Review

Neurofilaments and Beyond: Multi-Modal Biomarkers and the Hidden Biology of ALS

Neurofilaments et au-delà : biomarqueurs multimodaux et biologie cachée de la SLA

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Abstract | Résumé

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by progressive upper and lower motor neuron loss and death from respiratory failure within a few years of symptom onset. The marked clinical and biological heterogeneity of ALS has hindered the development of effective therapies, prompting intense interest in biomarkers that can improve diagnosis, prognostication, and trial efficiency. This review summarizes recent advances in fluid, imaging, genetic, and digital biomarkers of ALS, with a particular focus on neurofilament light chain as a leading candidate for prognostic and pharmacodynamic use. It highlights how current biomarkers, while promising, remain imperfect surrogates for underlying disease biology, and often expose fundamental gaps in understanding ALS pathogenesis. It argues that to fully realize the potential of biomarkers, the field must invest more heavily in mechanistic and longitudinal research that links biomarker dynamics to cellular pathways, genetics, and patient outcomes, ultimately enabling more rational, personalized therapeutic strategies.

La sclérose latérale amyotrophique (SLA) est une maladie neurodégénérative fatale caractérisée par la perte progressive des motoneurones supérieurs et inférieurs, entraînant le décès par insuffisance respiratoire quelques années après l'apparition des symptômes. L'hétérogénéité clinique et biologique marquée de la SLA a entravé le développement de thérapies efficaces, suscitant un intérêt considérable pour les biomarqueurs susceptibles d'améliorer le diagnostic, le pronostic et l'efficacité des essais cliniques. Cette revue résume les avancées récentes concernant les biomarqueurs liquides, d'imagerie, génétiques et numériques de la SLA, en mettant particulièrement l'accent sur la chaîne légère du neurofilament (NF-L), considérée comme un candidat majeur pour des applications pronostiques et pharmacodynamiques. Elle souligne que les biomarqueurs actuels, bien que prometteurs, demeurent des substituts imparfaits de la biologie sous-jacente de la maladie et révèlent souvent des lacunes fondamentales dans la compréhension de la pathogenèse de la SLA. Elle soutient que, pour exploiter pleinement le potentiel des biomarqueurs, le domaine doit investir davantage dans des recherches mécanistiques et longitudinales reliant la dynamique des biomarqueurs aux voies cellulaires, à la génétique et aux issues cliniques, afin de permettre l'élaboration de stratégies thérapeutiques plus rationnelles et personnalisées.

Keywords: Amyotrophic lateral sclerosis, biomarkers, neurofilament light chain, imaging, clinical trials, neurodegeneration, research priorities

Introduction

Amyotrophic lateral sclerosis (ALS) is the most common adult-onset motor neuron disease and presents with progressive weakness, muscle wasting, spasticity, and eventual respiratory failure (1). Median survival is approximately two to five years from symptom onset, although individual trajectories vary widely, from fulminant disease to slow progression over a decade (1). Currently approved disease-modifying therapies, including riluzole and edaravone, provide at best modest extensions in survival or slowing of functional decline, leaving an urgent unmet need for more effective treatments (2).

Heterogeneity in clinical presentation, genetics, and rate of progression complicates both diagnosis and therapeutic development (1). This variability reflects the multifactorial nature of ALS pathology, encompassing protein misfolding and aggregation, glutamate-mediated excitotoxicity, mitochondrial dysfunction, impaired RNA metabolism, and neuroinflammation, with the relative contribution of these mechanisms differing between patients. Diagnostic delay often exceeds 12 months, during which time patients may already have substantial motor neuron loss, reducing the window in which neuroprotective interventions could be effective (1). Delay is further compounded by the initially focal and frequently subclinical spread of neurodegeneration, the overlap of early symptoms with more common musculoskeletal or neuromuscular conditions, and the absence of specific biomarkers in routine clinical practice.

Traditional trial endpoints such as survival and functional scales are slow to change and are strongly influenced by baseline disease stage and progression rate, making large, lengthy, and expensive trials necessary to detect treatment effects (3). These challenges explain why robust biomarkers are central to the next phase of ALS research and why their development must go hand in hand with deeper study of disease mechanisms (3).

