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Review Article

Protein biomarkers for the diagnosis and prognosis of Amyotrophic Lateral Sclerosis

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ABSTRACT

Amyotrophic Lateral Sclerosis (ALS) is the most common motor neuron disease, still incurable. The disease is highly heterogenous both genetically and phenotypically. Therefore, developing efficacious treatments is challenging in many aspects because it is difficult to predict the rate of disease progression and stratify the patients to minimize statistical variability in clinical studies. Moreover, there is a lack of sensitive measures of therapeutic effect to assess whether a pharmacological intervention ameliorates the disease. There is also urgency of markers that reflect a molecular mechanism dysregulated by ALS pathology and can be rescued when a treatment relieves the condition. Here, we summarize and discuss biomarkers tested in multicentered studies and across different laboratories like neurofilaments, the most used marker in ALS clinical studies, neuroinflammatory-related proteins, $p75^{ECD}$, p-Tau/t-Tau, and UCHL1. We also explore the applicability of muscle proteins and extracellular vesicles as potential biomarkers.

1. Introduction

Motor neuron diseases designate a heterogeneous group of pathologies characterized by the selective and primary progressive involvement of motor neurons, a cell population that controls movement. Motor neurons are present in the primary motor cortex, brainstem, and the anterior horns of the spinal cord. Their progressive degeneration causes muscle cramps with fasciculations, atrophy, worsening paralysis with difficulties in walking and in the use of the upper limbs, leading to complete tetraplegia, dysarthria that evolves into anarthria, dysphagia which involves malnutrition, and respiratory failure-inducing death (Feldman et al., 2022; Goutman et al., 2022). Sensory symptoms are typically absent, while in a variable percentage of patients, there are clinical manifestations of cognitive involvement that can lead to a diagnosis of frontotemporal dementia (Feldman et al., 2022; van Es et al., 2017).

Motor neuron diseases are clinically variable: Amyotrophic Lateral Sclerosis is the most severe and is characterized by the involvement of both the upper and lower motor neurons (UMN and LMN); Primary Progressive Sclerosis with the exclusive participation of the upper motor neuron; Progressive Muscular Atrophy shows an elective degeneration of the lower motor neurons; the loss of the brainstem motor neurons induces progressive bulbar palsy. This subdivision is usually valid at the early stages of the disease. In the more advanced phases, the different diseases manifest with the typical symptoms of ALS (van Es et al., 2017).

ALS is a rare disease, presenting an incidence of 2–3 new cases per year per 100,000 inhabitants and a prevalence of 10–12 cases per 100,000 inhabitants; the risk of developing the disease increases with age, with a peak incidence between 65 and 75 years, it prevails in males, especially in the younger age groups. The estimated cumulative lifetime risk for developing ALS is 1:350 in men and 1:400 in women. Most cases are sporadic (90%), while 10% account for familial origin (Masrori and Van Damme, 2020).

In sporadic cases, 60% of the disease's risk is attributed to genetic mechanisms and 40% to environmental factors (sports, injuries, pesticides) (Al-Chalabi and Hardiman, 2013). Approximately 70% of patients present with limb weakness at disease onset, typically insidiously, asymmetrically, and distally, with subsequent progression in the same anatomical region and spread to the contiguous areas (Ravits and La Spada, 2009). Bulbar symptoms (difficulty in articulating speech,

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chewing, or swallowing) are present in 25% of patients, while the onset with involvement of the respiratory muscles is much rarer. Of note, extraocular movements are usually spared until the very advanced stages of the disease (Ravits and La Spada, 2009).

Despite the clinical heterogeneity, pathologically, ALS is characterized by increased cytoplasmic localization of phosphorylated TDP-43 (TAR DNA-binding protein 43) (Neumann et al., 2006). TDP-43 is a DNA and RNA binding protein with multiple functions comprising pre-mRNA splicing and translational regulation (Cordts et al., 2023). TDP-43 binds to thousands of RNAs in the cell (Lagier-Tourenne et al., 2010). The loss of function in RNA processing and its propensity to aggregate and form protein inclusions are mainly studied to understand ALS pathogenesis (Shefner et al., 2020; Vucic et al., 2021) better. Notably, this protein is also observed in 57% of patients with frontotemporal dementia and 50% of cases with Alzheimer's disease. The accumulation of intracellular inclusions is called the TDP-43 pathology (Neumann et al., 2006).

A single diagnostic test for ALS is not available. Currently, the diagnosis is based on a combination of anamnestic data, objective findings, and neurophysiological characteristics and on the exclusion of other potential pathologies that may partially mimic the clinical picture (e.g., trunk neoplasms, compressive myelopathy). (Goutman et al., 2022). Clinical examination reveals a combination of the typical signs of involvement of upper (paralysis, spastic hypertension, osteotendinous hyperreflexia) and lower motor neurons (fasciculations, muscle atrophy, hypotonia, and areflexia). The original diagnostic criteria defined in 1990 in El Escorial and their revisions in 1998 in Airlie House (USA) and 2006 in Awaji-shima (Japan) were primarily based on the presence of signs of simultaneous involvement of UMN and LMN in one or more of the four body regions: bulbar, cervical, thoracic, and lumbosacral. From the various combinations, the following sub-categories were now identified: "suspected," "possible," "probable," "probable with laboratory support," and "definite" (van Es et al., 2017). However, using these criteria was insufficient for an early diagnosis or to meet the requirements for inclusion in therapeutic trials (Vucic et al., 2021). In September 2019, the most recent Gold Coast diagnostic criteria were defined, simplifying and allowing an earlier diagnosis of ALS. However, the diagnosis is still clinically based on (Shefner et al., 2020).

2. ALS protein biomarkers

Biological markers, also called biomarkers, are measurable variables that signal a normal, natural process, the presence of a disease state, or the pharmacologic response to therapy. Different types of biomarkers have been described, such as molecular, radiographic, or physiological markers (Califf, 2018). The NIH-FDA has identified seven categories of biomarkers related to BEST (Biomarkers, EndpointS, and other Tools) Resources, each with its specific application. The types of biomarkers are 1. susceptibility/risk, 2. diagnostic, 3. monitoring, 4. prognostic, and 5. predictive, 6. pharmacodynamic/response, and 7. safety (FDA-NIH Biomarker Working Group, 2016). All these classes have a critical role in the whole developmental process of new therapies.

In ALS, new biomarkers are warranted to diagnose the pathology early. Diagnosing a person with ALS takes an average of one year, and the disease is confirmed in its progression, excluding ALS mimic syndromes (Goutman et al., 2022). Biomarkers are needed to stratify patients for enrollment in clinical trials to evaluate the effects of treatment in a homogeneous population, particularly in a complex disorder like ALS, characterized by a spectrum of variable disease trajectories. It is also necessary to use biomarkers to predict the rate of disease progression and track biological responses to therapy (Brooks et al., 2000).

Nowadays, different types of biomarkers have been studied and proposed for ALS. Biochemical, imaging, and neurophysiological techniques are applied to study this pathology. Here, we will summarize the most discussed and validated molecular markers among different laboratories and measured in biofluids associated with ALS.

2.1. Neurofilaments

Neurofilaments are intermediate filaments with a diameter of approximately 10 nm that are found as exclusively cytoskeletal scaffolds of neurons in both the central nervous system (CNS) and peripheral nervous system (PNS) (Zucchi et al., 2020). They are major structural components of giant, myelinated axons but can also be present in cell bodies, dendrites, and synapses (Gürth et al., 2023). Four different subunits are the constituents of the neurofilaments' heteropolymers: light chain, medium chain, heavy chain, and α-internexin or peripherin, considering CNS or PNS, respectively (Benatar et al., 2022). Neurofilaments stabilize neuronal axons and their radial growth, enabling high-velocity nerve conduction (Khalil et al., 2018). Their turnover is poorly understood; the ubiquitin-proteasome system and autophagy have been proposed as mechanisms for their degradation (Rao et al., 2023). However, the mechanisms inducing their release into the bloodstream are not entirely uncovered; possibly, it is linked to axonal damage or degeneration. Other factors, such as an increased synthesis or turnover of the neurofilament proteins, an altered transport, or exosome release, have also been considered (Verde et al., 2023). As a result, neurofilament fractions can be detected in CSF and blood. It emerged that the measure of neurofilaments could be regarded as a non-specific marker of neuroaxonal injury, regardless of the causal pathway of neuronal degeneration (Verde et al., 2021).

