CLINICAL INVESTIGATION

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

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Benjamin Chee Chuan Cheah[1,2], Steve Vucic[2,3] & Matthew Colm Kiernan*[1,2]

[1]Multi-disciplinary ALS Clinical Service, Prince of Wales Clinical School, University of New South Wales, Randwick, Sydney, NSW, Australia
[2]Neuroscience Research Australia, Randwick, Sydney, NSW, Australia
[3]Sydney Medical School, University of Sydney, Westmead, NSW, Australia
*Author for correspondence:
Tel.: +61 293 822 438
Fax: +61 293 822 437
E-mail: m.kiernan@unsw.edu.au

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 [2,7,8].

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*

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[13] genes have also been verified in ALS (see [14] for a recent review of familial ALS). More recently, hexanucleotide repeat expansions in the *C9ORF72* gene (chromosome 9p21) were identified as the most common genetic aberration in familial ALS, accounting for at least 23% of cases with an autosomal-dominant pattern of inheritance [15,16]. As such, the etiology of familial ALS is now understood in approximately 60% of cases, but how such mutations initiate motor neuron degeneration remains poorly defined.

Riluzole remains the only neuroprotective, disease-modifying therapy for ALS patients [17], affording a survival benefit of approximately 3 months [18]. Although the precise mechanisms of action remain poorly defined, riluzole appears to exert neuroprotection through influencing a host of different pathways, including Na^+- and Ca^{2+}-channel blockade and facilitating GABAergic neurotransmission [7]. Such effects are likely to confer neuroprotection through converging on the interruption of glutamatergic neurotransmission. Given that riluzole does not restore lost function, it is important that clinicians emphasize to patients that its therapeutic effects are not clinically perceptible, particularly in the setting of ongoing disease progression.

Advancing our understanding of ALS pathophysiology and improving treatment options have been hampered by lack of effective biomarkers [19]. Conversely, the dearth of biomarkers may also be explained by our limited understanding of the etiology and pathogenesis of sporadic ALS. The present review will summarize current understanding of ALS pathophysiology, with a focus on the current framework for diagnostic and treatment approaches, and the potential for incorporation of clinical biomarkers.

Biomarkers

The Biomarker Definitions Working Group defined a biomarker as 'a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathologic processes or pharmacologic responses to a therapeutic intervention [20].'

In essence, a biomarker represents any biological characteristic that may be quantified in physiological or diseased states. There remains a critical need to devise objective biomarkers in ALS to elucidate pathophysiological mechanisms, facilitate diagnosis and establish surrogate end points for use in clinical trials [21,22]. Most biomarkers belong to single categories, but some may have overlapping applications. To be clinically useful, a biomarker should have high intra- and inter-rater agreement [23].

Biomarkers related to ALS pathophysiology

The pathophysiology of sporadic ALS appears multifactorial, with likely genetic susceptibility factors and environmental triggers [2,7]. Comparison of biomarker 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 important, but often over looked consideration 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 of biomarkers. Despite convenience, this approach is underscored by the assumption that neurodegenerative disorders are associated with disrupted homeostasis in non-CNS tissues [22]. 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 [24]. 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. Many novel CSF biomarkers have emerged recently [25], with inflammatory chemokines receiving most attention to date [26] given that potential correlations with survival [27]. When designing CSF biomarkers studies, it is important to consider that multiple extraneous factors, including time of day that samples are collected, medication and diet, may influence CSF metabolite levels [22].

Neurophysiological biomarkers reveal cortical hyperexcitability in ALS

Biomarkers need not only exist as molecules dissolved in tissue or body fluids. Changes in cortical excitability, as assessed by transcranial magnetic stimulation (TMS) may serve as a biomarker of ALS. Using this technique, ALS patients demonstrate reduction in short interval intracortical inhibition (SICI), potentially reflecting diminution in $GABA_A$-mediated inhibitory circuits [28], combined with excessive activation of glutamate-mediated excitatory pathways in the motor cortex (Figure 1) [29]. The development of cortical hyperexcitability in ALS may suggest that corticomotoneurons have a reduced threshold for activation [30], perhaps acting as an upstream pathophysiological trigger of excitotoxic degeneration of lower motor neurons (LMNs) in the brainstem and spinal cord (a 'dying-forward' hypothesis) [8,31,32]. Nonetheless, it is also acknowledged that hypotheses describing a 'dying-back' pattern, or upper motor neuron (UMN) and LMN degeneration occurring as independent phenomena, have also been proposed [33].

