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REVIEW

Neuroimaging in Amyotrophic Lateral Sclerosis: Magnetic resonance techniques

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Abstract

Objectives: Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative motor neuron disease whose clinical presentation and evolution varies greatly among patients. The limited role of conventional Magnetic Resonance Imaging in ALS encourages the study of new advanced Magnetic Resonance Imaging (MRI) techniques. Our aim was to review the advances of MRI techniques applied to ALS and to analyze their contribution to the knowledge and monitoring of this disease.

Methods: We performed a Pubmed® database search with the following MeSH terms: "Amyotrophic Lateral Sclerosis", "Neuroimaging", "Magnetic Resonance Imaging". Articles from the last decade were preferentially included but previous important publications were added.

Results: Sixty-eight articles were considered in our revision article. Imaging methods were categorized as structural, functional and metabolic and their usefulness in detecting ALS related damage in brain and spinal cord was evaluated.

Conclusion: Advanced MRI techniques consistently prove that ALS is a multisystem disease, involving both motor and extra-motor neuronal areas. Primary motor cortex, corticospinal tract and corpus callosum are major regions involved, but several temporal, frontal and subcortical areas are also affected in ALS, as well as some neuronal networks related to sensoriomotor activity and cognition. Advanced neuroimaging techniques are providing a unique opportunity to study ALS and its underlying pathophysiology and disease course, which may enable the discovery of novel ALS drug targets.

Keywords: Amyotrophic Lateral Sclerosis, Neuroimaging, Magnetic Resonance Imaging.

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Introduction

Motor neuron diseases (MND) are a group of pathologies in which motor neurons are selectively affected. Amyotrophic lateral Sclerosis (ALS) is a member of this group, affecting both upper (UMN) and lower motor neurons (LMN) [1].

ALS is a progressive neurodegenerative disease [1] with an estimated incidence in caucasians of about 1.2-4.0 per 100,000 person-years [2]. ALS incidence increases with age, being more frequent between the 6th and 7th decades and in women more than men [3].

ALS aetiology is unknown in most cases; it is inherited in only 5–10% of cases (roughly one half have SOD1, TARDBP and C9orf72 mutations) [1]. Similar mutations have been sporadically found in non-hereditary forms [1].

The diagnosis of ALS relies mostly on its clinical characteristics, electromyography findings and imaging techniques. Nowadays, neuroimaging is mainly used to exclude mimic disorders [4, 5]. Despite conventional brain Magnetic Resonance Imaging (MRI) may show T2 motor cortex hypo-intensity [6, 7] and corticospinal tract hyper-intensity [8], these findings have low sensitivity and specificity for the diagnosis of ALS [9].

ALS clinical presentation is characterized by the presence of LMN signs (fasciculation, muscle atrophy and progressive muscle weakness) and UMN signs (spasticity and hyperreflexia) [1]. Usually ALS signs and symptoms are firstly observed on patients limbs, but dysarthria and dysphagia, may also be initially present, especially in those with bulbar-onset [1]. Besides these neurological findings, cognitive and behavioural deficits may be present. Indeed 50% of ALS patients have some kind of cognitive impairment in neuropsychological tests, while others (15%) have overt frontotemporal dementia [10]. To evaluate the level of functional impairment the Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) is frequently used. It measures physical impairment during daily life activities and is useful either in clinical practice as in clinical trials [11].

In spite of the well-recognized clinical features of this disease, ALS diagnosis, due to its significant clinical heterogeneity in its early stages, is mostly made 10–18 months after symptoms' onset [12].

Presently there are no specific therapeutic strategies that can completely halt the disease course. Riluzole, an anti-glutamatergic drug that acts by blocking NMDA receptors, is proven to reduce the excitotoxicity in ALS, modestly improving the prognosis [13]; its combination with multidisciplinary care and respiratory support can slow clinical deterioration and improve life quality [1]. Regardless of these therapeutic interventions, only a minority of patients (5–10%) survive for a decade or more: ALS life expectancy is usually less than 3 years and respiratory failure is the main cause of death [14, 15].

