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Advances in the application of MRI to amyotrophic lateral sclerosis

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Abstract

Importance of the field—With the emergence of therapeutic candidates for the incurable and rapidly progressive neurodegenerative condition of amyotrophic lateral sclerosis (ALS), it will be essential to develop easily obtainable biomarkers for diagnosis, as well as monitoring, in a disease where clinical examination remains the predominant diagnostic tool. Magnetic resonance imaging (MRI) has greatly developed over the past thirty years since its initial introduction to neuroscience. With multi-modal applications, MRI is now offering exciting opportunities to develop practical biomarkers in ALS.

Areas covered in this review—The historical application of MRI to the field of ALS, its state-of-the-art and future aspirations will be reviewed. Specifically, the significance and limitations of structural MRI to detect gross morphological tissue changes in relation to clinical presentation will be discussed. The more recent application of diffusion tensor imaging (DTI), magnetic resonance spectroscopy (MRS), functional and resting-state MRI (fMRI & R-fMRI) will be contrasted in relation to these more conventional MRI assessments. Finally, future aspirations will be sketched out in providing a more disease mechanism-based molecular MRI.

What the reader will gain—This review will equip the reader with an overview of the application of MRI to ALS and illustrate its potential to develop biomarkers. This discussion is exemplified by key studies, demonstrating the strengths and limitations of each modality. The reader will gain an expert opinion on both the current and future developments of MR imaging in ALS.

Take home message—MR imaging generates potential diagnostic, prognostic and therapeutic monitoring biomarkers of ALS. The emerging fusion of structural, functional and potentially molecular imaging will improve our understanding of wider cerebral connectivity and holds the promise of biomarkers sensitive to the earliest changes.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is the commonest clinical form of the wider neurodegenerative syndrome encompassed by the term motor neuron disease (MND) ¹. ALS has a consistent incidence of 1-2/100,000/year, and a lifetime risk estimated at 1 in 400. The condition is characterized by the progressive death of upper motor neurons (UMNs) of the cerebral primary motor cortex (PMC) and corticospinal tract (CST), in combination with degeneration of lower motor neurons (LMNs) whose origins lie in the brainstem and spinal anterior horns. Loss of motor neurons in general results in weakness, but specifically loss of

LMNs results in secondary wasting of the downstream musculature, and spasticity arises from loss of UMNs. This combined process leads inexorably to death, usually from respiratory failure as a result of diaphragm weakness. Although the median survival is consistently 3-4 years from symptom onset, a wide range is observed, with at least 10% of patients surviving beyond 10 years. ALS is largely a sporadic disorder, with the 10-15% of cases that are associated with a family history being clinically indistinguishable. Mutations of the superoxide dismutase-1 (*SOD1*) gene are found in 20% of familial ALS cases, with mutations in the RNA-processing genes *TDP-43* and *FUS* making up an additional 5-10%. It seems increasingly likely that there is a polygenetic profile of risk associated with apparently sporadic ALS more generally ².

The aetiology of ALS remains uncertain, though the presence of ubiquitinated cytoplasmic inclusions of the RNA processing protein TDP-43 currently defines the sporadic disorder neuropathologically. Furthermore, there is now acceptance of ALS having a multi-system extra-motor cerebral component to its pathology, characterized by clinicopathological overlap with frontotemporal dementia (FTD) ³. Several candidate molecular mechanisms, including RNA processing ⁴, now converge on a common pathway to motor neuronal cell death ⁵. The single licensed disease-modifying therapy for ALS to date – riluzole – has only a minimal effect on disease course ⁶.

Despite the earliest descriptions nearly 150 years ago ⁷, and the recognition of the simultaneous UMN and LMN involvement as its characteristic signature, ALS remains essentially a clinical diagnosis. Over the last 20 years, the mean diagnostic delay from symptom onset has remained approximately 12 months ⁸. This represents a sizeable proportion of the median survival. A fundamental lack of knowledge of the (presumably) larger population of people at risk of ALS is likely to be the greatest impediment to shortening this time. A greater awareness among primary care physicians and non-neurologists might be of some benefit in reducing the time to diagnosis ⁹. This diagnostic delay underlines the current paucity of easily-defined biomarkers in ALS. Despite numerous candidates from cerebrospinal fluid and blood ¹⁰, none has been validated for clinical use to date. Although several adverse prognostic factors, such as older age at onset and bulbar versus limb-onset to symptoms ¹¹, have been identified across large clinic-based databases of ALS cases, individual survival frequently transcends such factors. More robust and reliable prognostic biomarkers are therefore highly desirable. The ideal characteristics of an ALS biomarker are summarized in Table 1. Especially, non-invasive neuroimaging, such as magnetic resonance imaging (MRI), is emerging as a potential reliable biomarker for ALS.

Proton (¹H)-based MRI exploits the contrast generated by the differential water content of tissue sub-types and has continued to develop as a technique over the past 30 years of its clinical application to the human brain. As a non-invasive and relatively ubiquitous investigative tool, MRI has demonstrated particular potential as a source of biomarkers that may be sensitive to future therapeutic monitoring ¹⁰, as well as having diagnostic and prognostic value that rivals established and emerging neurophysiological techniques (Table 2).

2. The current diagnostic pathway in ALS

Although exhaustive lists of potential ‘mimics’ continue to appear in the literature, the combination of clinical UMN and LMN involvement with progressive weakness in the setting of preserved sensation has few tangible differential diagnoses, particularly when the disease begins in the bulbar territory (~25% of all cases). The revised (Airlie House) El Escorial (EE) criteria for ALS are based upon the demonstration of simultaneous UMN and LMN signs¹².

The onset of ALS symptoms is insidious, and it has been estimated that at least 30% of the anterior horn cells must be lost before weakness is apparent¹³. The sub-clinical detection of LMN involvement by electromyography (EMG) may be helpful in increasing diagnostic certainty by demonstrating involvement of multiple territories, but is not mandatory. The EE criteria, developed largely as a research tool, do not take into account the 10-20% of patients who may initially present with solely LMN signs on clinical examination, sometimes termed Progressive Muscular Atrophy (PMA), despite the neuropathological confirmation that many such patients clearly have CST involvement¹⁴ and are regarded by most clinicians as part of the ALS spectrum. The rare, but treatable, LMN-only auto-immune condition Multifocal Motor Neuropathy with Conduction Block (MFMNCB) is one of the few 'mimics' of PMA, and the demonstration of clear sub-clinical UMN involvement would allow its more rapid exclusion in those cases where EMG failed to demonstrate the conduction block.

Furthermore, the same EE criteria exclude the smaller proportion (~5%) of patients with UMN-only signs for the first 4 years of their disease course, termed Primary Lateral Sclerosis (PLS). PLS patients are distinguished through a consistently prolonged survival, frequently 10-20 years¹⁵. However these patients frequently develop LMN signs later in the course of the disease, and there is evidence of a shared neuropathological signature with ALS. PLS may be mis-diagnosed as one of the other UMN-only disorders, such as Hereditary Spastic Paraparesis or Primary Progressive Multiple Sclerosis.

At least 10% of ALS patients never fulfill more than 'possible ALS' according to EE criteria at the time of their death¹⁶. Questions continue to be raised about the value of the EE criteria, particularly in relation to clinical trial recruitment, where historically only those fulfilling criteria for 'probable' or 'definite' ALS were eligible. Even with recent amendments, EMG remains only 60% sensitive for ALS¹⁷.

Magnetic resonance imaging (MRI) has undoubtedly had its largest impact to date through the exclusion of other disorders (acknowledged in recent guidelines on the use of neuroimaging in ALS, see¹⁸). Cervical radiculomyelopathy and lumbosacral radiculopathy are the most commonly encountered potential ALS 'mimics' in those ~70% of patients with a limb-onset to their symptoms. However, in parallel with the recognition of ALS as a cerebral neurodegenerative disorder, MRI is now well-placed to fulfill a wider role in the diagnostic process through its ability to detect sub-clinical involvement of the UMN compartment in those with apparent LMN-only findings on examination, as well as the cerebral motor and extra-motor neuron involvement. In this way, MRI is poised to encompass the full range of phenotypes that come under the broader 'umbrella' term of MND.

3. MRI detects CST intensity changes in ALS

Standard T2-weighted MRI sequences, particularly FLAIR sequences, may reveal a striking hyperintensity of the CST in ALS. However, the sensitivity of such changes has been estimated at <40%¹⁹ and the specificity <70%²⁰, such that it was specifically mentioned as unhelpful in recent guidelines on the use of neuroimaging in MND¹⁸. To date there is no convincing evidence that such hyperintensity changes augur a more aggressive disease course (Figure 1). Notably, similar changes have been consistently described in the context of end-stage cirrhosis and liver transplantation. There may therefore be a particular vulnerability of these neuronal pathways, potentially with a common link to oxidative stress²¹, one of several converging pathways postulated in the pathogenesis of ALS⁵.

Changes in T2-relaxation (relaxometry) have been observed in a study of muscle in ALS and showed promise as a marker of denervation which may warrant reappraisal²², although

likely to be a secondary ‘downstream’ event in pathogenesis. More recently a reciprocal T2-based sequence known as R2* was applied in ALS as a surrogate marker for cerebral iron content, and found to be raised within the CST with potential implications for pathogenesis²³.

4. ALS as a wider cerebral neurodegenerative disorder

The nature and timing of the focal onset of symptomatic anterior horn cell pathology in ALS (which is typically within the brainstem, cervical or lumbo-sacral cord segments) remains uncertain in relation to overall pathogenesis. There is growing support for a simultaneous process²⁴, possibly reflecting an inherent human neocortical vulnerability²⁵ unmasked by the aging process or aggravated by an adverse genetic profile.

Extra-motor cerebral involvement in ALS was recognised as early as 1891 by Alois Alzheimer. Post-mortem studies have indeed confirmed the widespread cerebral degeneration of neurons in typical ALS cases²⁶⁻²⁸. After initial clinical observations on the coincidence of dementia and ALS, it is now recognised that cognitive impairments, largely of an executive nature, may be detected with detailed neuropsychological testing in as many as half of ALS patients. Only a small proportion (<5%) develop a frank dementia and this is indistinguishable from some forms of FTD (typically the behavioural or non-fluent sub-categories), and which may significantly pre-date the development of motor symptoms. Neuropathological studies later confirmed that ubiquitinated inclusions of TDP-43 are common to all cases of sporadic ALS and some types of FTD²⁹.

Studies with positron emission tomography (PET) demonstrated that frontal regions were involved in cases of ALS without dementia³⁰. More widespread areas of the brain were activated in ALS patients with documented cognitive impairments, during motor activity – termed a “boundary shift”³¹. Post-mortem studies have revealed that inhibitory neuronal populations are notably depleted in ALS³². This finding was supported by the finding of widespread reductions in the cerebral binding of the GABA_A receptor PET ligand [¹¹C]-flumazenil³³, later combined with transcranial magnetic stimulation (TMS) to demonstrate increased cortical excitability in ALS³⁴. Moreover, a study following unaffected individuals, at risk due to pathological mutations of the *SOD1* gene, revealed that such cortical hyperexcitability might be among the earliest detectable changes in pathogenesis³⁵, as well as precipitous LMN loss just prior to the first symptoms³⁶.

Given the weight of evidence that cerebral involvement is inherent in ALS, MRI is a uniquely practical tool to apply to the study of ALS and there are potentially multimodal applications.

5. Structural MRI detects subtle cerebral atrophy in ALS

Gross macroscopic atrophy is rarely a feature of ALS, with the exception of those cases with marked FTD-spectrum involvement, but may be observed focally in ALS³⁷, and particularly in the region of the motor cortex of those with PLS (Figure 2). A comparison of potential structural changes in individual patients against a template of an age-matched control group has been facilitated by the refinement of automated image analysis techniques. These co-register multiple images into a standard 3D space to compare voxel-by-voxel neuroanatomical differences with much greater sensitivity, in a technique known as voxel-based morphometry (VBM).

5. 1 Grey matter atrophy in ALS

In a VBM study of 24 heterogeneous ALS patients (without overt cognitive impairment), widespread regions of grey matter atrophy were detected in both motor (primary motor cortex) and non-motor regions (including the temporal lobes) compared with age-matched controls³⁸ (Figure 3), corroborating previous studies^{39,42}. Thinning of the precentral region of the neocortex has also been demonstrated⁴³, but it is noteworthy that atrophy of the primary motor cortex has not been a consistent finding in ALS VBM studies.

VBM also provides a distinction between patients with ALS and ALS-FTD, with the latter sub-group exhibiting a more pronounced fronto-temporal atrophy⁴⁴. Frontal lobe, as well as motor cortex atrophy has been particularly prominent in patients with PLS, presumably reflecting the UMN burden of this variant⁴⁵. Within the ALS category, a distinction was made in regional grey matter atrophy between shorter-lived sporadic and longer-surviving familial ALS patients homozygous for the 'D90A' mutation of the *SOD1* gene (despite similar disability). Those with the 'D90A' mutation had a more frontally-located pattern of grey matter atrophy⁴⁶. Thus, VBM might provide a novel means to identify ALS patients and early changes in those at risk, as well as insight into a differential diagnosis between closely related, potentially overlapping, clinical presentations.

5.2 White matter atrophy in ALS

Segmentation of MR images into grey and white matter based on their differential signal intensity, also affords the analyses of potential changes in the white matter, though diffusion tensor imaging offers a parallel and possibly more robust method to explore this compartment (see below). A study of 23 ALS patients reported extensive decreases in frontotemporal white matter volume using VBM even though only half of the ALS group exhibited detectable cognitive impairment⁴⁷, confirming the clinicopathological overlap with FTD. An earlier study reported white matter changes only in those with bulbar-onset ALS, who tend to have a higher burden of cognitive impairment⁴⁸.

6. Diffusion tensor imaging in ALS

To specifically investigate white matter and the directionality of fiber tracts, MRI-based diffusion tensor imaging (DTI) is apposite. DTI exploits the sensitivity of MRI to the movement of water and is a promising tool for the *in vivo* neuropathological study of neuronal pathways⁴⁹. Within intact neuronal pathways bounded by nerve membranes, water moves in a highly directional way (anisotropic), whereas in CSF and, to a lesser extent, damaged neuronal pathways, the movement of water is less restricted and hence multi-directional (isotropic). The spatial preference of water can be defined mathematically by a tensor comprising at least three 'eigenvectors' that define an ellipsoid. The axial (longitudinal or parallel) diffusivity (AD) is the eigenvector along the principal axis i.e. the neuronal tract. Radial diffusivity (RD) is the mean of the diffusivity in the two minor axes of the ellipse and reflects diffusion orthogonal to fibre tracts. An integration of all eigenvectors is termed the Fractional Anisotropy (FA, which is *reduced* with loss of neuronal pathway integrity) and is the most frequently used measure, whereas an average of all three is termed mean diffusivity (MD, which is *increased* with a loss of neuronal pathway integrity). RD and may be particularly sensitive to regions of secondary Wallerian-type demyelination⁵⁰. A larger number of diffusion-weighting directions acquired during the scan may significantly increase the sensitivity of DTI in ALS⁵¹.

The initial applications of DTI to ALS patients occurred over a decade ago⁵². This immediately confirmed the potential of DTI to delineate neuronal tract damage (specifically CST) *in vivo* providing henceforth a non-invasive measure of the features identified decades

earlier in post-mortem studies ^{26, 27}. Subsequent studies can be grouped according to the main neuronal pathway being inspected.

6.1 Corticospinal tract (CST)

The majority of DTI studies to date have used a region-of-interest method to compare groups based on well-described post-mortem pathology. The CST has been the most frequently studied neuronal pathway (reviewed in ^{10, 53, 54}). All published studies in this region have demonstrated the potential of DTI to discriminate ALS patients from healthy controls on the basis of changes in FA (and in a few cases also MD) at various levels of the CST. Less consistently, FA has been reported to correlate with disease duration ^{52, 55, 56}, and disability ^{57, 58}. These latter correlations suggest that DTI-based CST measures hold promise for future therapeutic monitoring. FA changes within the CST at baseline may be even able to predict disability at 6 months according to a preliminary study ⁵⁹. In a group of unaffected subjects 'at risk' of developing ALS due to *SOD1* mutation, FA changes in CST were reported even before the 'clinical horizon' ⁶⁰. However, replication of this biomarker and its potential as an identifier of 'at risk' patients is required.

6.2 Corpus callosum

Involvement of the corpus callosum (CC) had been observed neuropathologically in ALS ^{26, 27} and several DTI studies have specifically noted FA changes within the CC of patients ⁶¹⁻⁶⁴. In a high-field (3 Tesla) study of 24 heterogeneous ALS patients (many with few UMN signs and a wide range of disease duration), more consistent involvement of the CC was noted and, unlike the CST involvement, this appeared to be independent of the degree of UMN involvement clinically ³⁸. There was a more limited relationship between disability measures and CC changes than that noted by others ^{62, 63}, but CST changes were strikingly most marked rostrally and also bilaterally (despite correction for presumed hemisphere of disease onset), supporting the view that ALS may be a cerebrally-dominant pathological process ^{25, 65}. Although as yet unconfirmed, the involvement of the CC at an early stage of the disease process would be coherent with recent theories that have attempted to explain the nature of focality of onset and the subsequent rapid spread of ALS pathology ²⁴. This position is further corroborated by the largest FA changes being observed in the postero-central portion of the CC ³⁸ (Figure 4) that is known to contain fibres linking the two motor cortices ²⁶. Although FA changes within the CC have been proposed to discriminate PLS from ALS ⁶⁶, and CC atrophy also noted in PLS patients ^{45, 67}, it seems likely that such patients represent the extreme end of a continuum with more typical ALS.

Unfortunately, the involvement of the CC is not specific, with FA changes also demonstrable in the CC of those with Hereditary Spastic Paraparesis, a condition with occasional diagnostic overlap to those with PLS. Still, no CC-based FA changes were seen in patients with another potential 'mimic' of ALS, namely the LMN-only disorder of Kennedy's disease ⁶⁸. The ability to parcellate regions of the CC according to their wider cerebral projections ⁶⁹ makes this structure of further interest as a marker of wider cerebral involvement in ALS.

6.3 Extra-motor involvement and tractography

A few DTI studies have specifically studied extra-motor regions-of-interest in ALS and confirmed FA changes within the frontal regions ^{57, 61, 70}. A DTI study of familial ALS patients homozygous for the 'D90A' *SOD1* mutation, with similar disability to a group of sporadic ALS patients, but a markedly slower disease course, revealed significantly reduced extra-motor, as well as motor involvement ⁷¹. By probabilistic linking voxels with similar tensors from a 'seed point', it is possible to reconstruct fibre pathways in a technique related to DTI known as tractography. Although not quantitative to the same extent as DTI

measures at present, it permits the exploration of wider connectivity. DTI measures can be applied more quantitatively to such tracts in isolation, and have, for instance, confirmed that the uncinate fasciculus is involved in ALS patients⁷². In rare cases with extremely asymmetrical features clinically, it is even possible to perform within-subject comparisons to visibly demonstrate reduced integrity of individual tracts (Figure 5).

6.4 Longitudinal studies

The rapidly increasing physical disability associated with ALS, particularly orthopnoea as a result of diaphragm weakness, makes longitudinal studies using MRI challenging. However, these are vital to realise the goal of robust biomarkers useful for therapeutic monitoring. A study of 28 ALS patients, 7 of whom had repeat interval imaging, demonstrated that CST FA decreased over time⁵⁷. A study focusing on the cervical cord of 28 ALS patients, 17 of whom had a follow-up scan, noted longitudinal increase in MD as well as reduced FA, though not in the CST⁷³. Confirmatory studies are now a priority.

6.5 Spinal cord

The ability to study the spinal cord in ALS patients *in vivo*, particularly the anterior horns from whence the LMN cell bodies are located, would make MRI unrivalled in its coverage of the pathology. Although the resolution of MRI continues to improve (in part through higher field strengths, which brings its own difficulties through issues of greater field heterogeneity), the small diameter of the spinal cord and its surroundings (particularly bone), as well as breathing-mediated movement artifact make it very challenging. Novel methodology suggests that, as with the enormous developments in cerebral MRI over the past 20 years, these challenges will be overcome⁷⁴. There have already been some early successful applications of MRI to the cervical cord in ALS, with one study tightly linking changes in FA to disability⁷⁵. Radial diffusivity, as well as FA, appear to be most altered in the distal parts of the cord⁷⁶, which may support an alternative view that there is a significant 'dying back' process of the corticospinal tract involvement in ALS.

7. Magnetic resonance spectroscopy in ALS

MRS exploits the physical phenomenon underlying MRI, notably nuclear magnetic resonance (NMR). Metabolites can be detected using a NMR spectrum of a volume of tissue within a so-called voxel-of-interest (VOI). Although a variety of metabolites can be identified, *N*-acetylaspartate (NAA), often used as a marker of neuronal integrity, is most commonly investigated in neurodegenerative disease. NAA is often expressed as a ratio with creatine (Cr) which is a relatively constant marker that reflects cellular energy stores, or with choline (Cho) which is broadly a marker of increased cell turnover.

ALS patients with motor neuron disease show a decreased ratio of NAA:Cr in the PMC compared with controls⁷⁷ and the technique was used successfully to distinguish a small group of patients with PMA from patients with more typical ALS. There have been several other MRS studies in ALS (reviewed in^{10, 54}), with changes in the PMC linked to disability⁷⁸, disease progression^{79, 80}, and even therapeutic response^{81, 82}. Metabolic changes in the PMC appears to be an early feature after symptom onset⁸³ and potentially has greater sensitivity than neurophysiological techniques⁸⁴. The technique has been used to probe extra-motor involvement in ALS⁸⁵, and the study of specific ratios of metabolites might add sensitivity as well as reveal aspects of pathogenesis, for example a study in which NAA/(Cr + Cho) was seen to decrease longitudinally in grey but not white matter, and most significantly within the apparently less affected hemisphere⁸⁶.

To date, the sensitivity and specificity of ¹H-MRS for ALS has been limited (ranges quoted from ~50-70% for sensitivity and ~40-90% for specificity). This is potentially due to the low

resolution of the technique that only studies a single voxel. However, novel sequence design, such as chemical shift imaging (CSI), promises a more appropriate multi-slice whole-brain analysis. Higher field strengths will improve signal-to-noise and allow a better separation of metabolite peaks, such as glutamate and GABA, which may have more specific relevance to ALS pathogenesis. MRS can be based on ^{31}P instead where it provides an indirect measure of bioenergetics and neuronal membranes. It has been applied to muscle in ALS in a study showing reduced changes in energy metabolites in response to exercise, postulated to reflect intrinsically impaired muscle activation⁸⁷.

8. Functional MRI in ALS

The different paramagnetic properties of oxygenated and deoxygenated haemoglobin have been exploited in a technique known as Blood Oxygen Level-Dependent (BOLD) MRI. BOLD reflects regional changes in cerebral neuronal function in response to carefully timed tasks with gated image acquisition. To date, such functional MRI (fMRI) studies in ALS (reviewed in⁸⁸) have demonstrated qualitative differences in comparison with controls, generally showing an increased extent of regional activation, potentially indicating ongoing compensatory changes in related brain regions. The technique currently lacks sensitivity in terms of a biomarker and is highly dependent on the administration of a specific task which may be too difficult for physically disabled patients.

A more recent development focussing on cerebral blood flow (CBF) using MRI is Arterial Spin Labelling (ASL). In ASL, arterial blood within a volume of tissue just inferior to the cerebral hemispheres travelling cranially can be magnetically 'labelled' and 'tracked' as it perfuses the brain superiorly. Grey matter perfusion measured using the technique (and correcting for atrophy) was linked to disability in cases of ALS⁸⁰. Loss of cerebral neurons might require less supply of oxygenated blood to a given region and results in a region-specific decrease of CBF. The advantage of this approach is that patients can be evaluated even if they have significant physical impairment precluding task-based fMRI assessment.

8.1 The 'resting state'

Resting state functional MRI (R-fMRI) is a novel technique that studies fluctuations in the BOLD contrast with subjects completely at rest, in order to explore interactions between functional cerebral networks^{89, 90}. The technique has been used to demonstrate impaired connectivity in Alzheimer's disease⁹¹ and early studies have confirmed changes in the cerebral networks of ALS patients⁹². Changes in regional network connectivity might reflect the very earliest changes in neuronal functions prior to neurodegeneration. Combined with DTI, R-fMRI might provide an early screening protocol to identify subjects 'at risk' of developing ALS. However, significant further validation studies are required more generally with regard to this technique.

9. Post-mortem MRI

There has been virtually no examination of how *in vivo* MRI changes relate to post-mortem findings in ALS. The continued Brownian anisotropic motion of water molecules within intact neuronal pathways can still be sensitively detected in post-mortem brain using long acquisition times (e.g. 48 hours) with tissue suspended in specialised MRI-suitable oils. This has the potential to provide very detailed FA maps that can be subsequently compared with traditional neuropathology (Figure 6). Studies in multiple sclerosis have demonstrated that post-mortem MRI can detect active lesions not seen macroscopically with traditional neuropathology⁹³ (for review see⁹⁴). By establishing those *in vivo* changes that most accurately reflect subsequent neuropathology, post-mortem MRI studies have further potential to refine the acquisition and analysis of MRI sequences *in vivo*. Importantly, such

studies may also reveal the very late changes in the disease process when physical disability may preclude *in vivo* scanning.

10. Cellular/Molecular MRI

Identifying early events that either pre-dispose a person to develop ALS or the initial molecular events that trigger the neurodegeneration cascade remain the most challenging biomarkers to develop. However, being able to identify these early events might have the largest impact on delaying or reversing disease onset. The selective visualisation of cells or molecular processes using MRI *in vivo* is known as cellular/molecular MRI⁹⁵.

As ALS is characterised by a loss of cells through particular molecular processes, such as reactive oxygen species (ROS), it is apposite to investigate if these localised changes could be visualised *in vivo*. The brain redox state, for instance, can be monitored using 'Overhauser MRI'^{96, 97}. Sensitivity for a small number of cells being exposed to ROS might, however, require the use of specifically designed MRI contrast agents⁹⁸. It might even be possible to devise DNA-based MRI probes for the specific detection of gene transcription or mutations in *SOD1*^{99, 100}. MRI probes recognising ubiquitinated TDP-43 cell inclusions could provide a positive imaging marker akin to amyloid beta plaque imaging in Alzheimer's disease using PET¹⁰¹. Nevertheless, significant engineering challenges need to be resolved to allow such molecular MR imaging of neurodegenerative events in the human brain¹⁰².

A more immediate goal will be to identify tangible molecular targets that are easily accessible and provide a novel dimension of information regarding the disease. In most cases of ongoing degeneration, subtle changes in the blood-brain barrier (BBB) are often observed. This may also be a very early feature in the *SOD1* mouse model of ALS¹⁰³. Although these changes might be insufficient for a leakage of the BBB detectable with conventional MRI contrast agents, such as Gd-DTPA, the local expression of adhesion molecules, for instance, can be detected *in vivo* using ultrasmall iron oxide particles (USPIO) conjugated with antibodies that bind to activated endothelium in rodents¹⁰⁴. Although inflammatory markers might be of interest to monitor the effects of some therapeutic interventions, more specific molecules expressed only in areas undergoing motor neuron degeneration might find a diagnostic application in ALS.

An intravenous injection of larger superparamagnetic particles of iron oxide (SPIO) in contrast might inform the macrophage activity involved in clearing the debris of dying cells or axons, demonstrated *in vivo* in ALS patients using PET¹⁰⁵. As blood-borne macrophages are phagocytosing large particles, such as SPIO, these will report on the location of the labelled cell if it enters the brain. Human studies with SPIOs have so far been reported in patients with stroke¹⁰⁶ and multiple sclerosis¹⁰⁷, but would be informative in patients with ALS to chart the temporal progression of ongoing neurodegeneration. It is important to note though that macrophages already contain endogenous iron and hence can influence the MRI signal, albeit not at the cellular level.

11. Expert opinion

11.1 MRI is a credible biomarker source in ALS

MRI is a leading non-invasive, accessible tool to probe the motor (at present only UMN) and extra-motor lesion in ALS. It has tangible potential as a source for diagnostic and therapeutic monitoring biomarkers. DTI in particular holds the promise of highly sensitive detection of neuronal pathway damage, and in combination with R-fMRI may offer a unique insight into functional, as well as structural connectivity changes that may be among the

very earliest detectable. As new disease-modifying drugs are developed, these imaging approaches have a significant potential to detect therapeutic benefit even prior to symptom-onset, and lead to early 'Go-NoGo' decisions about candidate drugs.

11.2 The current challenges

The issue of disease heterogeneity in ALS is well-recognised in the context of clinical trials, and often cited as a potential reason for the apparent lack of efficacy of therapeutic agents to date. Although, many studies have included only patients fulfilling EE criteria for 'probable' or 'definite' ALS, in particular excluding those with PMA, more inclusive studies still demonstrate the ability of MRI to transcend such clinical categorisation and provide consistent biomarkers applicable across a wider spectrum of ALS. A sensitive MRI marker of LMN involvement remains aspirational at present.

Understanding the wider 'at risk' population in sporadic ALS, beyond those familial cases with single gene mutations e.g. *SOD1*, may allow the therapeutic window to move backwards, but this is a long-term aspiration requiring major developments in our understanding of motor neuronal cell biology. Currently, it appears that the therapeutic window occupies a very narrow time frame near to the 'clinical horizon' and prior to the development of significant disability. The use of MRI –generated biomarkers to narrow the current diagnostic delay could make accessing this window a realistic shorter-term proposition (Figure 7).

The absolute MRI contra-indication of cardiac pacemaker (or certain other embedded ferromagnetic materials) is rarely an issue, although significant claustrophobia may affect a small proportion of individuals and prevent MRI. More tangibly, the natural progression of ALS, particularly orthopnoea arising from diaphragm weakness, and the physical disability in getting on to the scan table, are significant barriers to longitudinal studies in the late stages of the disease.

The issue of controls is also a matter of debate. The diagnosis of ALS is rarely challenging by the time of presentation to an experienced neurologist (a feature of the referral delay at present). Rather than diagnostic biomarkers to separate the healthy from ALS patients, a comparison with tangible 'mimic disorders', such as cervical myeloradiculopathy, Multifocal Motor Neuropathy with Conduction Block (for PMA), or Primary Progressive Multiple Sclerosis (for PLS) is important to establish biomarkers of ALS. Current analyses involve groups of patients compared with similar-sized control groups, rather than single subject analysis. This clearly limits the wider application of MRI as a diagnostic or prognostic tool for individual patients. Nevertheless, this may be remedied by the development of normative control templates. Improvements in analysis methodology, along with the greater sensitivity that is likely to come with increasing field strengths will eventually lead to the wide-spread adoption of MRI biomarkers as the predominant tools to diagnose and monitor ALS. However the extensive image post-processing that is required at present for advanced MRI techniques will need to be standardised to permit multi-centre studies.

11.3 The next steps

In relation to MRI biomarkers with prognostic value in ALS, there has been an over-reporting of markers able to discriminate between limb and bulbar-onset patients, a distinction of limited value for an individual. Better markers will only be realised through routine longitudinal studies in ALS, which therefore need to begin at the earliest stages of the disease to gather multiple time points. It will also be important to integrate MRI assessments into future clinical trials (a point recognised in recent guidelines on the use of

neuroimaging in MND¹⁸), so that the potential for therapeutic monitoring in relation to standard surrogate markers of disability (e.g. ALSFRS-R) can be truly ascertained. International consensus on acquisition and analysis will be an essential component of this aspiration, and is particularly important to harness the full potential of neuroimaging. Such consensus may also pave the way for a centralised repository of scans for development of novel analyses and establish validated MRI biomarkers.

11.4 Future developments

The *post hoc* multi-modal analysis of MRI data in ALS (e.g. combining DTI and VBM measures) was shown in one study to improve both sensitivity and specificity to nearly 90%³⁸. However, pipelines for an efficient image processing need to be established to exploit this sensitivity and specificity in a clinically relevant time scale. The combination of DTI and R-fMRI offers particular interest in linking structure and function, and the development of quantitative tractography will be an important aspect of this. The '1000 Functional Connectomes Project' was a major international collaborative initiative that demonstrated the potential of R-fMRI, as well as the feasibility of international data pooling¹⁰⁸. Being able to quantitatively identify the subtle loss of network connectivity in the pre-clinical stages of neurodegenerative disorders may eventually provide a means to identify these conditions prior to the onset of any symptoms.

An era of molecular MRI might eventually lead to comprehensive assessment of neurodegeneration in ALS, including disease mechanisms and the monitoring of disease progression and therapeutics. It seems likely that neuroimaging, possibly in combination with markers from biofluids, is emerging as a tangible replacement for the current symptom-based assessments.

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Declaration of Interest

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Figure 1. Coronal T2-weighted MRI scans of the brain of a bulbar-onset ALS patient (A) and a patient scanned for headache (B). Both demonstrate hyperintensity of the corticospinal tract, which lacks sensitivity as well as specificity as a biomarker for ALS.

Figure 2.

3D-rendered MRI brain from two individuals. The first image is a patient with ALS and the rare association of progressive non-fluent aphasia, which revealed striking left temporal lobe atrophy (A, shown from below, arrow marks left temporal pole). The second is a patient with PLS in whom atrophy of the pre- and post-central gyri can be seen (B, shown from above, arrows mark central sulci). This sort of macroscopic atrophy is very uncommon in typical ALS, and semi-automated techniques such as voxel-based morphometry are required to detect regions of atrophy more sensitively.

Figure 3. Generic MRI brain slices with superimposed areas showing statistically significant regions of grey matter atrophy (blue, $p < 0.05$, corrected for multiple comparisons) in a group of 24 heterogeneous ALS patients compared with healthy age-matched controls using voxel-based morphometry. Changes are widespread beyond motor regions and confirm ALS as a multiple system neurodegenerative disorder that is known to have clinicopathological overlap with some types of frontotemporal dementia.

Figure 4.

Generic MRI brain slices with a superimposed standardised 'skeleton' of major white matter neuronal tracts (green). Statistically significant regions of reduced FA from a DTI study of a group of 24 heterogeneous ALS patients compared with healthy age-matched controls are shown (red-yellow). There are consistent changes noted within the corpus callosum.

particularly the posterocentral portion containing projections from both motor cortices. This supports the concept of a cerebrally-based process in the pathogenesis of ALS, even in those without significant clinical upper motor neuron involvement. Cerebral changes may even be among the earliest in the disease process.

Figure 5.

3D-rendered MRI brain viewed obliquely from right fronto-temporal aspect, with cut-out central section. This ALS patient had an unusual syndrome of rapidly progressive right hemiparesis and non-fluent aphasia with marked left temporal lobe atrophy. Diffusion tensor ‘probabilistic’ tractography was used to delineate the white matter tracts projecting into the temporal lobes. In this single-subject analysis it clearly demonstrated reduced tract integrity on the left (blue) compared with the right (red), and so the potential of the technique to explore wider connectivity in the brain non-invasively.

Figure 6.

DTI anisotropy maps (colour-coded for predominant fibre pathway direction, taken from ¹⁰⁹) performed in the same individual *in vivo* (left panel) and *post mortem* (right panel). Although in early development, this has potential to help refine MRI sequences *in vivo* so that they more accurately reflect histological changes, as well as to understand the very late changes in MRI appearances when a patient may be too disabled to be scanned *in vivo*.

Figure 7.

Schematic representation of events either side of the 'clinical horizon' where an individual first develops symptoms associated with ALS. There is currently an average delay of 1 year from this point to formal diagnosis, and a median survival of 2-3 years. There is a significant group with longer survival however, not all of whom have PLS. It is not clear when the degeneration of motor neurons begins, though evidence from pre-symptomatic familial cases with *SOD1* mutations suggests there are changes in both cortical hyperexcitability and LMN function in the months just prior to first symptoms. How cell death relates temporally to the formation of protein inclusions (e.g. TDP-43) is not certain either. Currently the optimal therapeutic window in sporadic ALS seems likely to be as near to the clinical horizon as possible, though greater knowledge of those 'at risk' may move this backwards with preventative strategies ultimately. The different biomarker stages are marked A-D.

A – biomarkers for those at risk of sporadic ALS

B – biomarkers for those 'at risk' of familial ALS with certain gene mutations e.g. *SOD1* or in earliest stages

C – diagnostic and prognostic biomarkers for ALS cases generally

D – monitoring biomarkers for ALS cases generally

Table 1

The ideal characteristics of a biomarker in ALS.

1. Sensitive and specific for the heterogeneous syndrome of ALS
2. Detectable prior to the onset of significant wasting/weakness
3. Discriminate between clinical phenotypes based on: <ul style="list-style-type: none"> a) Upper and lower motor neuron involvement e.g. PMA and PLS b) Patterns of regional involvement e.g. flail arm/leg, PBP c) Cognitive involvement i.e. ALS-MCI versus ALS-FTD d) Extremes of survival i.e. 'aggressive' (1-2 year survival from symptom onset) versus 'slow' (5-10 year) course
4. Able to predict regional involvement and the pattern of spread in advance: <ul style="list-style-type: none"> a) Bulbar dysfunction for early PEG b) Respiratory dysfunction for early NIV (possibly diaphragm pacing) c) Cognitive impairment
5. Change in a predictable way with disease progression
6. Sensitivity to confidently judge therapeutic response within weeks of challenge
7. Easily accessible and affordable technology
8. Practical to measure in the physically disabled patient

Table 2

The potential of MRI versus neurophysiology techniques to probe pathology and generate biomarkers in ALS.

Region of interest	MRI	EMG	TMS	MUNE
UMN lesion	+	–	+	–
Extra-motor cerebral lesion	+	–	–	–
LMN lesion	+	+	–	+
Type of biomarker				
Diagnostic	+	+	+	+
Prognostic	+	–	+	–
Therapeutic monitoring	+	–	–	+
Mechanistic	+	+	+	+

EMG – electromyography

TMS – transcranial magnetic stimulation

MUNE – motor unit number estimation