Potential utility of biomarkers in the diagnosis and treatment of amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a rare, neurodegenerative disease of the human motor system, with a median survival of 3 years from symptom onset. The age of onset is typically the 5th decade, with the clinical picture representing unrelenting progressive muscle weakness and resultant disability. Despite countless clinical trials examining novel medications, disease-modifying therapies remain limited, with riluzole affording a survival benefit of approximately 3 months. To date, the diagnosis and treatment of ALS have been hampered by lack of effective biomarkers. A diagnostic biomarker for ALS would enable prompt initiation of neuroprotective therapy, ideally when neural structures were intact and disease severity remained mild. Furthermore, a biomarker capable of monitoring disease progression may facilitate conduct of future clinical trials, by reducing sample size and trial duration. As such, the present review summarizes pathophysiology, diagnosis and treatment of ALS, with a focus on the development of effective biomarkers.

**Keywords:** amyotrophic lateral sclerosis • biomarker • clinical neurophysiology • clinical trial • diagnosis • neuroimaging • pathophysiology

First described by Charcot [1], amyotrophic lateral sclerosis (ALS) encompasses a group of neurodegenerative disorders pathologically characterized by motor neuron loss in the motor cortex, brainstem and spinal cord. With median survival of 3 years from symptom onset [2], ALS manifests as progressive weakness of muscles under voluntary control, with relative sparing of extra-ocular and pelvic floor muscles [3]. The disease usually begins in the limbs (limb-onset), but weakness may be first evident in the oropharyngeal muscles in 20% of patients (bulbar-onset) [4]. Rarely, ALS patients may present with dyspnea, signifying early respiratory muscle involvement [5]. Irrespective of site of disease onset, weakness of respiratory muscles culminates in respiratory failure, the principal cause of death.

Glutamate, the major excitatory neurotransmitter in the CNS, is a contributing factor to the demise of motor neurons in sporadic ALS (‘excitotoxicity hypothesis’) [6]. Excessive glutamatergic activation of ionotropic receptors may trigger an influx of Ca\(^{2+}\) into motor neurons, leading to activation of free radical-generating enzymes – Ca\(^{2+}\)-dependent proteases – ultimately precipitating neurodegeneration. Defects in axonal transport, mitochondrial dysfunction, neuroinflammation, ribonucleic acid splicing defects and accumulation of cytoplasmic inclusions have also been implicated in ALS pathogenesis [2,8].

The mechanisms of neurodegeneration in ALS remain complex and incompletely understood. Approximately 10% of patients report a family history of ALS, with mutations in the SOD-1 gene accounting for 20% of familial cases [9]. Furthermore, mutations in TARDBP, FUS [10], UBQLN2 [11], VCP [12] and OPTN...
The pathophysiology of sporadic ALS appears multifactorial, with likely genetic susceptibility factors and environmental triggers [6, 7]. Comparative marker levels amongst ALS patients, disease controls and healthy controls in case–control studies may enable further dissection of putative pathophysiological processes. In this respect, an approach in ongoing discussion considered in such case–control studies relates to disease time course, given that certain biomarkers may be elevated and more pathogenic at specific stages of the disease.

- **Tissue-based biomarkers**
  Peripheral blood would appear a likely source for biomarkers. Despite convenience, however, this approach is underscored by the assumption that neurodegenerative disorders are associated with disrupted homeostasis in non-CNS tissues [8]. Nevertheless, cytoplasmic aggregates of TAR DNA-binding protein 43 have been identified in circulating lymphocytes, suggesting that peripheral blood may act as a suitable surrogate for some biomarkers [9]. Cerebrospinal fluid (CSF) may also represent an attractive source for biochemical markers of ALS, given proximity to the CNS, although the invasive nature of obtaining CSF has tended to limit appeal, particularly from the patient perspective. In contrast, a number of CSF biomarkers, located at the nodes of Ranvier and intersegmental nodes, and have been used to investigate a wide range of peripheral nerve disorders [10]. Axonal excitability testing studies have specifically identified upregulation of persistent Na⁺ currents and Na⁺ conductances in peripheral motor axons of ALS patients [11]. Increased Na⁺ entry into motor axons may in turn enable membrane potential to reach threshold more frequently, resulting in spontaneous depolarization [12], and thereby implicating Na⁺ channels in the genesis of fasciculations, a universal feature of ALS. In addition to central changes, abnormalities of peripheral nerve excitability may have also been extensively documented in ALS. The contribution of persistent Na⁺ conductance to increased excitability of nervous tissue has been demonstrated in the peripheral motor nerves of ALS patients using axonal excitability techniques. Axonal excitability studies enable in vivo estimation of membrane potential and the function of constituent ion channels, located at the nodes of Ranvier and intersegmental nodes, and have been used to investigate a wide range of peripheral nerve disorders [10]. Axonal excitability testing has specifically identified upregulation of persistent Na⁺ conductances in peripheral motor axons of ALS patients [11]. Increased Na⁺ entry into motor axons may in turn enable membrane potential to reach threshold more frequently, resulting in spontaneous depolarization [12], and thereby implicating Na⁺ channels in the genesis of fasciculations, a universal feature of ALS.

- **Neurophysiological biomarkers reveal cortical hyperexcitability in ALS**
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- **Axonal Na⁺ conductances & ALS**
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- **Neuromuscular excitability studies**
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- **Neuroimaging may facilitate improved understanding of pathology**
  Neuroimaging studies, such as diffusion tensor imaging, have traditionally examined specific
regions of interest along the corticospinal tract in ALS patients, demonstrating disintegration of this major tract in ALS patients with and without clinical signs of UMN impairment (i.e., brisk reflexes and spasticity) [56]. However, more recent neuroimaging studies have evaluated a global understanding of cortical dysfunction in ALS. Transcallosal dysfunction has been detected in structural and functional neuroimaging studies [57]. The rationale for using graph theory as an alternative means of interpreting structural connectivity data has enabled further elucidation of how neurodegeneration may spread from one motor cortical region to another, in addition to reduced connectivity between the motor cortex and adjacent regions [58,59]. Nonetheless, a potential limitation of neuroimaging biomarkers may be an apparent disconnect with clinical features, such as rate of disease progression and disease time course, with mixed findings across studies [55–59].

NMR imaging & cortical dysfunction in ALS

Unlike traditional neuroimaging approaches that evaluate structure or function, 1H-NMR spectroscopy (1H-NMRS) exploits how proton-containing metabolites (e.g., myo-inositol, glutamate, glutamine, N-acetylaspartate, choline, creatine, and taurine) change with emerging disease. 1H-NMRS has also revealed reduction in this ratio in central motor regions consistent with neuronal loss [60–62]. 1H-NMRS has also revealed reduction in this ratio in asymptomatic individuals harboring mutations in the SOD1 gene, suggesting that a prodromal phase occurs in familial disease [63].

Diagnosing ALS & the role of biomarkers

In routine clinical practice, the diagnosis of ALS remains clinical, relying on the presence of clinical features, consistent with UMN and LMN impairment [4]. Patients usually present with a history of progressive weakness involving multiple regions of the body, in the absence of any structural lesion. By contrast, the revised El Escorial criteria are used to enroll patients for research studies, and require a combination of UMN and LMN signs within one or more of four body regions: cranial, bulbar, cervical (upper limbs), thoracoabdominal, and lumbosacral (lower limbs), conditional on the absence of other neurological disturbances [64]. Recent amendments to the criteria (the ‘Awaji criteria’), which consider electrophysiological signs of LMN dysfunction equivalent to clinical evidence of LMN involvement [65], have resulted in increased diagnostic accuracy [66], particularly in patients with bulbar-onset disease [67].

Given the lack of diagnostic biomarkers, diagnostic delay is common in sporadic ALS, up to 12 months after symptom onset [68], with initiation of established or experimental disease-modifying therapy inevitably delayed. An early diagnosis of ALS would facilitate commencement of neuroprotective therapy, and potentially bolster patient recruitment into clinical trials [69]. Neuroradiology is most warranted when neural structures are intact, and indeed, it has been surmised that by the time a patient presents to a neurologist, approximately 50% of motor neurons have already succumbed to disease. Early intervention may thereby maximize neuroprotective potential.