Nevertheless, neurofilaments in biofluids, mainly CSF and blood, have been assessed in different diseases, including acute neurological conditions, infections, and neurodegenerative and neuroinflammatory diseases (Simrén et al., 2022). However, neurofilaments are considered the elective biomarkers for ALS for the surprisingly high levels observed in patients (Verde et al., 2021) (Table 1). The most vulnerable cell type in ALS is spinal motor neurons, which display large and long myelinated axons and are enriched in neurofilament fragments. Mutations in NFH and peripherin genes have been found in a few ALS patients (Verde et al., 2021), although it is unknown if their presence correlates with increased neurofilament levels (Figlewicz et al., 1994; Gros-Louis et al., 2004).

Many studies have been conducted to assess the robustness of neurofilament fragment measures in biofluids in ALS, considering both the neurofilament light chain (NfL) and phosphorylated neurofilament heavy chain (pNfH) as diagnostic, prognostic biomarkers and potentially also as pharmacodynamic biomarkers (Benatar et al., 2020; Poesen and Van Damme, 2019). Initially, the detection of neurofilament fragments was confirmed in the CSF, where the concentration of both NfL and pNfH can be easily quantified, and their levels are significantly higher in ALS patients than in controls (Abu-Rumeileh et al., 2020; Wilke et al., 2019). NfL is considered less specific to ALS than pNfH, which was proposed as the best biomarker for ALS in CSF by Poesen et al., 2017 (Poesen et al., 2017). At the same time, NfL correlates with the ALSFRS score, suggesting NfL in the CSF is a marker of ALS severity (Brodovitch et al., 2021).

Since lumbar puncture is an invasive procedure and is not part of the standard clinical practice, the possibility of translating the neurofilament fragments measurement from CSF to blood has been investigated with the observation that NfL is forty times less present in the bloodstream compared to the CSF (Verde et al., 2021). Nevertheless, the advent of sensitive techniques, like Simoa, allowed for examining the NF concentration in blood in longitudinal studies (Khalil et al., 2018; Zucchi et al., 2020). Notably, a moderately strong correlation exists between CSF and serum NfL levels (Alagaratnam et al., 2021; Gille et al., 2019). In contrast, such a correlation was not confirmed for pNfH levels (Benatar et al., 2019). The latter result is probably caused by the low dynamic range of the ELISA used to measure serum pNfH concentrations. ELISA methodological limitations resulted in more than 50% of the samples being outside the analytical curve, performed in a small cohort of patients (ten ALS, ten FTD, and ten control subjects) (Wilke et al., 2019).

Moreover, serum pNfH was reported not to add prognostic value to

Table 1
Summary of studies in which neurofilaments have been measured since 2016. Abbreviations: IPN=Inflammatory Peripheral Neuropathy, NHC=Neurologically Healthy Controls, eGFR=estimated Glomerular Filtration, PLS=Primary Lateral Sclerosis, MN=motor neurons, MND=motor neuron disease, OND=other neurodegenerative diseases.

Reference	Biofluid	Cohort of patients	Methodology	Take home message
Oeckl et al. (2016)	CSF	75 ALS 75 controls	ELISA	CSF NfL and pNfH are increased in ALS patients from fifteen centers
De Schaepdryver et al. (2018)	CSF Blood	85 ALS 31 ALS mimics 215 disease control	ELISA Optimized ELISA with biotin/streptavidin	CSF pNfH is higher in ALS patients and correlates with survival Serum pNfH correlates with CSF pNfH Serum pNfH ALS patients significantly overlap with levels of disease controls and ALS mimics pNfH does not associate with upper/lower MN dysfunction Serum pNfH has a weak but significant correlation with disease progression and survival
Poesen et al., 2017	CSF	220 ALS 316 OND 50 ALS mimics	pNfH (Biovendor, Brno, CzechRepublic; RD191138300R) NfL (UmanDiagnostics AB, Umea, Sweden; UD51001)	pNfH is a diagnostic biomarker pNfH and NfL levels correlate with the extent of UMN and LMN involvement.
Benatar et al. (2018)	CSF Blood	17 ALS 84 individuals at risk for ALS 10 phenoconverters 34 HC	Electrochemiluminescence immunoassey Electrochemiluminescence immunoassey	CSF NfL levels are higher in ALS patients than in controls and at-risk individuals and remain stable over time. Levels increase over HC in phenoconverters twelve months before symptoms onset Serum NfL levels are higher in ALS patients than in controls and at-risk individuals and remain stable over time Levels increase over HC in phenoconverters 12 months before symptoms onset
Gille et al. (2019)	Blood	149 ALS 19 ALS mimics 82 disease control	CSF NfL levels: enzyme-linked immunosorbent kit (UmanDiagnostics, Umea) Sweden Serum NfL levels: electrochemiluminescent assay	Strong correlation between serum NfL and CSF NfL levels Serum NfL correlates with UMN degeneration and disease progression rate
Verde et al. (2019)	Blood	124 ALS 44 disease control 50 controls 65 OND	Simoa	Serum NfL is higher in ALS than all other conditions (cut-off 62 pg/mL, 85.8% sensitivity, 81.8% specificity), except for Creutzfeldt-Jakob disease Serum NfL correlates with disease progression; higher levels are associated with shorter survival NfL remains stable over the disease course
Benatar et al. (2019)	CSF Blood	22 ALS 79 individuals at risk for ALS 14 phenoconverters 34 controls	Enzyme-linked immunosorbent assay Enzyme-linked immunosorbent assay	Longitudinal study, analysis of pNfH before and after phenoconversion CSF NfL and pNfH can discriminate against ALS patients CSF and serum pNfH levels are poorly correlated Serum NfL increases longitudinally before symptoms onset, and this has also been seen for pNfH In both serum and CSF: 6–12 months prior in SOD1 A4V mutation carriers, 2 and 3.5 years in FUS c.521del6 mutation and C9orf72 HRE, respectively Serum NfL distinguish between ALS and HC controls and at-risk individuals; pNfH levels overlap between the three groups
Thouvenot et al. (2020)	Blood	207 ALS 21 HC	Simoa	Serum NfL is higher in ALS patients; levels> 15 pm/mL discriminate ALS patients and control (94% severity, 100% specificity) Serum NfL is a strong and independent prognostic factor of death in ALS at the diagnosis and positively correlates with the ALSFRS-R rate of decline
Wilke et al. (2019)	CSF Blood	10 ALS 10 FTD 14 controls	Homebrew Simoa, commercially available Simoa, and ELISA Homebrew Simoa, commercially available Simoa, and ELISA	In CSF, pNfH concentrations are strongly correlated between the three analytical approaches CSF pNfH levels can differentiate ALS patients from controls Serum pNfH has a strong correlation between the two Simoa assays but not between Simoa and ELISA Serum pNfH correlates with CSF pNfH if measured by the two Simoa approaches
Abu-Rumeileh et al. (2020)	CSF	80 ALS 46 ALS mimics 43 HC	Enzyme-linked immunosorbent assay kits	CSF NfL levels are higher in ALS than in ALS mimics and control There is a positive correlation between NfL levels and the extent of LMN involvement; there is no significant correlation with UMN
Benatar et al. (2020)	Blood	229 ALS 20 PLS 11 progressive muscular atrophy	Simoa	Baseline serum NfL, but not pNfH, predicts the future ALSFRS-R slope and survival: clinically validated biomarker Serum NfL, and perhaps pNfH, potential pharmacodynamic biomarkers
De Schaepdryver et al. (2020)	Blood	383 ALS	-	Serum NfL is higher in women Serum NfL shows a weak significant correlation with disease progression rate; high serum NfL is associated with shorter survival
Dorst et al. (2020)	Blood	100 ALS	Simoa	Serum NfL positively associates with progression rate Fast progressors have higher NfL median levels and prognostic biomarkers
Falzone et al. (2020)	Blood	219 MND	ELISA	Serum pNfH is a negative independent prognostic factor of survival Pyramidal, bulbar, and classic phenotypes have higher pNfH

(*continued on next page*)

Table 1 (*continued*)

