

The adult-onset neurodegenerative disorder amyotrophic lateral sclerosis (ALS) is life-shortening for majority of those affected [1]. Therapeutic trials relying on traditional endpoints such as tracheostomy-free survival, or significant reduction in the slope of decline of the revised ALS Functional Rating Score (ALSFRS-R), entail costly studies which last up to 18 months. Rapid advances in the understanding of the molecular biology of ALS are making the prospect of disease-modifying therapy increasingly likely, so that the need for objective markers of disease activity has never been greater.

ALS and frontotemporal dementia (FTD) lie at the ends of a clinical spectrum. Nearly all cases of ALS and half of those with FTD share a pathological signature comprising neuronal and glial cytoplasmic inclusions of the 43kDa transactive response DNA-binding protein (TDP-43). However, variably phosphorylated and truncated forms of TDP-43 are found across different tissue compartments in ALS. As a result, antibody specificity and limited sensitivity of biofluid immunoassays currently make this a very challenging *in vivo* biomarker target. [2]. A broader range of biomarker candidates have nonetheless arisen by extending beyond the signature of ALS at the cellular level, to both structural and functional changes at the level of the motor *system* and its wider frontotemporal connections. Biomarkers have a range of possible roles in ALS, but

most frequently published are those purported to have diagnostic or therapeutic monitoring potential. Among the frontrunners such as neurofilaments, the focus is now on how candidates address key barriers to ALS therapy development.

The future of neurofilaments

Multiple studies, spanning more than two decades [3], have shown that neurofilament light chain (NfL) and phosphorylated heavy chain (pNfH) are significantly raised in cases of ALS compared to healthy controls [4], presumed to reflect axonal degeneration. However, the question facing the ALS specialist in the clinic is not whether the individual before them is healthy. The average delay of one year from symptom onset to reaching an ALS specialist means that the majority of cases will provide a clear history of progressive weakness, with clinically detectable signs of upper and lower motor neurone degeneration. The diagnosis of ALS can then be safely made entirely on clinical grounds, with few plausible structural or metabolic mimicking causes to concern the experienced neurologist [5]. The resistant diagnostic delay in ALS is multi-factorial. It may involve initial denial of symptoms, compounded by failure of the primary care physician to recognize nervous system involvement, or the gravity of a disease they may encounter once in their career. As such, the yield from an ALS diagnostic biomarker with near 100% sensitivity and specificity

applied in the primary care setting would be minimal. It is clear that raised CSF neurofilaments are found in a broad range of CNS inflammatory as well as neurodegenerative disorders, and the application of blood-based testing in primary care across a range of potentially neurological symptoms is an exciting wider possibility worthy of investigation.

Of greatest impact for the diagnostic pathway in ALS is a biomarker relevant to clinical presentations that cause diagnostic difficulty for the specialist. These are typically more slowly-progressive, with only lower motor neurone signs detectable on examination, and regionally-limited weakness e.g. single limb. Such individuals, despite harboring TDP-43 pathology, may be denied enrollment in therapeutic trials because they do not fit existing criteria that are reliant on clinical upper motor neurone signs [6], even though sub-clinical corticospinal tract pathology is accepted to be present [7,8]. Undoubtedly, CSF neurofilament levels do seem to correlate most strongly with upper motor neurone pathology [9,10].

Diagnostic biomarker studies have thus begun to address the performance in distinguishing ALS from relevant disease, rather than healthy controls. There is cause for optimism from a large single-centre study involving a range of disease controls [11] and multi-centre studies of the early symptomatic period [12].

However, the diagnostic performance of neurofilaments against

both upper and lower motor neurone-predominant ALS presentations still needs specifically testing. Ideally this will be a multi-centre study, focusing on cases selected as initially uncertain to ALS specialists (as the gatekeepers to trial enrollment).

Diagnostics aside, it is the wealth of evidence that CSF neurofilament levels reflect the rate of disability progression in ALS that is driving increasing hope of an objective outcome measure in trials. The observation that CSF NfL levels measured longitudinally remain relatively stable for an individual, strengthens the appeal as a pharmacodynamic marker [13]. Applied in this way, a significant reduction in group mean CSF neurofilament level in response to treatment over a period of only a few months might provide justification for proceeding to a larger phase III study or help to guide optimal dosing. Conversely, failure to demonstrate lowering of group mean level might allow a faster “no-go” decision and a smaller group size.

The appeal of a more minimally-invasive biomarker is obvious, but the sensitivity of blood neurofilament assay has so far proved significantly lower in comparison to CSF. Urinary sampling would be potentially much more convenient, and urinary levels of the extracellular domain of the neurotrophic receptor p75 are a promising new candidate biomarker of disease progression in ALS [14], though with possibly greater variability in the slope of serial

measurements compared to neurofilaments. Glial markers, in particular those reflecting neuroinflammatory responses, are another area of growing interest with the identification of CSF chitinases thought to reflect macrophage activation. Like neurofilaments, they appear to show a strong relationship with rate of ALS progression [15-17].

Systems-level physiological biomarkers

ALS involves a non-random disintegration of the motor system and its frontotemporal connections, with significant phenotypic heterogeneity [18]. Although the downstream muscle wasting effects of lower motor neurone degeneration are often the most clinically apparent, ALS has become recognised as involving a significantly brain-based pathology operating at a network level. Advanced MRI has been the leading tool to explore these functional as well as structural changes *in vivo* [19,20].

ALS is consistently associated with cortical hyperexcitability. In this regard, short-interval paired transcranial magnetic stimulation has high sensitivity and specificity [21], but application beyond ALS diagnostics is uncertain. The 'downstream' neurophysiological marker of motor unit quantification, in its most advanced form as the Motor Unit Number Index (MUNIX), appears to be more sensitive than the current gold standard marker of disease

progression (ALSFRS-R) [22]. Though limited to the study of the lower motor neurone component of ALS pathology, MUNIX is sensitive to clinically unaffected muscles [23,24] and a leading therapeutic monitoring biomarker candidate as such.

Capturing the subtler changes in neurophysiology, which might provide much earlier proof-of-principle in novel drug studies, requires tools more sensitive to the motor system as a whole.

Magnetoencephalography (MEG) in ALS have demonstrated profound changes in cortical neuronal oscillations in the beta band frequency during motor activity. Evidence of milder changes were also found in asymptomatic carriers of ALS-causing genetic mutations [25]. Moreover, it has been possible to link the activity of cortical and lower motor neurone systems through MEG-based measures of corticomuscular coherence [26]. The sensitivity to change of these emerging biomarkers is not yet known, and the limited availability of hardware currently constrains its translational potential. However, the same principles of cortical physiology are being explored through developments in the analysis of more routinely accessible electroencephalographic data [27].

Genetic biomarkers

One in 10 cases of ALS and FTD are associated with an intronic hexanucleotide GGGGCC repeat expansion (HRE) in *C9orf72*.

Developments in the synthesis and delivery of anti-sense oligonucleotides are poised to bring these and other monogenetic forms of ALS and FTD within reach of therapeutic trials [28]. Such trials, independent of the ultimate goal of a therapeutic response, will benefit greatly from biomarkers that provide evidence of effective target engagement. In the case of *C9orf72* HRE carriers, repeat-associated non-AUG (RAN) translation produces dipeptide repeat proteins (DPRs). While a direct role in pathogenesis is currently uncertain, DPRs are detectable in the CSF of asymptomatic as well as affected carriers [29], and therefore the leading biomarker candidate.

The unprecedented pace of discovery in neuroscience makes the consideration of preventative therapy administered to pre-symptomatic individuals at high risk of neurodegenerative disorders a tangible aspiration. It will depend on building high confidence in the neuroprotective effects of drugs without sole reliance on lengthy study periods based solely on rates of symptomatic conversion. This vision may require novel combinations of molecular and physiological biomarkers, and ALS makes a strong case for this combined cell and system approach.

References

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