What counts as a biomarker in ALS?

Biomarkers are measured indicators of normal biological, pathogenic processes, or responses to therapeutic interventions (4). In ALS, candidate biomarkers span multiple categories, including diagnostic biomarkers, which help distinguish ALS from mimicking conditions, prognostic biomarkers, which predict disease courses independent of treatment, and predictive and pharmacodynamic biomarkers, which indicate likely response to therapy or capture biological effects of an intervention (4). For example, cerebrospinal fluid or blood neurofilament light chain (NfL) supports both diagnosis and prognosis, higher baseline NfL levels predict faster functional decline and shorter survival, and reductions in NfL or mutant protein levels in response to a targeted therapy can serve as pharmacodynamic readouts in clinical trials (1, 5-7).

Biomarkers can be derived from blood and cerebrospinal fluid (CSF), neuroimaging, electrophysiology, genetics, and digital or computational measures such as speech and movement patterns (5). The recent literature emphasizes that no single biomarker is sufficient; instead, multi-modal panels integrating fluid, imaging, electrophysiological, and digital measures are likely needed to capture the complexity of ALS and to link underlying biology with clinical outcomes (3, 5-7).

Fluid biomarkers: neurofilaments and beyond

Neurofilament light chain (NfL) has emerged as the most extensively validated fluid biomarker in ALS (8). NfL is a structural protein of myelinated axons, released into CSF and blood when axonal damage occurs. In ALS, CSF and blood NfL concentrations are several-fold higher than in healthy controls and ALS mimics, with levels often approximately 4–10 times those seen in controls, and higher NfL values are associated with faster functional decline and shorter survival (8). Ultrasensitive assays now allow reliable measurement of NfL in serum, which strongly correlates with CSF concentrations, making it practical for repeated sampling in large cohorts and clinical trials (9). Large cohort studies show that baseline serum NfL levels predict subsequent decline in ALS Functional Rating Scale–Revised (ALSFRS-R) scores and overall survival, indicating robust prognostic utility (9).

In addition, reductions in NfL in response to experimental therapies, most notably SOD1-lowering antisense oligonucleotides, have been used as pharmacodynamic readouts, contributing to regulatory decisions and supporting its use as a surrogate of target engagement and possibly neuroprotection (8, 10). Consensus papers now argue that there is compelling evidence for NfL as a prognostic and response biomarker in ALS therapy development, even though formal regulatory qualification is still in progress (10). However, NfL also illustrates the limits of our current understanding. Elevated NfL reliably signals axonal injury but does not specify which molecular pathways are active or why some patients with high NfL progress more slowly than others (6, 9). This gap underscores the need for mechanistic studies linking NfL kinetics to cell-type-specific pathology, genetics, and other biomarkers, rather than relying on NfL as a black-box marker of neurodegeneration.

Other protein and metabolic markers

Beyond neurofilaments, multiple protein and metabolic biomarkers are under investigation. CSF phosphorylated neurofilament heavy chain (pNfH) may have slightly better specificity for differentiating ALS from mimics in early disease, though serum pNfH has shown more variable performance (7). This likely reflects that pNfH in CSF more directly reflects neuroaxonal damage within the central nervous system, whereas blood concentrations are influenced by additional biological and analytical factors leading to greater variability in serum pNfH performance. The TAR DNA-binding protein 43 (TDP-43), the major protein component of pathological inclusions in most ALS cases involved in RNA processing, gene expressing and neuron function, can be detected in plasma and CSF, where higher levels appear to be associated with faster disease progression and may decline as disease advances, suggesting possible roles as both monitoring and prognostic markers (11).