Reference	Biofluid	Cohort of patients	Methodology	Take home message
				than LMN and UMN involvement Higher serum pNfH levels in C9orf72 MND patients compared to non-C9orf72 MND Higher levels but not significant in MND patients with cognitive dysfunction
Behzadi et al. (2021)	CSF	234 ALS 44 ALS mimics	NfL: validated ELISA pNfH: in-house-developed ELISA	CSF NfL and pNfH levels are significantly higher in ALS patients and negatively correlated with survival
	Blood	9 controls	Simoa	Plasma NfL is significantly higher in ALS patients than controls Plasma NfL is significantly higher in ALS patients with bulbar onset than spinal onset Plasma NfL is significantly higher in patients with C9orf72HRE mutation than in patients with SOD1 mutation and worse survival Plasma NfL negatively correlates with survival and can differentiate between short and extended survival
Brodovitch et al. (2021)	CSF	20 ALS 17 IPN	R-PLEX Human Neurofilament L Antibody Set from MesoScale Discovery	CSF NfL is the best marker of ALS severity CSF NfL + CSF ICAM-1 + serum IFN-gamma: improved diagnostic performance
	Blood	60 ALS 94 HC 43 IPN	R-PLEX Human Neurofilament L Antibody Set from MesoScale Discovery	Serum NfL marker of disease progression rate
Kläppe et al. (2022)	CSF	150 ALS 28 ALS mimics 108 HC	UmanDiagnostics' sandwich enzyme-linked immunoassay (Umeå, Sweden; cat number 10–7001)	CSF NfL slowly decreases and becomes stable after that CSF NfL performs better than hs-cTnT as a diagnostic and prognostic biomarker
Vacchiano et al. (2021)	CSF	171 ALS 60 ALS mimics	Simoa	CSF NfL is higher in ALS patients, particularly in fast progressors than intermediate- and slow-progressors CSF NfL is a better diagnostic marker CSF NfL levels are associated with the number of body regions displaying UMN signs
	Blood	171 ALS 60 ALS mimics	Simoa	Plasma NfL is higher in ALS patients, particularly in fast progressors than intermediate- and slow-progressors Plasma NfL levels are associated with the number of body regions displaying UMN signs Longitudinally, plasma NfL levels remain stable
Thompson et al. (2022)	CSF	258 ALS 80 OND 101 HC	Meso Scale Discovery R-PLEX electrochemiluminescence platform	CSF NfL does not provide any significant advantage over plasma NfL
	Blood	258 ALS 80 OND 101 HC	Meso Scale Discovery R-PLEX electrochemiluminescence platform	Plasma NfL is associated with survival Plasma NfL increases the power in clinical trials
Verde et al. (2023)	Blood	209 ALS 46 NHC	Simoa SR-X platform (Quanterix, Lexington, MA, United States)	Serum NfL levels can discriminate between ALS and NHC Females have higher serum NfL levels Serum NfL levels are higher with both UMN and LMN involvement, showing a predominance of UMN Negative correlation between serum NfL and eGFR

NfL (Benatar et al., 2020). As a cautionary note, blood NfL shows significant inter-individual variability (Simrén et al., 2022) and correlates with age (Benatar et al., 2018). This correlation is less pronounced but still present in ALS patients (Verde et al., 2023), with higher values in female ALS patients than males (De Schaepdryver et al., 2019; Thouvenot et al., 2020; Verde et al., 2023).

Concerning the involvement of UMN and LMN, both pNfH and NfL measured in the CSF correlate with the extension of the CNS regions involved in the degeneration (Abu-Rumeileh et al., 2020; Poesen et al., 2017). Peripherally, serum NfL levels are higher in patients displaying both UMN and LMN degeneration (20), even though serum NfL concentration exhibits a stronger correlation with clinical signs of UMN involvement (Gille et al., 2019; Verde et al., 2023). UMN and LMN's specific contribution to the neurofilament fractions detectable in biofluids is still under investigation.

From a prognostic point of view, NFs were measured in carriers of ALS genes in a longitudinal study and compared to sporadic patients and controls. NfL levels did not differ between patients with familial and sporadic ALS (Verde et al., 2023) but from the pre- to the symptomatic phase. ALS patients show significantly higher serum and CSF NfL levels than at-risk individuals and control (Benatar et al., 2018). Nevertheless, a progressive increase of NF levels in both CSF and serum in at-risk individuals carrying ALS mutation was also reported; for instance, in SOD1 mutation carriers, the increase was observed twelve months before the onset of the symptoms, while in patients with FUS c.521del6 mutation or C9orf72 hexanucleotide repeat expansion (HRE) carriers up to 3.5 years before phenoconversion (Benatar et al., 2019). Further studies will be needed to determine if the length of the presymptomatic phase is proportional to that of the symptomatic stage. Finally, fast-progressor patients show higher median NfL levels at baseline than slow-progressors (Dorst et al., 2020).

Neurofilaments are nowadays quantified in clinical trials. One of the best examples of their use in clinical settings is the study of patients treated with Tofersen, an antisense oligonucleotide against SOD1. NfL levels were reduced in the plasma of treated patients (Miller et al., 2022).

2.2. *(Neuro)Inflammation-related biomarkers*

Nowadays, neuroinflammation is recognized as a pathological feature of ALS (De Marchi et al., 2023), even if it is still unclear whether it emerges because of neurodegeneration or as a pathogenic trigger that precedes neuronal loss. At the early stages of the disease, neuroinflammation may be involved in maintaining brain homeostasis through anti-inflammatory responses. Specifically, this phase is characterized by the presence of reactive CNS microglia and by the infiltration of immune cells from the periphery. There is an increase in regulatory T cells, upregulation of anti-inflammatory cytokines, and

activation of neuroprotective microglia cells. Subsequently, a proinflammatory response is elicited, with more effector T cells and cytokines and activated neurotoxic microglia cells (Vucic, 2019). Inflammatory biomarkers may be of great interest in predicting disease progression, stratifying patients, and monitoring drug responsiveness in clinical trials, even though they are not specific to ALS. The most studied inflammatory markers will be briefly presented.

2.2.1. Chitinases

Chitinases are a family of enzymes in the innate immunity cells; their expression is increased by inflammatory states (Kumar and Zhang, 2019). Chitotriosidase 1 (CHIT1) and chitinase-like proteins, namely chitinase-3-like protein 1 (YKL-40) and chitinase-3-like protein 2 (CHI3L2), exhibit high CSF levels in ALS patients, discriminating from healthy control subjects and patients affected by other neurodegenerative diseases (Dreger et al., 2022; Thompson et al., 2018; Thompson and Turner, 2019; Vu et al., 2020). CHIT1 and YKL-40 levels correlate with the disease progression rate and severity, and they are more abundant in fast than slow progressors (Andrés-Benito et al., 2018; Gille et al., 2019; Steinacker et al., 2018; Varghese et al., 2020; Vu et al., 2020). Finally, CSF CHIT1 displays the strongest correlation with NF levels, while YKL-40 ameliorates the prediction power of NFs in the survival of ALS patients (Masrori et al., 2022; Steinacker et al., 2021). In longitudinal studies, CHIT1 and YKL-40 levels are comparable to control groups at the asymptomatic stage of carriers of ALS-causing genetic mutation, with a rapid increase at symptom onset (Gray et al., 2020; Oeckl et al., 2019, 2018).

CHIT1 and YKL-40 levels were also investigated in serum and plasma. Still, no significant differences were observed among ALS patients and control (Gille et al., 2019; Steinacker et al., 2018), making the diagnostic accuracy of chitinases alone inferior to that of neurofilaments. However, in some studies, combining all three chitinases improves their diagnostic performance (Thompson et al., 2018).

Notably, one-third of Europeans manifest a duplication of the *CHIT1* gene that results in lower levels and activity of CHIT1 (Thompson and Turner, 2019). Particularly, homozygous carriers of *CHIT1* 24-bp duplication polymorphism have undetectable CHIT1 levels that result in a complete enzyme activity deficiency, while heterozygous and wild-type carriers have similar CHIT1 levels in CSF (Steinacker et al., 2021). This polymorphism has been proven to not correlate with axonal degeneration and disease severity in ALS patient studies (Oeckl et al., 2019). Still, it may be relevant to assess the presence of the polymorphism in future studies based on this neuroinflammation marker as a potential confounding factor.

2.2.2. GFAP

Glial fibrillary acidic protein (GFAP) is another protein linked to neuroinflammation, specifically astrogliosis, a feature observed in ALS patients (Vargas and Johnson, 2010). GFAP is also the most abundant astrocyte cytoskeletal protein. Conflicting results have been reported until now regarding its performance as a biomarker. In one study, the concentration of GFAP in CSF was higher in ALS patients than in other neurological conditions, suggesting a trend between its levels and the disease duration (Benninger et al., 2016). Oppositely, Oeckl reported no significant increase in GFAP levels in CSF of ALS patients (Oeckl et al. (2019). Considering GFAP concentration in serum instead, a significant increase was noted in ALS patients, with a weak or no diagnostic performance in discriminating ALS patients from controls (Falzone et al., 2022; Verde et al., 2023). Its serum levels are linked to cognitive and behavioral impairment in ALS patients (Falzone et al., 2022), with higher values in females (Verde et al., 2023). Further studies are needed to define its use as a biomarker for ALS.