Alternatively, it could be argued that reduction in SICI in ALS patients may represent an adaptive process, facilitating cortical plasticity and thereby functional compensation in response to ongoing disability, as suggested by combined TMS and functional MRI studies [34,35]. Indeed, suppression of inhibitory circuits occurs in a range of neurological conditions associated with ongoing motor cortical reorganization [36–41]. On the other hand, incongruent with the observation that reduced SICI may reflect cortical plasticity is maintenance of SICI in pure LMN disorders, such as spinobulbar muscular atrophy or Kennedy's disease [42].

Axonal Na^+ conductances & ALS

In addition to central changes, abnormalities of peripheral nerve excitability 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 internodal segments, and have been used to investigate a wide range of peripheral nerve disorders [43]. Axonal excitability testing has specifically identified upregulation of persistent Na^+ conductances in peripheral motor axons of ALS patients [44–47]. Increased Na^+ entry into motor axons may in turn enable membrane potential to reach threshold more frequently, resulting in spontaneous depolarization [47], and thereby implicating Na^+ channels in the genesis of fasciculations, a universal feature of ALS.

In addition to the generation of fasciculations, raised cytoplasmic Na^+ concentrations may lead to neurodegeneration. Elevations of cytoplasmic Na^+ concentration may result in reverse operation of the Na^+/Ca^{2+} exchanger as a compensatory change, which under normal circumstances extrudes Ca^{2+} from the intracellular space in exchange for Na^+. Reverse operation may produce deleterious elevations in intra-axonal Ca^{2+} concentrations, with resultant initiation of degradative cascades [48]. In further support of the potential injurious role of Na^+ in axonal degeneration, pharmacological enhancement of Na^+ entry into mammalian white matter exacerbated tissue destruction, while an environment devoid of Na^+ was protective against anoxic damage [49].

Raised cytoplasmic Na^+ concentrations may also lead to neurodegeneration through alternative pathways. Specifically, increased Na^+ concentration may increase energy demands through increased activity of the Na^+/K^+ ATPase [50]. Moreover, progressive loss of motor units in ALS may result in compensatory overuse of surviving motor units, thereby further increasing demand for ATP. Na^+/K^+ ATPase activity may appear extinguished in α-motor neurons from transgenic *SOD-1* mice [51], although it would appear overactive in patients with sporadic disease [50].

While the implications of increased persistent Na^+ conductances in ALS would appear almost certain, the origins, and clinical and pathophysiological consequences of K^+ channel dysfunction in ALS remain elusive. Axonal excitability studies have repeatedly shown that ALS patients demonstrate impairments in K^+ conductances, principally reflected by reduction in S2 accommodation in depolarizing threshold electrotonus (Figure 2A) and increased superexcitability in recovery cycle (Figure 2B) [45,46].

Neuroimaging may facilitate improved understanding of cortical dysfunction

Neuroimaging studies, such as diffusion-tensor imaging, have traditionally examined specific

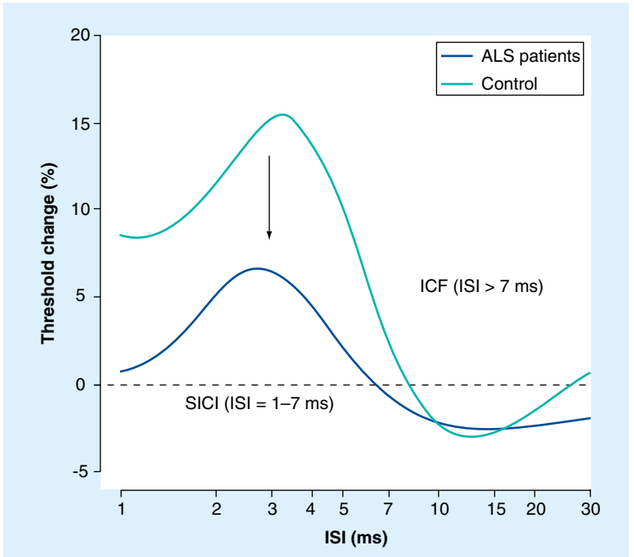

Figure 1. Abnormalities in cortical excitability in amyotrophic lateral sclerosis patients compared with healthy controls. SICI, reflecting $GABA_A$ receptor-driven processes mediated by inhibitory interneurons, is reduced in ALS patients.
ALS: Amyotrophic lateral sclerosis; ICF: Intra-cortical facilitation; ISI: Inter-stimulus interval; SICI: Short interval intracortical inhibition.