Markers of muscle and systemic metabolism also show promise (11, 12). Plasma creatinine, a proxy for muscle mass and metabolism, declines over time in ALS and has shown lower variability than ALSFRS-R in some studies, supporting its potential as a surrogate endpoint for disease progression (11). Inflammatory proteins and complement components, as well as cytokine profiles reflecting peripheral immune activation, correlate with disease severity and rate of decline, but reproducibility across cohorts and platforms remains a major challenge (12). Recent work has also explored retroviral elements such as HERV-K (the Human Endogenous RetroVirus-family K, that can become reactivated in neurons in ALS), oxidative stress markers, and metabolic signatures, yet these remain at earlier stages of validation (3, 12). Together, these fluid biomarkers highlight a critical point: they reveal that ALS is not purely a motor neuron problem but a systemic disorder involving immune, metabolic, and muscle changes, and thus motivate further research into how these systems interact over the course of the disease.

Imaging biomarkers: seeing ALS in the brain and spinal cord

Neuroimaging offers a complementary window into ALS pathology (13). Structural MRI consistently demonstrates atrophy of the primary motor cortex and corticospinal tract, along with involvement of extra-motor regions in many patients, particularly those with cognitive or behavioral impairment (1, 13). Diffusion tensor imaging (DTI) metrics, such as reduced fractional anisotropy along the corticospinal tract and corpus callosum, correlate with disease severity and can differentiate ALS from

controls at the group level (13).

More advanced techniques, including functional MRI and PET, are uncovering alterations in functional connectivity and metabolic activity across motor and frontotemporal networks (13). PET tracers targeting neuroinflammation (e.g. TSPO ligands) or synaptic markers can detect microglial activation and synaptic loss in vivo, potentially serving as mechanistic and pharmacodynamic biomarkers (13). However, variability across scanners, protocols, and analysis pipelines, along with limited accessibility and high cost, has slowed translation into routine clinical practice and large multicenter trials (13).

The current generation of imaging biomarkers thus underscores both progress and limitation: they visualize ALS-related changes in living patients, but often lack disease specificity, standardization, and individual-level predictive power (13). Addressing these issues will require coordinated, longitudinal imaging studies linked tightly to fluid biomarkers, genetics, and detailed clinical phenotyping (3, 5, 13).

Genetic and digital biomarkers

Genetic testing is now an integral component of ALS evaluation in many centers, particularly for patients with a family history or early onset (1). Pathogenic variants in ALS-associated genes, such as the superoxide dismutase 1 gene (SOD1), which encodes the SOD1 enzyme that protects cells from oxidative stress, and C9orf7, a hexanucleotide repeat expansion, can inform counselling and, in selected contexts, guide therapeutic decisions as gene-targeted approaches emerge (1,3,8). Although only a minority of ALS cases have a clear family history and known pathogenic variants explain only a fraction of apparently sporadic disease, such genetic findings still represent an important entry point for precision therapies and mechanistic studies (1,3,5,6). From a biomarker perspective, these variants act as risk and stratification markers, defining biologically distinct subgroups for trial enrichment and mechanistic work (3,5-6)

Digital biomarkers, derived from speech analysis, kinematic assessments, wearable sensors, and smartphone-based tasks, represent a rapidly expanding frontier (5-14). Early studies suggest that quantitative measures of speech rate, articulatory precision, limb acceleration, and fine motor control can capture subtle changes before they are apparent on traditional scales, offering high-frequency, low-burden longitudinal monitoring (14). Nevertheless, most digital biomarkers remain in proof-of-concept stages, and their validation across devices, languages, and real-world environments is incomplete (5,14). Importantly, the emergence of genetic and digital biomarkers reinforces the need for intensive basic and translational work: genetic markers raise mechanistic questions about how specific variants drive degeneration, while digital outputs must be linked back to underlying neurobiology if they are to do more than describe surface-level function (14, 15).

Biomarkers in clinical trials: success and limitations

The integration of biomarkers into ALS clinical trials has accelerated over the past decade (3). NfL is now frequently included as a secondary or exploratory endpoint, enabling early readouts of biological effect that may precede changes in ALSFRS-R or survival (3,8,10). Systematic reviews emphasize that biomarkers can improve trial design by enabling the following three aspects (3). Enrichment of cohorts for faster progressors, increasing statistical power (3). Stratification based on biology (e.g. genetic status, baseline NfL) to reduce heterogeneity (3,8,10). Finally, adaptive designs where interim biomarker changes inform continuation, modification, or termination of trial arms (3).

Recent trials have used biomarker-based inclusion criteria, such as minimum NfL levels or specific genetic variants, and have interpreted NfL reductions as supportive evidence of target engagement and potential efficacy (8, 10). These developments represent a shift towards more efficient, biology-driven trials and demonstrate how biomarker research can directly impact therapy development (3).

At the same time, biomarker use in trials highlight several gaps (3-12). Many candidate biomarkers show promising associations but lack standardized assays, reference ranges, or clear thresholds for clinical decision-making (3, 12). Moreover, a biomarker's responsiveness to treatment does not guarantee that modifying the measured process will yield meaningful clinical benefit, especially in a multifactorial disease like ALS (15). Future work must therefore embed mechanistic studies and multi-marker panels within trials, so that biomarker dynamics are interpreted in the context of pathways and networks rather than isolated analytes (3,15).

How biomarkers reveal that ALS is under-studied

A central theme emerging from the biomarker literature is that our current tools, while increasingly sensitive, often raise more questions than they answer about ALS biology (6, 12). For instance, NfL clearly reflects axonal damage, yet current research lacks a detailed understanding of why some genetic backgrounds or environmental exposures yield higher or lower NfL at similar clinical stages, or how NfL trajectories differ between phenotypes such as bulbar-onset and limb-onset disease (6, 8).

Similarly, inflammation-related biomarkers reveal robust immune activation, but the relative contributions of peripheral versus central immune responses and the balance between neuroprotective, and neurotoxic glial states, remain incompletely defined (12). Imaging shows widespread network involvement beyond the motor system; however, a full understanding of how these changes relate to cognitive and behavioral symptoms, as well as non-motor manifestations such as pain, weight loss, and autonomic dysfunction, remains lacking (1, 13).

These gaps reflect a broader issue: ALS has historically received less research attention and funding than more prevalent neurodegenerative diseases, despite its devastating prognosis (1).

To move beyond descriptive biomarkers towards truly mechanistic, predictive, and actionable tools, the field must invest more in longitudinal, multi-modal cohort studies that track patients from pre-symptomatic or very early stages through the disease course, integrating fluid, imaging, genetic, and digital data (3, 5). Complementary experimental models are equally important: patient-derived cell-based systems and induced pluripotent stem cell–derived motor neurons and glia, as well as genetically engineered animal models, can be used to test how ALS-associated variants or environmental stresses influence candidate biomarkers and to dissect cell-type specific pathways that are difficult to resolve in vivo (5, 6, 15). More investments should as well be considered for basic research linking biomarker alterations to cellular pathways in neurons and glia, using models that incorporate patient-specific genetics (15). Diverse, global cohorts to understand how ancestry, environment, and healthcare access shape biomarker profiles and ALS risk are likewise important to consider (1,3). In this sense, biomarkers are not just tools for trials; they are signals pointing to where ALS remains under-studied and where deeper investigation is most urgently needed (3,12,15).

Conclusion

Biomarker research has transformed the conceptual and practical landscape of ALS by providing objective measures of neurodegeneration, prognosis, and therapeutic response, with neurofilament light chain at the forefront (8, 9). Fluid, imaging, genetic, and digital biomarkers collectively demonstrate that ALS is a multisystem, heterogeneous disease and are beginning to enable more efficient, biology-driven clinical trials (1, 3, 5, 13, 14). At the same time, the limitations and unanswered questions surrounding these biomarkers expose how much remains unknown about ALS pathogenesis, progression, and response to treatment (6, 8, 12, 13, 15). To fully realize the promise of biomarkers, future ALS research must emphasize longitudinal, multi-modal studies, mechanistic work that ties biomarker dynamics to cellular pathways, and inclusive cohorts that capture global diversity (3, 5, 15). Such efforts will not only refine biomarker panels but also deepen our understanding of ALS itself, ultimately supporting the development of rational, personalized combination therapies and, crucially, offering patients earlier diagnosis, better prognostication, and more effective treatment options.

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