2.2.3. Cytokines

Chitinases and GFAP are not the only candidate biomarkers of inflammation studied in biofluids of ALS patients; indeed, type I interferons (IFNs), interleukins (ILs), and tumor necrosis factor (TNF) have gained attention. These innate immune system components highlight the importance of neuroinflammation, but more in general, of a systemic inflammatory response.

In recent years, several studies have analyzed many soluble factors in a multiplexing setting, obtaining a different combination of significantly altered cytokines in the biofluids of ALS patients (Dreger et al., 2022; Staats et al., 2022). Notably, cytokines cannot outperform the solid prognostic value for survival obtained by the analysis of neurofilaments, mainly due to the high inter-subject variability and the different pathways regulating cytokines release (Huang et al., 2020a). Cytokine levels correlate with age, which could result from ALS's pathogenic mechanism and the aging process (Olesen et al., 2020). In patients with a hexanucleotide repeat expansion in the *C9orf72* gene, there is a correlation between higher IL-1β plasma levels and survival (Olesen et al., 2020). Patients with a short survival exhibit higher levels of TNF-α and IL-1β in plasma and higher levels of TNF- α in CSF (Olesen et al., 2020). Another study found a significant increase of the proinflammatory cytokines MCP-1, MIP-1α, and IL-18 in CSF and MCP-1 and IL-18 in the blood of ALS patients. Finally, a significant correlation was not observed between central and peripheral cytokine levels (Huang et al., 2020b).

Because of proinflammatory cytokines releases, such as IL-6, IL-1β, and TNF-α, hepatocytes produce acute phase proteins (APPs). APPs include soluble CD14 (sCD14), lipopolysaccharide-binding protein (LBP), and C-reactive protein (CRP). All these three proteins have been reported to be elevated in the serum of ALS patients (Beers et al., 2020). sCD14 was also increased in the CSF and urine of ALS patients (Beers et al., 2020). Ferritin, creatine kinase (CK), complement C3, and complement C4 were also analyzed with an increase for plasma CK, serum ferritin, and C3 (Thompson et al., 2022), but further validations are needed.

2.2.4. Neopterin

Another marker of systemic proinflammation is neopterin, a small molecule released in response to IFN-γ from monocytic cells, such as macrophages and dendritic cells in the periphery and microglia cells in the CNS (Shepheard et al., 2022). Neopterin is, therefore, a marker of cell-mediated inflammation, and its release correlates with reactive oxygen species production (Sucher et al., 2010).

Neopterin urinary levels at baseline are higher among ALS patients than healthy controls and other neurological disease patients and inversely correlate with ALSFRS-R scores (Lunetta et al., 2020; Shepheard et al., 2022). Neopterin levels progressively increase during the disease (Shepheard et al., 2022), suggesting it is a potentially valuable marker for monitoring the effects of drugs targeting the immune system.

2.3. Other biomarkers

2.3.1. $\underline{p75^{ECD}}$

The neurotrophin receptor ($p75^{NTR}$) is a member of the superfamily of tumor necrosis factor receptors that bind neurotrophins (and their respective pro-neurotrophins), a family of closely related proteins composed, among others, by nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT3), and neurotrophin-4 (NT4) (Chao, 1994). It mediates opposite functions, such as neuronal growth, synapse plasticity, and apoptosis (Sankorrakul et al., 2021). P75 is highly expressed postnatally during development, with a drastic downregulation (Chen et al., 2017). Its expression is restored after injury or in motor neurons and Schwann cells in postmortem tissue of ALS patients (Shepheard et al., 2017). The soluble extracellular domain of the neurotrophin receptor ($p75^{ECD}$) is cleaved after injury and excreted in urine; its urinary detection is indicative of motor neuron degeneration (Shepheard et al., 2014). Urinary $p75^{ECD}$ levels are significantly higher in ALS than in patients affected by other neurological diseases and healthy controls, with a negative correlation between urinary $p75^{ECD}$ and baseline ALSFRS-R (Jia et al., 2017). Its levels are

significantly higher in fast-progressing ALS patients than in slow-progressing patients (Jia et al., 2017), and it increases following disease progression and motor function decline (Shepheard et al., 2017).

These characteristics make $p75^{ECD}$ a valuable marker for monitoring disease progression. Evaluating its levels in urine has clear advantages, considering that it is a biofluid that is readily accessible, non-invasive, easy to collect, and allows frequent sampling (Shepheard et al., 2017). This evidence must be validated in more extensive independent studies because $p75^{ECD}$ is not highly specific for ALS. It would also be interesting to monitor its levels in FTD and ALS mimics patients (Jia et al., 2017).

2.3.2. p-Tau/t-Tau

Tau is a microtubule-associated protein stabilizing neuronal microtubules and promoting nerve growth (Wang and Mandelkow, 2016). A subset of neurodegenerative diseases, called tauopathies, are characterized by aggregates of hyperphosphorylated tau, mainly in paired helical filaments and neurofibrillary tangles (Wang and Mandelkow, 2016). In recent years, both total tau (t-tau) and phosphorylated tau (p-tau) have gained attention as biomarkers mainly for Alzheimer's disease (AD) and other tauopathies, and the ratio p-tau/t-tau is considered an established marker in frontotemporal dementia (FTD) spectrum (Abu-Rumeileh et al., 2020).

Tau protein has also been investigated in biofluids of ALS patients, and surprisingly, its levels were found to be altered compared to controls. In CSF, t-tau levels have been reported to be higher in ALS patients than in controls and ALS mimics (Abu-Rumeileh et al., 2020). Higher t-tau levels were found in ALS patients with bulbar onset (Petrozziello et al., 2022). Among the multiple phosphorylation sites present in the protein, tau phosphorylated at threonine 181 (p-tau181) is the predominant one. Even though no difference was detected in CSF p-tau181, the ratio p-tau181/t-tau is reduced in ALS patients (Scarafino et al., 2018). Notably, a correlation between the increased t-tau and decreased p-tau181/t-tau ratio and ALS progression rate was assessed with the ALSFRS-R, suggesting that they may be used as predictive biomarkers (Petrozziello et al., 2022).

Serum t-tau levels are significantly lower in the patients than in the healthy control (Falzone et al., 2022). Instead, plasma p-tau181 levels were significantly higher in ALS patients than in controls but still lower than in AD patients (Vacchiano et al., 2023), poorly discriminating between these neurodegenerative diseases (Cousins et al., 2022). Plasma p-tau181 levels correlate with lower motor neuron (LMN) signs in cervical, thoracic, and lumbosacral regions (Cousins et al., 2022). The absence of a correlation between p-tau181 levels in CSF and plasma/-serum may underline the peripheral origin of its increased amount (Gong et al., 2022; Vacchiano et al., 2023). Longitudinally, increased plasma p-tau181 levels have been reported over time, with a marked trend in fast progressors (Vacchiano et al., 2023).

In conclusion, tau protein does not outperform the predictive value of survival and the diagnostic performance of neurofilaments; anyway, it may be considered a valuable biomarker to integrate into the monitoring procedure as a complement of neurofilaments.

2.3.3. UCHL1

Ubiquitin carboxyl-terminal hydrolase isozyme L1 (UCHL1) is expressed in the cytoplasm of neurons and is involved in the ubiquitin-proteasome system (Falzone et al., 2022). It is a multifunctional protein with hydrolase and ligase activity (Barschke et al., 2020).

CSF levels of UCHL1 are significantly higher in ALS patients than in other neurodegenerative disorders patients (Li et al., 2020). CSF UCHL1 levels increase in fast-progressor ALS patients (Zhu et al., 2019) and discriminate between C9orf72 gene mutation (C9-ALS) carriers and C9-ALS patients with behavior variant FTD signs from C9-FTD and controls (Barschke et al., 2020).

The serum concentration of UCHL1 does not differentiate ALS from other neurological disorders and ALS mimics (Falzone et al., 2022). Still, it shows a significant difference with healthy controls, even if conflicted results have also been reported (Li et al., 2020).

2.4. Muscle biomarkers

Cardiac troponin T (cTnT) is a component of the sarcomere of striated muscles in the heart that regulates excitation-contraction coupling and is released by disintegrated myocytes (Mueller et al., 2013). The expression of cTnT and cardiac troponin I (cTnI) is highly tissue-specific, and their detection in serum is commonly used as an indicator of myocardial infarction (Mueller et al., 2013). Surprisingly, cTnT levels were found elevated in the peripheral blood of more than half of ALS patients in a cohort of subjects without evidence of cardiac diseases (Mach et al., 2016). It has been reported that cTnT concentration correlates with disease progression (Mach et al., 2016) and increases longitudinally (Casmiro and Graziani, 2019; Kläppe et al., 2022). ALS had higher values than ALS mimics patients and healthy controls, with maximum concentration in non-bulbar ALS patients (Kläppe et al., 2022). There was no correlation between cTnT in serum/plasma and NfL (Kläppe et al., 2022) CSF levels and pNfH (Castro-Gomez et al., 2021). Considering this evidence and that cTnI serum levels remain stable in ALS patients, peripheral skeletal muscle is believed to be a possible source of cTnT (Castro-Gomez et al., 2021).

Regarding the exact nature of measured cTnT, it has been hypothesized that its re-expression in diseased skeletal muscle (Castro-Gomez et al., 2021). However, it has also been proved that there could be a cross-reaction of the immunoassay for cTnT with skeletal TnT (Schmid et al., 2018). Considering that the role of cTnT in ALS and its origin are still unclear, it would be interesting to better investigate this marker in ALS patients, considering its increasing levels over time and potential utility in monitoring the disease.

Interestingly, cTnT levels also correlate with another marker of muscle cell damage that can be measured in the serum of ALS patients: creatinine kinase isoenzyme MB (CK-MB) (Kittipeerapat et al., 2023). CK is an enzyme involved in energy metabolism; the isoenzyme CK-MB is mainly expressed in the myocardium and minimally in skeletal muscles (Kittipeerapat et al., 2023). As cTnT, CK-MB was also elevated in more than half of ALS patients in the analyzed cohort, with higher levels recorded in patients with spinal onset (Kittipeerapat et al., 2023). Other studies also measured total CK in the blood of ALS patients, which is broadly expressed by various cell types. ALS patients reported higher concentrations, particularly spinal-onset patients (Ceccanti et al., 2020). The increased CK levels in ALS patients have been linked to lower motor neuron loss and muscle denervation (Tai et al., 2018).

2.5. Extracellular vesicles

Extracellular vesicles (EVs) are small membranous nanoparticles ubiquitously released from the cell. They are a heterogeneous group of stable particles carrying nucleic acids, lipids, and proteins of different sizes and origins (Hill, 2019). EVs mediate intercellular communication; they reach and fuse with neighboring or distant cells (Basso and Bonetto, 2016), even though the exact up-take mechanisms in the receiving cells have yet to be completely elucidated. EVs have been isolated from different biofluids, such as blood, CSF, saliva, urine, and milk. The possibility of detecting them in biofluids and that EV cargo reflects the parental cell composition favors EV applicability as a biomarker (Ferrara et al., 2018).

As ALS is a multisystem disease, circulating EVs may be very informative about the pathological state of the patients. Moreover, they could mirror early pathological events (Barbo and Ravnik-Glavač, 2023). Plasma EVs themselves have been proposed as biomarkers for disease diagnosis and prognosis. Combining their biophysical parameters and the presence of two specific proteins in their cargo, namely HSP90 and cyclophilin A, and exploiting machine learning techniques, it was possible to propose EVs as diagnostic and prognostic biomarkers discriminating fast- and slow-ALS progressors (Pasetto et al., 2021).

Moreover, the leukocyte-derived microvesicles are over-represented in the blood of ALS patients and enriched in misfolded SOD1 protein (Sproviero et al., 2019). A few studies analyzed EVs isolated from CSF of ALS patients. Being CSF physically close to the cells of the central nervous system, we can speculate that its EV content is representative of alterations occurring when neuronal degeneration starts. Considering the size distribution of CSF EVs, no differences were reported between ALS patients and controls.

Still, the proteomic analysis revealed a defect in the proteostasis (Thompson et al., 2020). Similarly, in another study investigating the mRNA content of CSF EVs, several genes were deregulated in ALS patients compared to healthy controls, mainly involved in the ubiquitin-proteasome pathway, oxidative stress response, and unfolded protein response (Otake et al., 2019). Moreover, the transcriptomic analysis of blood EVs revealed a difference between the mRNA composition of small and large EVs in ALS patients (Sproviero et al., 2022). Among microRNAs in ALS patients, miR-15a-5p and miR-193a-5p showed a diagnostic potential and were associated with disability progression (Saucier et al., 2019). miRNAs have also been analyzed in neuron-derived EVs, a subpopulation of plasma EVs immunoprecipitated with L1CAM/CD171 showing thirty deregulated molecules between ALS patients and healthy controls (Katsu et al., 2019). In another study, the comparison between EVs from the frontal cortex, spinal cord, and blood of ALS patients and healthy controls revealed miR-342–3p as increased and miR-1254 as decreased in ALS (Lo et al., 2021). Of note, there has yet to be a consensus on specific RNA, messengers, or microRNAs as biomarkers to measure to follow the disease progression. Although EVs are an attractive source of biomarkers, many challenges are still open in the EV field. The lack of consensus and standardization on protocols for the isolation and analysis of EVs makes inter-laboratory data comparison difficult (Théry et al., 2018).

3. Conclusions

Nowadays, many different molecules have been proposed as candidate biomarkers (Fig. 1). Although various studies have been conducted to validate them as promising diagnostic or prognostic markers, none are currently used in clinics. It is reasonable that a single marker would not be able to address the whole complexity of this pathology. Therefore,

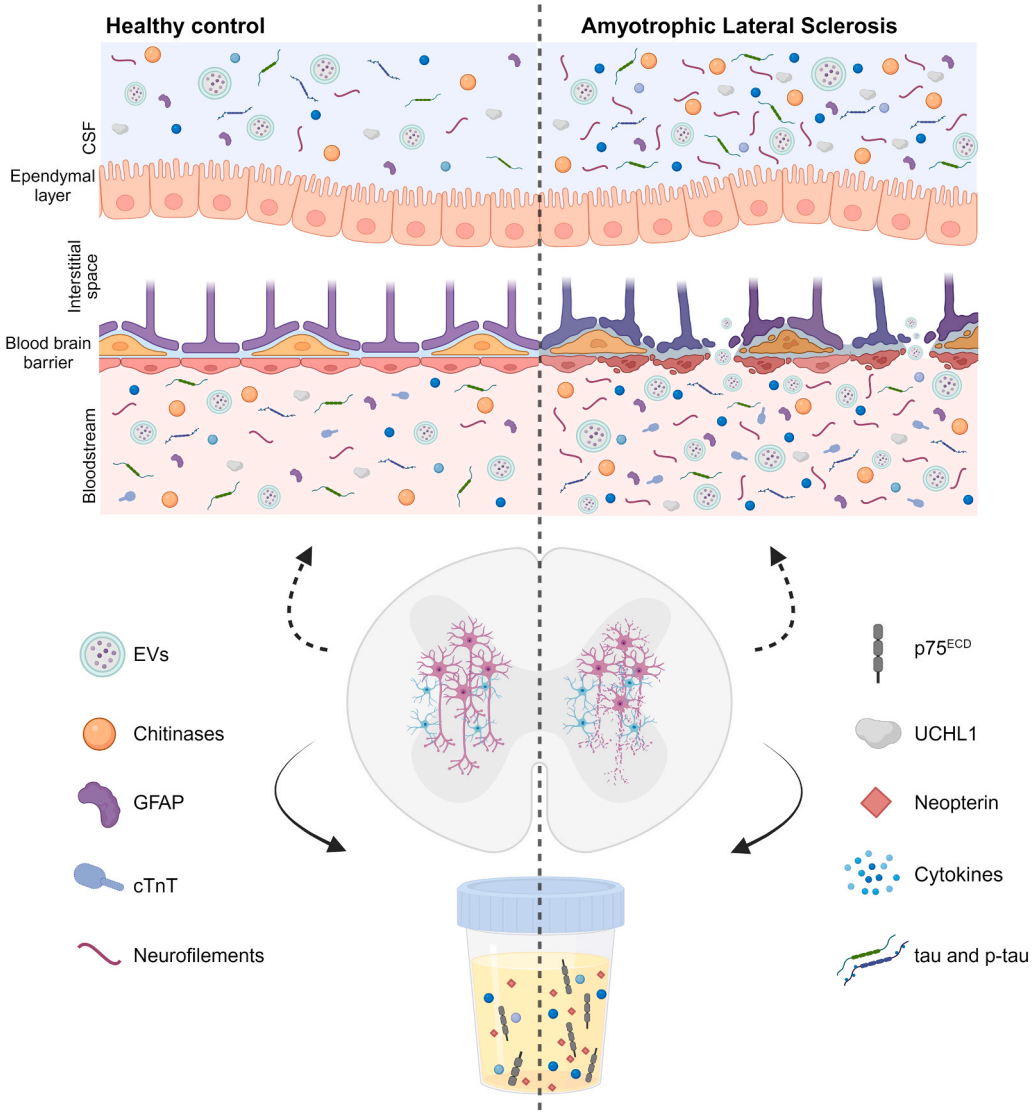

Fig. 1. Biochemical biomarkers for ALS. Schematic representation of the different biomarkers measured in ALS patients' biofluids (CSF, blood, and urine) with varying proportions in the pathology and healthy control context. EVs=extracellular vesicles; cTnT= cardiac troponin T; UCHL1 = ubiquitin carboxyl-terminal hydrolase isozyme L1; GFAP = glial fibrillary acidic protein; $p75^{ECD}$ = extracellular domain of the neurotrophin receptor.
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there is a joint interest in combining different biomarkers in a multiplexing system to obtain information deriving from other mechanisms involved in the neurodegenerative process. Among those proposed, blood- and urine-based biomarkers are favored, requiring less or non-invasive sampling and may be arranged in biochemical assays usable in clinical practice and repeated routinely.

Currently, neurofilaments are the most promising biomarkers for ALS. Many different groups and centers have validated them, and their measurement is reproducible and standardized. However, it is crucial to consider that its release is the outcome of motor neuron degeneration. In this review, we have listed several additional molecules investigated as candidate biomarkers. The joint effort to arrange multicenter studies for their final validation is notable for all of these. For any candidate marker, it is essential to be validated in many patients, in multiple centers, with a fixed sampling procedure, analysis, and the definition of standard endpoints.

For the future, it is auspicious to define biomarkers that recapitulate early changes happening in the presymptomatic stage when no apparent symptoms or signs of the disease are manifest.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data Availability

No data was used for the research described in the article.

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References

Abu-Rumeileh, S., Vacchiano, V., Zenesini, C., Polischi, B., de Pasqua, S., Fileccia, E., Mammana, A., Di Stasi, V., Capellari, S., Salvi, F., Liguori, R., Parchi, P., 2020. Diagnostic-prognostic value and electrophysiological correlates of CSF biomarkers of neurodegeneration and neuroinflammation in amyotrophic lateral sclerosis. J. Neurol. 267, 1699–1708. https://doi.org/10.1007/s00415-020-09761-z.

Alagaratnam, J., von Widekind, S., De Francesco, D., Underwood, J., Edison, P., Winston, A., Zetterberg, H., Fidler, S., 2021. Correlation between CSF and blood neurofilament light chain protein: a systematic review and meta-analysis. BMJ Neurol. Open 3, e000143. https://doi.org/10.1136/bmjno-2021-000143.

Al-Chalabi, A., Hardiman, O., 2013. The epidemiology of ALS: a conspiracy of genes, environment and time. Nat. Rev. Neurol. 9, 617–628. https://doi.org/10.1038/nrneurol.2013.203.

Andrés-Benito, P., Domínguez, R., Colomina, M.J., Llorens, F., Povedano, M., Ferrer, I., 2018. YKL40 in sporadic amyotrophic lateral sclerosis: cerebrospinal fluid levels as a prognosis marker of disease progression. Aging 10, 2367–2382. https://doi.org/10.18632/aging.101551.

Barbo, M., Ravnik-Glavač, M., 2023. Extracellular vesicles as potential biomarkers in amyotrophic lateral sclerosis. Genes 14, 325. https://doi.org/10.3390/genes14020325.

Barschke, P., Oeckl, P., Steinacker, P., Al Shweiki, M.R., Weishaupt, J.H., Landwehrmeyer, G.B., Anderl-Straub, S., Weydt, P., Diehl-Schmid, J., Danek, A., Kornhuber, J., Schroeter, M.L., Prudlo, J., Jahn, H., Fassbender, K., Lauer, M., van der Ende, E.L., van Swieten, J.C., Volk, A.E., Ludolph, A.C., Otto, M., 2020. Different CSF protein profiles in amyotrophic lateral sclerosis and frontotemporal dementia with *C9orf72* hexanucleotide repeat expansion. J. Neurol. Neurosurg. Psychiatry 91, 503–511. https://doi.org/10.1136/jnnp-2019-322476.

Basso, M., Bonetto, V., 2016. Extracellular vesicles and a novel form of communication in the brain. Front. Neurosci. 10. https://doi.org/10.3389/fnins.2016.00127.

Beers, D.R., Zhao, W., Neal, D.W., Thonhoff, J.R., Thome, A.D., Faridar, A., Wen, S., Wang, J., Appel, S.H., 2020. Elevated acute phase proteins reflect peripheral inflammation and disease severity in patients with amyotrophic lateral sclerosis. Sci. Rep. 10, 15295. https://doi.org/10.1038/s41598-020-72247-5.

Behzadi, A., Pujol-Calderón, F., Tjust, AE., Wuolikainen, A., Höglund, K., Forsberg, K., Portelius, E., Blennow, K., Zetterberg, H., Andersen, PM., 2021. Neurofilaments can differentiate ALS subgroups and ALS from common diagnostic mimics. Sci Rep. 11 (1), 22128 https://doi.org/10.1038/s41598-021-01499-6. PMID: 34764380; PMCID: PMC8585882.

Benatar, M., Wuu, J., Turner, M.R., 2022. Neurofilament light chain in drug development for amyotrophic lateral sclerosis: a critical appraisal. Brain awac394. https://doi.org/10.1093/brain/awac394.

Benatar, M., Wuu, J., Andersen, P.M., Lombardi, V., Malaspina, A., 2018. Neurofilament light: a candidate biomarker of presymptomatic amyotrophic lateral sclerosis and phenoconversion. Ann. Neurol. 84, 130–139. https://doi.org/10.1002/ana.25276.

Benatar, M., Wuu, J., Lombardi, V., Jeromin, A., Bowser, R., Andersen, P.M., Malaspina, A., 2019. Neurofilaments in pre-symptomatic ALS and the impact of genotype. Amyotroph. Lateral Scler. Front. Degener. 20, 538–548. https://doi.org/10.1080/21678421.2019.1646769.

Benatar, M., Zhang, L., Wang, L., Granit, V., Statland, J., Barohn, R., Swenson, A., Ravits, J., Jackson, C., Burns, T.M., Trivedi, J., Pioro, E.P., Caress, J., Katz, J., McCauley, J.L., Rademakers, R., Malaspina, A., Ostrow, L.W., Wuu, J., Consortium, C.Re.A.Te, 2020. Validation of serum neurofilaments as prognostic and potential pharmacodynamic biomarkers for ALS. Neurology. https://doi.org/10.1212/WNL.0000000000009559.

Benninger, F., Glat, M.J., Offen, D., Steiner, I., 2016. Glial fibrillary acidic protein as a marker of astrocytic activation in the cerebrospinal fluid of patients with amyotrophic lateral sclerosis. J. Clin. Neurosci. 26, 75–78. https://doi.org/10.1016/j.jocn.2015.10.008.

Brodovitch, A., Boucraut, J., Delmont, E., Parlanti, A., Grapperon, A.-M., Attarian, S., Verschueren, A., 2021. Combination of serum and CSF neurofilament-light and neuroinflammatory biomarkers to evaluate ALS. Sci. Rep. 11, 703. https://doi.org/10.1038/s41598-020-80370-6.

Brooks, B.R., Miller, R.G., Swash, M., Munsat, T.L., World Federation of Neurology Research Group on Motor Neuron Diseases, 2000. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotroph. Lateral Scler. Other Mot. Neuron Disord. 1, 293–299. https://doi.org/10.1080/146608200300079536.

Califf, R.M., 2018. Biomarker definitions and their applications. Exp. Biol. Med. (Maywood) 243, 213–221. https://doi.org/10.1177/1535370217750088.

Casmiro, M., Graziani, A., 2019. Serum troponin T in patients with amyotrophic lateral sclerosis. Acta Neurol. Belg. 119, 285–288. https://doi.org/10.1007/s13760-017-0855-y.

Castro-Gomez, S., Radermacher, B., Tacik, P., Mirandola, S.R., Heneka, M.T., Weydt, P., 2021. Teaching an old dog new tricks: serum troponin T as a biomarker in amyotrophic lateral sclerosis. Brain Commun. 3, fcab274. https://doi.org/10.1093/braincomms/fcab274.

Ceccanti, M., Pozzilli, V., Cambieri, C., Libonati, L., Onesti, E., Frasca, V., Fiorini, I., Petrucci, A., Garibaldi, M., Palma, E., Bendotti, C., Fabbrizio, P., Trolese, M.C., Nardo, G., Inghilleri, M., 2020. Creatine kinase and progression rate in amyotrophic lateral sclerosis. Cells 9, 1174. https://doi.org/10.3390/cells9051174.

Chao, M.V., 1994. The p75 neurotrophin receptor. J. Neurobiol. 25, 1373–1385. https://doi.org/10.1002/neu.480251106.

Chen, Z., Donnelly, C.R., Dominguez, B., Harada, Y., Lin, W., Halim, A.S., Bengoechea, T. G., Pierchala, B.A., Lee, K.-F., 2017. p75 is required for the establishment of postnatal sensory neuron diversity by potentiating ret signaling. Cell Rep. 21, 707–720. https://doi.org/10.1016/j.celrep.2017.09.037.

Cordts, I., Wachinger, A., Scialo, C., Lingor, P., Polymenidou, M., Buratti, E., Feneberg, E., 2023. TDP-43 proteinopathy specific biomarker development. Cells 12, 597. https://doi.org/10.3390/cells12040597.

Cousins, K.A.Q., Shaw, L.M., Shellikeri, S., Dratch, L., Rosario, L., Elman, L.B., Quinn, C., Amado, D.A., Wolk, D.A., Tropea, T.F., Chen-Plotkin, A., Irwin, D.J., Grossman, M., Lee, E.B., Trojanowski, J.Q., McMillan, C.T., 2022. Elevated plasma phosphorylated tau 181 in amyotrophic lateral sclerosis. Ann. Neurol. 92, 807–818. https://doi.org/10.1002/ana.26462.

De Marchi, F., Tondo, G., Corrado, L., Menegon, F., Aprile, D., Anselmi, M., D'Alfonso, S., Comi, C., Mazzini, L., 2023. Neuroinflammatory pathways in the ALS-FTD continuum: a focus on genetic variants. Genes 14, 1658. https://doi.org/10.3390/genes14081658.

De Schaepdryver, M., Lunetta, C., Tarlarini, C., Mosca, L., Chio, A., Van Damme, P., Poesen, K., 2020. Neurofilament light chain and C reactive protein explored as predictors of survival in amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 91 (4), 436–437. https://doi.org/10.1136/jnnp-2019-322309. Epub 2020 Feb 6. PMID: 32029541.

De Schaepdryver, M., Jeromin, A., Gille, B., Claeys, KG., Herbst, V., Brix, B., Van Damme, P., Poesen, K., 2018. Comparison of elevated phosphorylated neurofilament heavy chains in serum and cerebrospinal fluid of patients with amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 89 (4), 367–373. https://doi.org/10.1136/jnnp-2017-316605. Epub 2017 Oct 20. PMID: 29054919.

De Schaepdryver, M., Goossens, J., De Meyer, S., Jeromin, A., Masrori, P., Brix, B., Claeys, K.G., Schaeverbeke, J., Adamczuk, K., Vandenberghe, R., Van Damme, P., Poesen, K., 2019. Serum neurofilament heavy chains as early marker of motor neuron degeneration. Ann. Clin. Transl. Neurol. 6, 1971–1979. https://doi.org/10.1002/acn3.50890.

Dorst, J., Schuster, J., Dreyhaupt, J., Witzel, S., Weishaupt, J.H., Kassubek, J., Weiland, U., Petri, S., Meyer, T., Grehl, T., Hermann, A., Jordan, B., Grosskreutz, J., Zeller, D., Boentert, M., Schrank, B., Prudlo, J., Winkler, A.S., Gorbulev, S., Roselli, F., Dupuis, L., Otto, M., Ludolph, A.C., 2020. Effect of high-caloric nutrition on serum neurofilament light chain levels in amyotrophic lateral sclerosis. J. Neurol. Neurosurg. Psychiatry 91, 1007–1009. https://doi.org/10.1136/jnnp-2020-323372.

Dreger, M., Steinbach, R., Otto, M., Turner, M.R., Grosskreutz, J., 2022. Cerebrospinal fluid biomarkers of disease activity and progression in amyotrophic lateral sclerosis. J. Neurol. Neurosurg. Psychiatry 93, 422–435. https://doi.org/10.1136/jnnp-2021-327503.

van Es, M.A., Hardiman, O., Chio, A., Al-Chalabi, A., Pasterkamp, R.J., Veldink, J.H., van den Berg, L.H., 2017. Amyotrophic lateral sclerosis. Lancet 390, 2084–2098. https://doi.org/10.1016/S0140-6736(17)31287-4.

Falzone, Y.M., Domi, T., Mandelli, A., Pozzi, L., Schito, P., Russo, T., Barbieri, A., Fazio, R., Volontè, M.A., Magnani, G., Del Carro, U., Carrera, P., Malaspina, A., Agosta, F., Quattrini, A., Furlan, R., Filippi, M., Riva, N., 2022. Integrated evaluation of a panel of neurochemical biomarkers to optimize diagnosis and prognosis in amyotrophic lateral sclerosis. Eur. J. Neurol. 29, 1930–1939. https://doi.org/10.1111/ene.15321.

Falzone, YM., Domi, T., Agosta, F., Pozzi, L., Schito, P., Fazio, R., Del Carro, U., Barbieri, A., Comola, M., Leocani, L., Comi, G., Carrera, P., Filippi, M., Quattrini, A., Riva, N., 2020. Serum phosphorylated neurofilament heavy-chain levels reflect phenotypic heterogeneity and are an independent predictor of survival in motor neuron disease. J Neurol. 267 (8), 2272–2280. https://doi.org/10.1007/s00415-020-09838-9. Epub 2020 Apr 18. PMID: 32306171; PMCID: PMC7166001.

FDA-NIH Biomarker Working Group, 2016. BEST (Biomarkers, EndpointS, and other Tools) Resource. Food and Drug Administration (US), Silver Spring (MD),.

Feldman, E.L., Goutman, S.A., Petri, S., Mazzini, L., Savelieff, M.G., Shaw, P.J., Sobue, G., 2022. Amyotrophic lateral sclerosis. Lancet 400, 1363–1380. https://doi.org/10.1016/S0140-6736(22)01272-7.

Ferrara, D., Pasetto, L., Bonetto, V., Basso, M., 2018. Role of extracellular vesicles in amyotrophic lateral sclerosis. Front Neurosci. 12, 574. https://doi.org/10.3389/fnins.2018.00574.

Figlewicz, D.A., Krizus, A., Martinoli, M.G., Meininger, V., Dib, M., Rouleau, G.A., Julien, J.-P., 1994. Variants of the heavy neurofilament subunit are associated with the development of amyotrophic lateral sclerosis. Hum. Mol. Genet 3, 1757–1761. https://doi.org/10.1093/hmg/3.10.1757.

Gille, B., De Schaepdryver, M., Dedeene, L., Goossens, J., Claeys, K.G., Van Den Bosch, L., Tournoy, J., Van Damme, P., Poesen, K., 2019. Inflammatory markers in cerebrospinal fluid: independent prognostic biomarkers in amyotrophic lateral sclerosis. J. Neurol. Neurosurg. Psychiatry jnnp- 2018–319586. https://doi.org/10.1136/jnnp-2018-319586.

Gille, B., De Schaepdryver, M., Goossens, J., Dedeene, L., De Vocht, J., Oldoni, E., Goris, A., Van Den Bosch, L., Depreitere, B., Claeys, KG., Tournoy, J., Van Damme, P., Poesen, K., 2019. Serum neurofilament light chain levels as a marker of upper motor neuron degeneration in patients with Amyotrophic Lateral Sclerosis. Neuropathol Appl Neurobiol. 45 (3), 291–304. https://doi.org/10.1111/nan.12511. Epub 2018 Jul 18. PMID: 29908069.

Gong, Z., Gao, L., Lu, Y., Wang, Z., 2022. CSF p-tau as a potential cognition impairment biomarker in ALS. Front. Neurol. 13, 991143 https://doi.org/10.3389/fneur.2022.991143.

Goutman, S.A., Hardiman, O., Al-Chalabi, A., Chió, A., Savelieff, M.G., Kiernan, M.C., Feldman, E.L., 2022. Recent advances in the diagnosis and prognosis of amyotrophic lateral sclerosis. Lancet Neurol. 21, 480–493. https://doi.org/10.1016/S1474-4422(21)00465-8.

Gray, E., Oeckl, P., Amador, M.D.M., Andreasson, U., An, J., Blennow, K., Bowser, R., De Schaepdryver, M., Heslegrave, A., Kuhle, J., Maceski, A., Koel-Simmelink, M., Lamari, F., Lombardi, V., Malaspina, A., Nilsson, I., Poesen, K., Salachas, F., Steinacker, P., Teunissen, C.E., Van Damme, P., Zetterberg, H., Ludolph, A., Jeromin, A., Turner, M.R., Otto, M., 2020. A multi-center study of neurofilament assay reliability and inter-laboratory variability. Amyotroph. Lateral Scler. Front. Degener. 21, 452–458. https://doi.org/10.1080/21678421.2020.1779300.

Gros-Louis, F., Larivière, R., Gowing, G., Laurent, S., Camu, W., Bouchard, J.-P., Meininger, V., Rouleau, G.A., Julien, J.-P., 2004. A frameshift deletion in peripherin gene associated with amyotrophic lateral sclerosis. J. Biol. Chem. 279, 45951–45956. https://doi.org/10.1074/jbc.M408139200.

Gürth, C.-M., do Rego Barros Fernandes Lima, M.A., Macarrón Palacios, V., Cereceda Delgado, A.R., Hubrich, J., D'Este, E., 2023. Neurofilament levels in dendritic spines associate with synaptic status. Cells 12, 909. https://doi.org/10.3390/cells12060909.

Hill, A.F., 2019. Extracellular vesicles and neurodegenerative diseases. J. Neurosci. 39, 9269–9273. https://doi.org/10.1523/JNEUROSCI.0147-18.2019.

Huang, D., Yue, F., Qiu, J., Deng, M., Kuang, S., 2020. Polymeric nanoparticles functionalized with muscle-homing peptides for targeted delivery of phosphatase and tensin homolog inhibitor to skeletal muscle. Acta Biomater. 118, 196–206. https://doi.org/10.1016/J.ACTBIO.2020.10.009.

Huang, F., Zhu, Y., Hsiao-Nakamoto, J., Tang, X., Dugas, J.C., Moscovitch-Lopatin, M., Glass, J.D., Brown, R.H., Ladha, S.S., Lacomis, D., Harris, J.M., Scearce-Levie, K., Ho, C., Bowser, R., Berry, J.D., 2020. Longitudinal biomarkers in amyotrophic lateral sclerosis. Ann. Clin. Transl. Neurol. 7, 1103–1116. https://doi.org/10.1002/acn3.51078.

Jia, R., Shepheard, S., Jin, J., Hu, F., Zhao, X., Xue, L., Xiang, L., Qi, H., Qu, Q., Guo, F., Rogers, M.-L., Dang, J., 2017. Urinary extracellular domain of neurotrophin receptor p75 as a biomarker for amyotrophic lateral sclerosis in a Chinese cohort. Sci. Rep. 7, 5127. https://doi.org/10.1038/s41598-017-05430-w.

Katsu, M., Hama, Y., Utsumi, J., Takashina, K., Yasumatsu, H., Mori, F., Wakabayashi, K., Shoji, M., Sasaki, H., 2019. MicroRNA expression profiles of neuron-derived extracellular vesicles in plasma from patients with amyotrophic lateral sclerosis. Neurosci. Lett. 708, 134176 https://doi.org/10.1016/j.neulet.2019.03.048.

Khalil, M., Teunissen, C.E., Otto, M., Piehl, F., Sormani, M.P., Gattringer, T., Barro, C., Kappos, L., Comabella, M., Fazekas, F., Petzold, A., Blennow, K., Zetterberg, H., Kuhle, J., 2018. Neurofilaments as biomarkers in neurological disorders. Nat. Rev. Neurol. 14, 577–589. https://doi.org/10.1038/s41582-018-0058-z.

Kittipeerapat, N., Fabian, R., Bernsen, S., Weydt, P., Castro-Gomez, S., 2023. Creatine kinase MB isoenzyme is a complementary biomarker in amyotrophic lateral sclerosis. IJMS 24, 11682. https://doi.org/10.3390/ijms241411682.

Kläppe, U., Chamoun, S., Shen, Q., Finn, A., Evertsson, B., Zetterberg, H., Blennow, K., Press, R., Samuelsson, K., Månberg, A., Fang, F., Ingre, C., 2022. Cardiac troponin T is elevated and increases longitudinally in ALS patients. Amyotroph. Lateral Scler. Front. Degener. 23, 58–65. https://doi.org/10.1080/21678421.2021.1939384.

Kumar, A., Zhang, K.Y.J., 2019. Human chitinases: structure, function, and inhibitor discovery. In: Yang, Q., Fukamizo, T. (Eds.), Targeting Chitin-Containing Organisms, Advances in Experimental Medicine and Biology. Springer, Singapore, Singapore, pp. 221–251. https://doi.org/10.1007/978-981-13-7318-3_11.

Lagier-Tourenne, C., Polymenidou, M., Cleveland, D.W., 2010. TDP-43 and FUS/TLS: emerging roles in RNA processing and neurodegeneration. Hum. Mol. Genet. 19, R46–R64. https://doi.org/10.1093/hmg/ddq137.

Li, R., Wang, J., Xie, W., Liu, J., Wang, C., 2020. UCHL1 from serum and CSF is a candidate biomarker for amyotrophic lateral sclerosis. Ann. Clin. Transl. Neurol. 7, 1420–1428. https://doi.org/10.1002/acn3.51141.

Lo, T., Figueroa-Romero, C., Hur, J., Pacut, C., Stoll, E., Spring, C., Lewis, R., Nair, A., Goutman, S.A., Sakowski, S.A., Nagrath, S., Feldman, E.L., 2021. Extracellular vesicles in serum and central nervous system tissues contain microRNA signatures in sporadic amyotrophic lateral sclerosis. Front. Mol. Neurosci. 14, 739016 https://doi.org/10.3389/fnmol.2021.739016.

Lunetta, C., Lizio, A., Gerardi, F., Tarlarini, C., Filippi, M., Riva, N., Tremolizzo, L., Diamanti, S., Dellanoce, C.C., Mosca, L., Sansone, V.A., Campolo, J., 2020. Urinary neopterin, a new marker of the neuroinflammatory status in amyotrophic lateral sclerosis. J. Neurol. 267, 3609–3616. https://doi.org/10.1007/s00415-020-10047-7.

Mach, L., Konecny, T., Helanova, K., Jaffe, A.S., Sorenson, E.J., Somers, V.K., Reeder, G. S., 2016. Elevation of cardiac troponin T in patients with amyotrophic lateral sclerosis. Acta Neurol. Belg. 116, 557–564. https://doi.org/10.1007/s13760-015-0596-8.

Masrori, P., Van Damme, P., 2020. Amyotrophic lateral sclerosis: a clinical review. Eur. J. Neurol. 27, 1918–1929. https://doi.org/10.1111/ene.14393.

Masrori, P., De Schaepdryver, M., Floeter, M.K., De Vocht, J., Lamaire, N., D'Hondt, A., Traynor, B., Poesen, K., Van Damme, P., 2022. Prognostic relationship of neurofilaments, CHIT1, YKL-40 and MCP-1 in amyotrophic lateral sclerosis. J. Neurol. Neurosurg. Psychiatry 93, 681–682. https://doi.org/10.1136/jnnp-2021-327877.

Miller, T.M., Cudkowicz, M.E., Genge, A., Shaw, P.J., Sobue, G., Bucelli, R.C., Chiò, A., Van Damme, P., Ludolph, A.C., Glass, J.D., Andrews, J.A., Babu, S., Benatar, M., McDermott, C.J., Cochrane, T., Chary, S., Chew, S., Zhu, H., Wu, F., Nestorov, I., Graham, D., Sun, P., McNeill, M., Fanning, L., Ferguson, T.A., Fradette, S., 2022. Trial of antisense oligonucleotide tofersen for SOD1 ALS (VALOR and OLE Working Group). N. