [29,136]. Only few studies have described the MRI changes in presymptomatic phase in ALS patients with specific genetic mutations [137]. A presymptomatic cerebral imaging study described a series of cortical and subcortical changes up to 10 years before the anticipated onset of symptoms in individuals with FTD related to certain genetic mutations [137].While presymptomatic cerebral imaging study has revealed that gray matter atrophy and white matter microstructural alterations, can be discerned in *C9orf72* carriers up to 25 years, particularly in individuals younger than 40 years, before the age of onset of symptoms of their relevant [138], a spinal cord imaging study conducted on the same cohort found that WM atrophy was exclusively observed in presymptomatic C9orf72 carriers older than 40 years [110]. Which in turn suggest the corticofugal propagation of the disease.

Conclusion and futures avenues

In conclusion, spinal cord imaging holds promising potential for the advancement of a biomarker in ALS. This is also can be further supported with the introduction of ultrahigh filed MRI and particularly noteworthy is the ongoing development of automated open-source software, such as the Spinal Cord Toolbox, to overcome part of technical hurdles.

However, it's important to emphasize that spinal cord imaging should not be viewed in isolation but as part of a comprehensive, multimodal approach to biomarker development. The synergy between spinal cord imaging and these diverse biomarkers can lead to a multi-faceted view of the disease, improving diagnostic accuracy, monitoring disease progression, and evaluating therapeutic responses.

Future research directions should focus on validating these potential biomarkers in larger cohorts and across different stages and subtypes of ALS in a longitudinal studies design. Moreover, research can benefit from combining these imaging biomarkers with clinical variables and molecular markers to improve patient care and therapeutic clinical trial design.

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