In comparison to clinical trials and case-control studies, studies of diagnostic accuracy are infrequently undertaken, and their methodology not widely promulgated, despite the formulation of criteria for design and conduct of such studies [70]. It is not frequently understood that measures of diagnostic accuracy (i.e., sensitivity and specificity) reflect the composition of the target clinic population, and are not intrinsic to the clinical test under scrutiny [71,72]. To mimic the incidence of disease in a clinic population in a diagnostic accuracy study, recruitment of controls should arise exclusively from the same clinic environment as patients with the disease of interest. Moreover, healthy controls from outside the clinic population should not be enrolled into diagnostic accuracy studies as this would inflate test specificity. Given such properties of a diagnostic accuracy study, the utility of a diagnostic biomarker would differ between general neurology and specialist neuromuscular clinics, with the latter generally associated with a higher incidence of ALS. Moreover, the biomarker under scrutiny should also be investigated in patients with a disease time course similar to those in whom it would be applied in clinical practice, otherwise ‘spectrum bias’, whereby the diagnostic accuracy of a biomarker is inflated because it is tested in patients with severe disease, may develop.

Serum and CSF biomarkers have been investigated for ALS, but to date none have been successfully applied to clinical practice [4]. Although conventional MRI sequences appear to be of little diagnostic value in ALS [73], emerging neuroimaging biomarkers demonstrate the potential given that they are insensitive, in vivo exploration of structural and functional changes in the CNS, especially in the early stages of disease [74]. Specifically, diffusion-tensor imaging, which quantifies water diffusion along white matter tracts, may be of greater diagnostic potential than conventional MRI sequences given its increased sensitivity to corticospinal tract abnormalities [75]. In contrast to neuroimaging modalities, clinical neurophysiology may represent less costly avenues for pursuit. The triple stimulation technique of TMS may be used for identifying UMN abnormalities in patients not fulfilling ‘probable’ or ‘definite’ criteria for ALS [76]. Furthermore, investigation of cortical hyperexcitability using a novel threshold-tracking approach to TMS has shown that, compared with patients with ALS, spinal cord disorders, cortical hyperexcitability appears unique to ALS [77,78]. Capitalizing on the early occurrence of cortical hyperexcitability in ALS, threshold-tracking TMS may be useful in distinguishing ALS from mimicking disorders. Nonetheless, despite the existing body of literature, there remain few studies that have prospectively assessed the diagnostic accuracy of putative biomarkers in ALS in a multicenter environment.

Surrogate end points

According to the Biomarker Definitions Working Group, a surrogate end point is defined as a variable that is intended to substitute for a clinical end point. A surrogate end point is expected to predict clinical benefit (or harm or lack of benefit or harm) based on epidemiologic, therapeutic, pathophysiologic or other scientific evidence [79].

Increasing public pressure for new, promising drugs to receive expedited approval ensures there are many proponents for use of biomarkers as surrogate end points in clinical trials. Use of surrogates as surrogate end points is perhaps the most difficult application, given the assumptions involved (Figure 3). Surrogate end points may be of greatest value during early-phase trials when the objective is to demonstrate ‘proof-of-concept’. Surrogate end points enable assessment of putative mechanisms of disease, particularly useful in the context of multifactorial diseases, such as ALS [80].

ALS functional rating scale—revised

Functional scales (e.g., maximum voluntary isometric contraction and ALS functional rating scale—revised (ALSFRS-R) and respiratory function (e.g., forced vital capacity) have largely replaced survival as primary outcome measures in ALS trials. Although survival is a clinically relevant, unambiguous outcome in ALS trials, death as a primary end point dictates that trials be of long duration and that large numbers of patients are recruited to ensure that sufficient deaths
End points related to the pharmacology of an experimental therapy
Surrogate end points may also include those that are specific to the experimental therapy in question. For instance, a Phase III trial of celecoxib failed to demonstrate lowering of CSF prostaglandin E1 levels [94]. Moreover, no clinical benefit was observed in this trial. On the other hand, positive effects on a blood biomarker (increased histone deacetylation) were reported in a Phase II trial of sodium phenylbutyrate, although no attenuation in functional decline was observed [6]. These studies have highlighted that blood biomarkers were obtainable in a clinical setting, but the feasibility of performing sequential short-term punctures in large patient cohorts remains questionable.