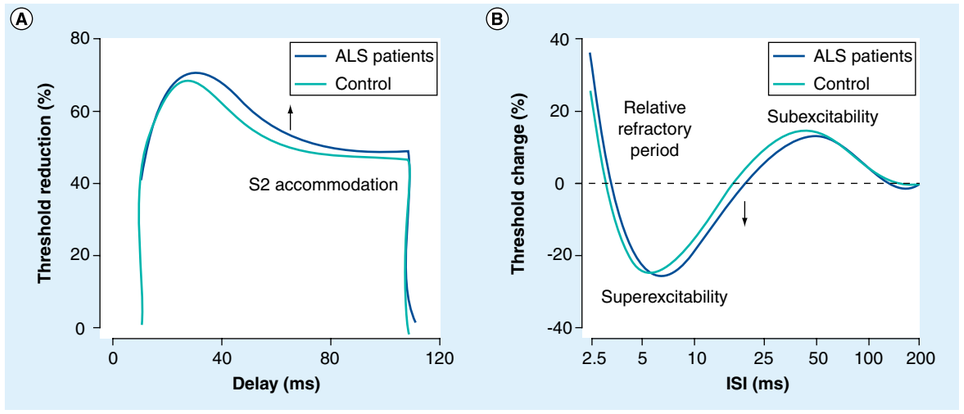

Figure 2. Abnormalities in peripheral nerve excitability in amyotrophic lateral sclerosis patients compared with healthy controls. (A) Depolarizing threshold electrotonus, reflecting the electrotonic changes in axonal membrane potential in response to subthreshold conditioning stimulation. S2 accommodation is reduced in the ALS patients compared, as indicated by a downward shift in the depolarizing threshold electrotonus trace (delays of 40–110 ms), suggesting dysfunction of internodal slow K^+ channels. **(B)** Changes in depolarizing threshold electrotonus are mirrored in the recovery cycle, which describes the recovery of axonal membrane excitability following depolarization, with changes in ALS manifesting as increased superexcitability (defined by where the recovery cycle is below the zero line).
ALS: Amyotrophic lateral sclerosis; ISI: Inter-stimulus interval.

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) [52]. However, more recent neuroimaging studies have enabled a global understanding of cortical dysfunction in ALS. Transcallosal dysfunction has been detected in structural and functional neuroimaging studies [53–55]. The emergence of 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 [56,57]. 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, ^{1}H-NMR spectroscopy (^{1}HNMRS) exploits how proton-containing metabolites react to magnetic radiation to probe *in vivo* biochemical pathology in the CNS. ^{1}HNMRS estimates the concentrations of different molecules in any given region of interest in the CNS. Metabolites of relevance to neurodegenerative disorders are N-acetylaspartate, a molecule present in the neuronal cytoplasm, and choline-containing compounds, which are markers of plasma membrane integrity, with the N-acetylaspartate:choline ratio typically reported to reduce within subject variability. ALS patients demonstrate reduction in the N-acetylaspartate:choline ratio in central motor regions consistent with neuronal loss [60–62]. ^{1}HNMRS has also revealed reduction in this ratio in asymptomatic individuals harboring mutations in the *SOD-1* 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: craniobulbar, 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]. Neuroprotection 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 [70,71]. To mimic the incidence of disease in a clinic population in a diagnostic accuracy study, recruitment of controls should arise exclusively from the same clinical 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.

Many serum and CSF biomarkers have been investigated for ALS, but to date none have been successfully applied to clinical practice [22]. Although conventional MRI sequences appear to be of little diagnostic value in ALS [72], emerging neuroimaging biomarkers demonstrate diagnostic potential given that they enable noninvasive, *in vivo* exploration of structural and functional changes in the CNS, especially in the early stages of disease [73]. 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 [74].

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 [75]. Furthermore, investigation of cortical hyperexcitability using a novel threshold-tracking approach to TMS has shown that, compared with patients with ALS-mimic disorders, cortical hyperexcitability appears unique to ALS [42,76,77]. Capitalizing on the early occurrence of cortical hyperexcitability in ALS, threshold-tracking TMS may be useful in distinguishing ALS from mimic disorders [78]. 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 biomarker 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 [20].'

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 biomarkers 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 [71].

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

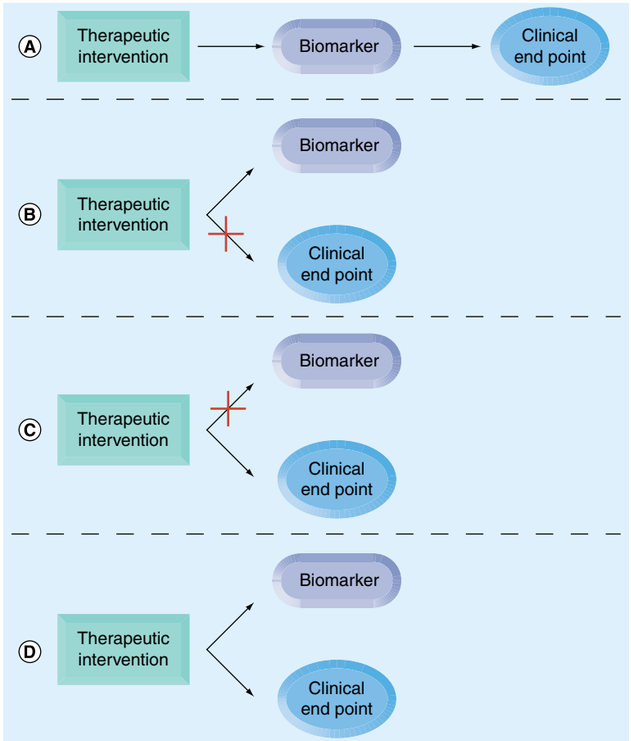

Figure 3. Effects of therapeutic interventions on biomarkers and clinical end points in drug trials. (A) The therapeutic intervention affects the biomarker, which is causally related to the clinical outcome. This would be considered a suitable biomarker. **(B)** The therapeutic intervention modulates 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 [7]. On the other hand, use of functional scales as primary outcome measures may reduce sample size and trial duration [71]. 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 [79].

■ 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 E_2 levels [80]. 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 [81]. These studies have highlighted that blood biomarkers were obtainable in a clinical setting, but the feasibility of performing sequential lumbar 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 [82]. 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 [83], celecoxib [80,84] and memantine [85], 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 [86]. 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.

■ Neurophysiological index

As a simple measure of peripheral disease burden, the neurophysiological index (NI; calculated using the formula: compound muscle action potential amplitude [mV] × F-wave frequency [%]/distal motor latency) may be a suitable alternative to MUNE [87]. 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 [88]. NI was also employed in a trial of memantine, declining at a linear rate, similar to MUNE [85], 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 [89].