Currently there are, also, no well-established biological markers of progression or prognosis, even though older age and bulbar-onset are considered factors which lead to worse prognosis [16].

In the last twenty-years an effort has been made to clarify pathophysiological mechanisms and to find new diagnostic methods in order to improve ALS dramatic prognosis [17]. New neuroimaging techniques, allowing investigation of anomalies in micro-structure, biochemistry and neuronal networks in brain and spinal cord [17], have been uncovering important data about ALS pathophysiology.

The aim of this review is to present the advances of MRI techniques applied to ALS and to analyse their contribute to the knowledge and monitoring of this disease.

Methods

We performed a Pubmed® database search for the following MeSH terms "Amyotrophic Lateral Sclerosis", "Neuroimaging" and "Magnetic Resonance Imaging", and included research papers from the last decade that had an available English abstract. Case reports and animal experiments studies were excluded. We also included further articles, namely older publications with high relevance. The authors used Endnote X7® for bibliography management.

Results

After selection by title and abstract, and full text analysis we considered 68 articles in our revision article. For better understanding, we are going to present advanced brain MRI techniques results in three major categories – structural MRI, functional MRI, MRI spectroscopy; spinal cord imaging will be briefly mentioned at the end (**Table 1**).

Structural MRI

Whole brain analysis techniques have been developed to quantify and segment grey and white mater morphology with T1-weighted images. These advanced techniques are surface and voxel based morphometry. The first is known as "cortical thickness measure" because it allows decomposition of cortical volume into both thickness and surface area and respects cortical topology [18]. The second, voxel base morphometry, allows the regional assessment of grey-matter (GM) and white matter density (WM) [5]. Other recent techniques include tensor based morphometry (TDM), diffusion-tensor imaging (DTI), q-ball imaging (QBI) and quantitative susceptibility mapping (QSM). TDM is designed to obtain a map of tissue loss over time [19], demonstrating a good potential to access the atrophic longitudinal changes in motor and extra-motor areas. DTI, based on the diffusion of water molecules, allows the study of the neuronal tract integrity and can be evaluated via indices such as mean diffusivity (MD), a measure of the magnitude of diffusion, and fractional anisotropy (FA), that quantifies the preferential direction of water diffusion along fibre tracts, reflecting the degree of alignment of cel-

Table 1. Description of the MRI advanced techniques.

Advanced analysis MRI techniques		Description	Variables of study
Structural MRI [5]	Surface Based Morphometry (SBM) [17]	Enables detailed structure analysis through acquisition of high resolution tree dimensional imagens at 1mm in each dimension [17]	(SBM) Cortical Thickness
	Voxel Based Morphometry (VBM) [17]		(VBM) Grey and white matter volume loss
	Magnetization Transfer (MT) [29]	Capable to detect microstructural neurodegeneration in the cerebral cortex, like intra or extracellular matrix alterations, gliosis and axonal density-changes.	Microstructural neurodegeneration
	Tensor Based Morphometry (TBM) [19]	Allows mapping of tissue loss over time so with good potential to access the atrophic longitudinal changes.	Tissue loss measuring
	Diffusion tensor Imaging (DTI) [20]	Pretends to detect abnormal structure of the SNC through evaluation of the diffusion behaviour of water molecules. When neuronal tissues are damage mean diffusivity will increase and fractional anisotropy decreases	Mean Diffusivity (MD), measure of the magnitude of water diffusion through the tissues. Fractional Anisotropy (FA) a measure of the preferable axis of water diffusion
	Q-ball Imaging (QBI) [21]	Fiber tracking package, using a multi-tensorial model	Tract length; Fiber volume and density; Generalized fractional anisotropy
	Quantitative susceptibility Mapping (QMS) [22]	Method designed to distinguish paramagnetic species (such as iron) from diamagnetic species (such as calcium) since the first ones appear hyper-intense and the others hypo-intense.	Hyper-intensity of tissues
Function MRI [5]	Task-associated	Based on a contrast imaging blood oxygenation level-dependent (BOLD) during a given external stimulus/task called Task-associated fMRI or by called Resting-state fMRI	Activity pattern
	Resting state (rs-fMRI)	Assesses spontaneous fluctuations in BOLD mapping neuronal circuits at a resting state point	Functional Connectivity (FC)
Magnetic resonance imaging spectroscopy (MRI-S) [18]		MRI-S allows quantification of cerebral tissue metabolites in a non-invasive manner.	Several tissue Metabolites
Spinal Cord MR [18]		Study of spinal cord anomalies with the same techniques used in the brain such as structural MRI, DTI and MRS.	Morphology and biochemistry of spinal cord

lular structures [20]. Because pathological processes reduce the barriers that restrict the movement of water molecules, as a consequence, MD increases and FA decreases. QBI, unlike DTI, uses a multi-tensorial model [21] to assess WM tracts microscopic and macroscopic characteristics. Finally, QSM is a newer imaging technique that quantifies magnetic susceptibility of tissues, making it capable to distinguish paramagnetic species (such as iron, which appear hyper-intense) from diamagnetic species (such as calcium, which appear hypo-intense) [22].

Surface-based (SBM) and voxel-based MRI morphometry (VBM)

Surface based MRI morphometry studies suggest that Primary Motor Cortex (PMC) thinning is present in ALS patients and might be a sensitive marker of diagnosis and prognosis, since PMC and temporal lobe accelerated thinning correlates with rapidly progressive disease [23]. Moreover, frontotemporal and parietal thinning is also observed in ALS patients, even with normal cognitive functions, being more severe in those patients with cognitive impairment and FTD [24].

Studies using Voxel-based MRI have shown ALS related significant GM loss in several regions of the frontal lobe, especially PMC [25]. Further VBM studies revealed that GM loss extends to premotor cortex, frontal and temporal gyrus and occipital regions even in mildly disabled patients [26, 27]. The Magnetization Transfer (MT) imaging is capable to detect intra- or extracellular matrix alterations, gliosis and axonal density-changes, making it more sensitive to early structural damage, not so well detected by VBM weighted on T1 [28]. Through association of MT to VBM several neurodegenerative changes in premotor and some extra-motor frontotemporal areas,

also helps supporting the hypothesis of a multi-structure involvement in ALS [29].

For a better understanding of ALS mechanisms correlation between clinical parameters and imaging data is important. Both SBM and VBM anomalies have positive correlation to clinical signs: UMN bulbar signs correlate with thinning on bulbar segment in the motor cortex, and limb UMN signs correlate with thinning at the limb segment in the motor cortex [27, 30]. Further evidence is given by left inferior primary motor cortex thinning, in SBM, correlated with worse ALSFRS-R [30].

Tensor based morphometry (TBM)

Progression of GM atrophy was found in motor regions but also in other cortical and subcortical motor areas and extra-motor frontal regions, supporting other imaging techniques findings [31]. Few studies have been made using this technique, however its peculiar ability to show longitudinally altered imaging patterns may be useful for monitoring disease progression and therapeutic strategies [31].

Diffusion Tensor Imaging (DTI)

Post-mortem histopathological studies have demonstrated widespread cerebral white matter tract damage in ALS and this alteration can now be detected non-invasively using diffusion tensor imaging [18].

DTI uses several analysis strategies; one is voxelwise analysis with Tract-Based Spatial Statistics (TBSS) [32, 33]. DTI with TBSS shows widespread corticospinal tract [34], corpus callosum and posterior limb of internal capsule damage [35, 36]. Moreover, reduction in FA in the corticospinal tract correlates with disease progression [37].

A number of alternative techniques have been proposed to improve WM analysis. Since WM is organized as individual tracts, TBSS has poor ability to distinguish certain adjacent white matter tracts, presenting low anatomical specificity [38]. Tract-Specific Analysis (TSA), may help to overpass this issue, due to the ability to construct skeletons for individual tracts so each single white matter tract can be analysed. With this technique it is also possible to assess macroscopic properties of tracts (like tract size and shape) along with the usual microscopic features, FA and MD. TSA supports the findings of TBSS that are reduced corticospinal tract connection to PMC and primary somatosensory cortex along with reduced thickness in the internal capsule of the corticospinal tract [38] .

Few DTI studies have investigated alterations of cortical and subcortical GM structures. The performed studies show that several subcortical circuits, especially thalamus, amygdala and hippocampus have increased MD values, suggesting microstructural damage in many subcortical areas that may be related to behavioural impairment [39].

Studies searching for GM and WM changes have shown degeneration involving both the widespread cortices and the underlying WM fibres [37]. One example is the combined longitudinal assessment through VBM and DTI techniques which revealed damage in frontotemporal lobe and the adjacent white matter [26, 40].

Q-ball imaging (QBI)

QBI is another tract analysis method that may give new diagnostic and prognostic clues [21]. Studies with this technology show decreased fibre density and volume, and increased tract length in corpus callosum and left corticospinal tract, supporting the callosal involvement as a consistent feature of most ALS variants [21]. Besides, callosal involvement is significantly related to both pyramidal dysfunction and disease disability [21].

Quantitative Susceptibility Mapping (QSM)

The few studies made with this technique show that QSM has a greater diagnostic accuracy for ALS when compared to T2-weighted conventional MRI imaging [22]. Its ability to distinguish paramagnetic (iron) from diamagnetic (calcium) species gives to this technique an advantage in earlier detecting ALS cortical related anomalies; thus it may be used as a supplement to the MRI evaluation in clinical practice [22].

Functional MRI (fMRI)

This neuroimaging method is based on a contrast imaging blood oxygenation level-dependent (BOLD) [18]. There are two main types of fMRI: task-associated fMRI, if associated with an external stimulus/task and resting-state fMRI, if the focus is on the evaluation of spontaneous fluctuations in BOLD [5].

Task associated fMRI

When asked to perform a simple finger flexion task, ALS patients have cortical and subcortical regions of increased activation [41]. Basal ganglia, brainstem, and cerebellum are some of these areas and this hyper-activation pattern has been associated to recruitment of subcortical motor structures to compensate cortical and spinal motor neurons loss [42]. Also, when evaluating the regions of hyper-activation in non-primary motor cortices, with VBM, they appear atrophic due probably to loss of inhibitory interneurons [43]. Further conclusions, in this field, were taken in longitudinal studies: in the initial stages, ALS patients have hyper-activation of primary sensorimotor area, premotor cortices, supplementary motor area, cerebellar and basal ganglia regions. With disease progression, after 3 months, a decrease activity pattern begins to appear in sensoriomotor cortex (SMC), which correlates with patient clinical status [44]. The main explanation for this changing in activation pattern is that, in early disease development, a compensatory hyper-activation and recruitment of additional areas is dominant, but ongoing neurodegeneration causes compensatory mechanisms to breakdown resulting in decreased activity levels and clinical impairment [44, 45] .

fMRI may help distinguish different ALS phenotypes that are probably associated with different neurodegen-

erative patterns. In task-associated studies during vertical tongue movements, patients with bulbar signs showed significant decrease in cortical activation, but not in patients without bulbar signs. Besides, during the hand movement there was an increased cortical activity, regardless of site of onset and presence of bulbar signs [46]. Therefore, different pathophysiological mechanisms are present in patients with and without bulbar involvement. Other studies also show similar results [47] but further evidence is needed.

Traditionally considered as a neurodegenerative disease selectively affecting the motor-system, ALS is now conceptualized as a multisystem disorder also affecting emotional and cognitive domains [5]. Alteration in the processing of emotional stimuli is supported by fMRI when presenting affective pictures to ALS patients [48]. Longitudinal assessment of cognitive functions shows hippocampal hyperactivity when no memory impairments are present, but in later stages hippocampal dysfunction arises, presenting hippocampal hyperactivity as a possible compensation process to overcome early cognitive lesions, that present as hippocampal dysfunction arises [44].

Resting state fMRI

Resting-state functional MRI (rs-fMRI) evaluates temporally correlated spontaneous low-frequency fluctuations in BOLD MRI signal, originated from several widespread functional distinct networks [49]. Since no task is imposed on the subject, changes in the network cannot mean difficulty to perform a task. Besides, it allows investigation of several networks at the same time. One of that networks is the default mode network (DMN) which is conceptualized as a stand-alone cognitive network, most active when individuals are left to think to themselves and during mental explorations like remembering, consider hypothetical social interactions, and thinking about his own future [50]. Another often reported network is the sensorimotor network (SMN), important to action–execution, comprising supplementary motor area, SMC, and secondary somatosensory cortex [51].

As concerns motor network, no altered connectivity is found in early ALS stages, but in later ALS stages several either increased or decreased Functional Connectivity (FC) areas are found[50]. In SMN, pre-motor area has decreased FC and in DMN widespread diminished FC was found, especially in lateral prefrontal cortex, posterior cingulated cortex and inferior parietal cortex [52]. This last discovery means that decreased FC is present at the "core hub" of DMN. DMN regions appear to be responsible for working memory, tasks of sustained attention, problem solving, perception, recognition and recall of written language and this finding may explain the reason for impairment of high level executive functions in ALS [53]. Nevertheless, DMN decreased FC is not a consistent feature. In other studies, some frontal areas and left precuneus, areas within the DMN, as well as fronto-parietal network (source of attentional control) show increased FC [54, 55] and this finding may be justified by compensatory activity to maintain cognitive performance. Further support of this idea is given by the observation of left medial frontal gyrus activity negative correlation with disease progression rate [55], and parietal increased connectivity positive correlation with better executive functions [54]. In contrast, increased cerebellum-superior parietal lobule FC is related to higher disease severity, showing the need for more studies assessing the correlation of resting FC with clinical disease progression indexes [50].

DTI and rs-fMRI combined allows the assessment of FC in patients with or without damage of the corticospinal tract. Patients with no corticospinal tract damage in DTI have increased functional connectivity to left SMC. On the contrary, if DTI detects damage in corticospinal tract, FC is decreased in some connections to SMC [56]. Besides, reduced FC in SMC was related to disease severity, supporting that increased FC plays a compensating role for (limited) structural damage and might exhaust with disease progression [56]. Similar studies support coupling of structural and functional degeneration [57].

Magnetic resonance imaging spectroscopy (MRI-S)

MRI-S allows quantification of cerebral tissue metabolites non-invasively [18]. The evaluated parameters include, among others, N-acetylaspartate (NAA), a marker for neuronal function, total creatine (Cr), that reflects cellular energy reserves and total Choline (Cho), an indicator of cell membrane turnover [58]; Decreased NAA/Cho ratio is consistent with neuronal dysfunction [59] and NAA/Cr ratio may act as a biomarker of neuronal integrity [60]. This indexes have been the most widely studied due to their simple spectral pattern and high concentrations in CNS [18].

Quantification of cerebral metabolites in motor cortex of ALS patients reveals reduced concentrations of NAA and reduced NAA/Cho and NAA/Cr ratios probably meaning neuronal dysfunction and loss of neuronal integrity [61]. Moreover, the levels of these metabolites drop out over time in correlation with clinical impairment, possible serving as good prognosis markers [61, 62]. Several extra-motor areas, show decreased NAA/Cr and NAA/Cho ratios reinforcing the already mentioned theory that ALS is a multi-structure disorder of the brain [58].

Spinal cord MRI

Dying-back theory suggests that early degeneration is more likely to be captured at the spinal anterior horn rather than in the brain [63]. The pitfall is that imaging of the spinal cord can be difficult because of technical details, especially because of small dimensions of cross-sectional area. Notwithstanding, advanced MRI methods have been very useful in the study of this structure.

In morphometric studies, positive association between muscle deficits and local spinal cord atrophy was found, suggesting that atrophy may be a sensitive biomarker for LMN degeneration [64].

DTI parameters from individual cervical segments support dying-back theory, since in early ALS, more distal segments have larger anomalies comparing to proximal [65] and, in longitudinal evaluations, FA reduces and MD increases [66], which can serve as biomarkers of ALS progression.

In MRI-S of spinal cord, pre-symptomatic carriers of SOD1 mutations show reduction in NAA/Cr ratio and in myosinositol concentrations [67] raising potential for early detection of the disease. These indices are also positively correlated with ALSFRS in ALS patients; therefore they can serve as prognosis biomarkers [68].

Discussion

Primary motor cortex appears to be, as anticipated, one of the most affected brain regions in ALS degeneration. Thinning and loss of grey matter at several motor areas, specially primary motor cortex are consistent findings in SBM, VBM, MT and TBM studies [23, 25, 29, 31]. Corticospinal tract and corpus callosum degeneration are also an evident feature in DTI and QBI [21, 34-36, 38]. Besides, MRI-S demonstrates neuronal degeneration through demonstration of decreased levels in certain cerebral and spinal cord metabolites [61, 62, 68].

Until now ALS is seen as a typically motor related disease, however, neuroimaging findings also support extra-motor involvement in ALS, with thinning and reduced density in several frontal and temporal regions when structural imaging techniques are applied [24, 26, 27, 29, 31], and altered diffusion pattern in thalamus, amygdala and hippocampus (subcortical areas) [39]. Moreover, abnormal activity in hippocampus [44] and during emotional stimulus [48] and at resting state in DMN, SMN and frontoparietal network [52, 54, 55] are also consistent findings. Abnormal metabolites concentrations in several and widespread cortical regions also support extra-motor involvement [58].

Neuroimaging, especially functional studies, also supports current ALS pathophysiology knowledge. In an early disease stage, atrophy corresponds to loss of inhibitory interneurons, by a not well established cause, resulting in a hyper-activation of cortical and subcortical areas in a compensatory manner; as the disease progresses ongoing neurodegeneration causes compensatory mechanisms to breakdown resulting in decreased activity levels and clinical impairment becomes more evident [42, 44, 45, 54-56].

Some potential disease progression biomarkers can be appointed: cortical thinning in PMC and temporal lobe [23], reduced FA in corticospinal tract [37], reduced concentrations of NAA and reduced NAA/Cho and NAA/Cr ratios in PMC [61, 62]; at spinal cord altered MD and FA [66], and reductions in NAA/Cr ratio and myosinositol concentrations [68]. Likewise, frontotemporal and parietal thinning might be a marker for development of cognitive dysfunction [24] and NAA/Cr ratio and myosinositol reduction at MRI-S of spinal cord are potential markers for screening and early detection of ALS familiar forms [67].

Conclusion

Advanced neuroimaging techniques provide an unique opportunity to study ALS noninvasively. They contribute to the knowledge on ALS about its pathophysiology, motor and extra-motor systems involvement, disease course, and may improve early stage disease diagnosis.

In the future we hope that MRI advanced techniques in ALS may be easily integrated into routine clinical practice in a multimodal approach enhancing our capacity to enrol patients at earlier stages of the disease in clinical trials. Additionally, further research in this field will help clarify new targets for pharmacotherapy development.

Abbreviations

ALS: Amyotrophic lateral Sclerosis; ALSFRS-R: Amyotrophic Lateral Sclerosis Functional Rating Scale; Cho: Choline; Cr: Creatinine; DMN: Default mode network; DTI: Diffusion Tensor Imaging; FA: Fractional anisotropy; fMRI: Functional Magnetic Resonance Imaging; GM: Grey-matter; LMN: lower motor neurons; MD: Mean diffusivity; MND: Motor neuron diseases; MRI: Magnetic Resonance Imaging; MRI-S: Magnetic resonance imaging spectroscopy; MT: Magnetization Transfer; NAA: N-acetylaspartate; PMC: Primary Motor Cortex; QBI: q-ball imaging; QSM: Quantitative susceptibility mapping; rs-fMRI: Resting-state functional MRI; SBM: Surface-based; SMC: sensoriomotor cortex; TBSS: Tract-Based Spatial Statistics; TBM: Tensor based morphometry; TDM: Tensor based morphometry; TSA: Tract-Specific Analysis; UMN: Upper motor neurons; VBM: Voxel-based MRI morphometry; WM: White matter

Competing interests

The authors declare no conflict of interest.

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