Motor unit number estimation
A number of electrophysiological biomarkers of disease progression have been investigated as potential surrogate end points. Motor unit number estimation (MUNE) is an electrophysiological technique that enables estimation of the number of motor units present in a peripheral nerve [86]. MUNE has an intuitive interpretation, as it is a direct measure of disease time course, although a technical limitation is there being many different methods for its calculation. MUNE was incorporated as a secondary outcome measure in trials of creatine monohydrate [93], celecoxib [90,94] and memantine [95], but over recent years has fallen out of favor due predominantly to inherent variability across and within patients, and its time-consuming nature. The re-appearance of MUNE as a biomarker in ALS clinical trials may occur with the multipoint incremental approach, which is associated with high test-re-test reliability, increased patient compliance and shorter test duration [96]. Moreover, the assumptions underlying multipoint incremental MUNE may be valid in terms of ALS pathophysiology, given that progressive increases in single motor unit potential associated with collateral reinnervation may be captured by this technique.

Neuropathological index
As a simple measure of peripheral disease burden, the neuropathological index (NI) calculated using the formula: compound muscle action potential (CMAP) / F-wave frequency [97] is simple to perform and the motor unit number is obtained by multiplying the number of motor units in a cross-section of the nerve fiber. NI has been used to track disease progression in a Phase II trial of dex-pramipexole, demonstrating a nonsignificant reduction in slope of decline in post-treatment measurements compared with lead-in assessment [98]. NI was also employed in a trial of memantine, declining at a linear rate, similar to MUNE [99], with no difference in the rates of decline between treatment arms noted. Furthermore, NI may be recorded as frequently as every 4 weeks, particularly relevant when there is increasing demand for trials to be of shorter duration [96].

Figure 3. Effects of therapeutic interventions on biomarkers and clinical end points in drug trials. (A) The therapeutic intervention affects the biomarker, which is causally related to the clinical outcome. This would be considered a suitable biomarker. (B) The therapeutic intervention modulates the biomarker, without altering the clinical end point, which is independent of the biomarker. In this instance, the biomarker is not an effective surrogate. (C) The therapeutic intervention influences the clinical end point, without modulating the biomarker. The biomarker is thus insensitive to the intervention, and its use as a primary outcome measure would lead to false-negative findings. (D) The therapeutic intervention modifies both biomarker and clinical end point, although the biomarker is unrelated to the clinical end point. The biomarker may correlate with the clinical end point in one study; however, this relationship may not be replicated in a trial investigating a different medication.

are accrued for statistical inference [8]. On the other hand, use of functional scales as primary outcome measures may reduce sample size and trial duration [95]. Furthermore, they have straightforward interpretation and predict survival. Nonetheless, functional scales are not without limitations, as some have debated that certain medications may detrimentally influence functional capacity, without attenuating survival. Finally, an alternative to monitoring decline is to record time to 6-point reduction in ALSFRS-R, although this approach was associated with reduced statistical power compared with conventional longitudinal analyses involving ALSFRS-R [96].

Potential utility of biomarkers in amyotrophic lateral sclerosis

Figure 4. Summary of amyotrophic lateral sclerosis pathophysiology, pathological processes related to mitochondrial dysfunction in amyotrophic lateral sclerosis and sites of action of dex-pramipexole. Failure of cytoplasmic mitochondria induces increased susceptibility to glutamate-mediated excitotoxicity, perturbations in motor neuronal energy production and apoptosis. Axonal mitochondrial dysfunction may lead to disrupted axonal transport and disturbances in membrane potential along the entire length of an axon. The proposed driver of mitochondrial dysfunction in amyotrophic lateral sclerosis is loss of matrix metalloproteinases due to formation of the mitochondrial permeability transition pore, comprising VDAC-1, ANT and CyPD. Given the lack of protective histones within mitochondria, DNA mutations may occur as a result of oxidative damage, leading to reduced cytochrome C oxidase expression. Translocation of the Bax/Bak channels into the outer mitochondrial membrane increases permeability to cytochrome C, perpetuating apoptosis. The mitochondrial permeability transition pore enables transit of molecular species, including apoptotic enzymes, cytochrome C and caspases 3 and 9, between the mitochondrial matrix and cytosolic space, causing expansion and rupture of the outer mitochondrial membrane.

Disruption of these enzymes into the cytosol results in apoptosis, failure of ATP synthase, and consequent collapse of ATP production. Mitochondrial dysfunction is also associated with increased production of reactive oxygen species. In patients with familial amyotrophic lateral sclerosis, cytoplasmic aggregates of SOD-1 may directly inhibit conductance of VDAC-1, thereby reducing the supply of ADP to mitochondria for ATP generation. ADP: Mitochondrial membrane potential; ROS: Reactive oxygen species.
Electrical impedance myography

Electrical impedance myography is a non-invasive, painless, quantitative technique that relies upon the application and surface measurement of high-frequency, low-intensity electrical current, thereby enabling measurement of the electrical resistance of a target skeletal muscle. Unlike electromyographic techniques that evaluate the electrical potential of an excitable tissue, electrical impedance myography assesses the flow of electrical current through resting muscle. In simple terms, healthy, intact skeletal muscle is associated with a certain level of electrical resistance because of its unique architecture, which comprises aligned muscle fibers in a low-fat environment. The occurrence of denervation, as occurs in ALS, is associated with atrophy and concurrent increases in adiposity. Such changes may lead to increased tissue resistance that may be detected by electrical impedance myography. Of particular relevance to early-phase ALS trials was the association of electrical impedance myography with smaller sample sizes, compared with manual muscle testing, ALSFRS-R and maximum voluntary contraction assessment. However, further testing, particularly validation, is required.

Future perspective

The greatest problem associated with the majority of ALS biomarker studies relates to small sample size. To address this problem, a multicenter, case–control study aiming to collect 650 blood samples, 300 CSF samples and 600 DNA samples from four groups, ALS patients, patients with pure LIMN or UMN syndromes, patients with other neurological disorders and healthy subjects, is currently being undertaken. The scheduled date of completion has been listed as June 2012. A separate longitudinal observational study is also being undertaken to validate the utility of electrical impedance myography against established measures of disease progression in ALS. This study has a recruitment target of 120 patients, with a scheduled date of completion of December 2011.

...future science group

Potential utility of biomarkers in amyotrophic lateral sclerosis

Review: Clinical Trial Outcomes

Claire P. Cheah, Vucic & Kiernan

Executive summary

Amyotrophic lateral sclerosis (ALS) is a rare, neurodegenerative disease of the human motor system, with median survival of 3 years from symptom onset.

Objective biomarkers are needed for ALS to elucidate pathophysiological mechanisms, facilitate diagnosis and establish robust efficacy of treatments. Although the use of surrogate end points in clinical trials may be underpinned by strong assumptions, their use may expedite completion of clinical trials in ALS. Surrogate end points in ALS include functional measures, respiratory function and electromyographic biomarkers.

The revised El Escorial criteria for ALS remain a research tool for enrolling patients into research studies. Despite their improved specificity, they are based on clinical features and may not be sufficient to identify participants currently understanding about ALS.

Although threshold-tracking TMS demonstrates diagnostic potential in identifying ALS in a mixed neuromuscular cohort, it has not been validated in ALS. In practice, ALS is a clinical diagnosis, although additional investigations are undertaken to exclude alternative diagnoses. The diagnostic utility of amyotrophic lateral sclerosis (ALS) biomarker studies relates to small sample size.

Future perspective

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Future perspective

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Details the most recent discovery concerning a novel gene mutation responsible for familial ALS (published concurrently with [15]).

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