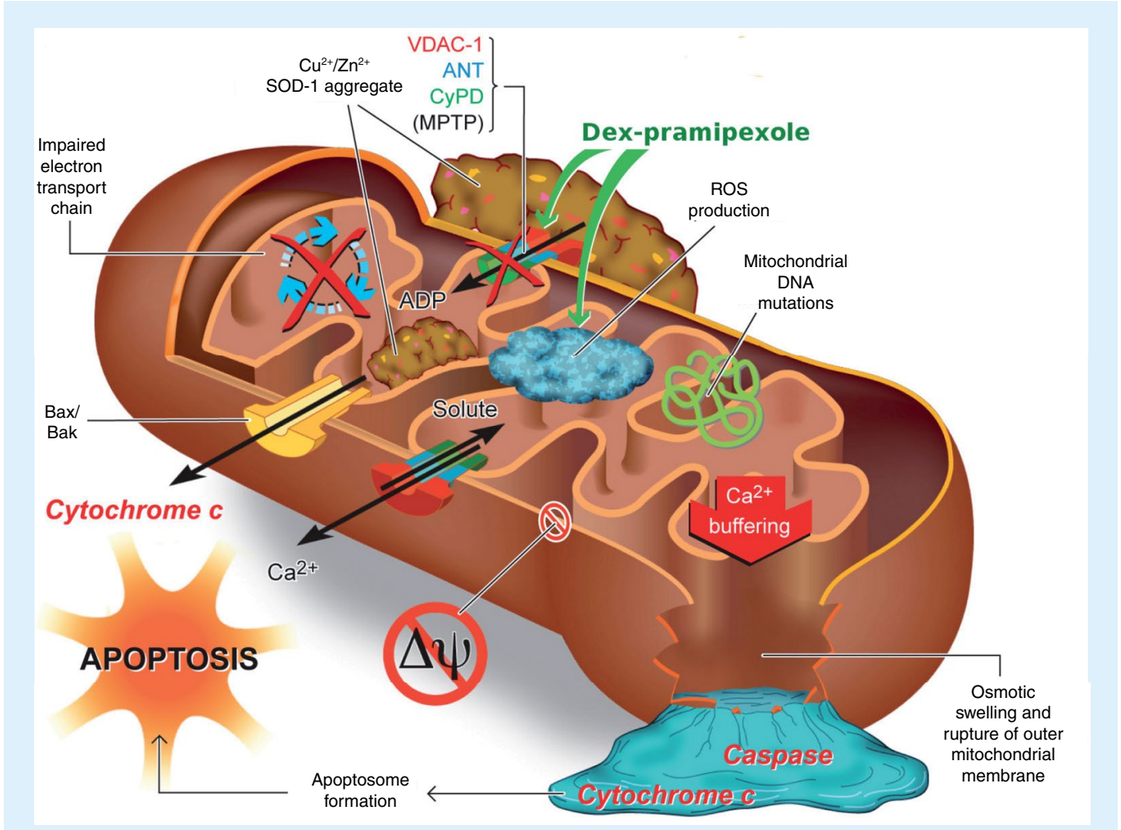

Figure 4. Summary of amyotrophic lateral sclerosis pathophysiology, pathological processes related to mitochondrial dysfunction in amyotrophic lateral sclerosis and sites of action of dex-pramipexole. Failure of cytoplasmic mitochondria induces increased susceptibility to glutamate-mediated excitotoxicity, 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, potentially 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. Displacement 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 *SOD-1* 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.
ΔΨ: Mitochondrial membrane potential; ROS: Reactive oxygen species.

■ Electrical impedance myography

Electrical impedance myography is a noninvasive, 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 [90]. Unlike electrophysiological techniques that evaluate the electrical potential of an excitable tissue, electrical impedance myography assesses the flow of electrical current through restricted portions of skeletal 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 cylindrical myocytes 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 [91]. 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 isometric contraction [92]. 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 [22]. 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 LMN or UMN syndromes, patients with other neurological disorders and healthy subjects, is currently being undertaken [101]. 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 [102]. This study has a recruitment target of 120 patients, with a scheduled date of completion of December 2011.

Much interest is developing around a medication currently in Phase III development for ALS. The clinical efficacy of dex-pramipexole, the R(+) enantiomer of the anti-Parkinsonian drug, pramipexole, is being investigated in an international clinical trial for which 943 ALS patients have been recruited [103]. Although the precise pharmacological behavior of dex-pramipexole remains poorly characterized, it likely inhibits neuronal apoptosis by interfering with mitochondrial permeability transition and free radical production (Figure 4) [93]. Mitochondrial permeability transition is a state wherein the mitochondrial membrane potential is lost [94]. Maintenance of the mitochondrial membrane potential is essential for driving ATP production, and its loss thereby results in disruption of mitochondrial and cellular homeostasis, as well as leading to apoptosis. Dex-pramipexole has been repeatedly associated with attenuation of functional decline and improved respiratory function in ALS patients [88]. Importantly, this treatment effect was demonstrated in a placebo-controlled, Phase II trial in a dose-dependent manner, with 300 mg/day the optimal dose [95]. The primary end point of the Phase III trial represents a novel approach to understanding ALS disease progression in that it represents a joint rank of ALSFRS-R adjusted for mortality, with slow vital capacity and muscle strength as secondary end points. The scheduled date of completion for this trial is February 2013.

The development of effective biomarkers is likely to facilitate progress in our understanding of ALS, but methodological issues remain. Regardless of how a biomarker will be used, future techniques must exploit rapid advances in computing power to implement advanced bioinformatic strategies tailored to disentangle signal from noise. In doing so, it may be possible to overcome the deluge of data arising from biomarker studies, as exemplified by the increasing popularity of genome-wide association [96] and exome-sequencing studies [12]. Nonetheless, such endeavors in isolation will not be sufficient to advance the current understanding about ALS. Only co-coordinated approaches, by means of multicenter collaborations, will ensure that sufficient patients are recruited for case–control studies, diagnostic accuracy studies and therapeutic trials, so that questions concerning the utility of specific biomarkers may be unequivocally answered in ALS. From a regulatory perspective, biomarker discovery based on high-quality evidence will ensure more efficient translation from benchtop to bedside [97].

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 surrogate end points for use in clinical trials.
- In practice, ALS is a clinical diagnosis, although additional investigations are undertaken to exclude alternative diagnoses. The revised El Escorial criteria for ALS remain a research tool for enrolling patients into research studies. Despite their improved sensitivity towards early disease over the original El Escorial criteria, the Awaji criteria may be perceived as cumbersome in nature.
- Diagnostic accuracy studies of biomarkers in ALS remain few and far between, and their methodology is not well understood by the research community.
- Neuroimaging biomarker studies are transitioning towards more global approaches to understanding cortical dysfunction in ALS.
- Threshold-tracking TMS demonstrates diagnostic potential in identifying ALS in a mixed neuromuscular cohort.
- 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 electrophysiological biomarkers.

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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, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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- ■■ of considerable interest

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■■ **Details the most recent discovery concerning a novel gene mutation responsible for familial amyotrophic lateral sclerosis (ALS), with emphasis on Finnish ALS patients.**

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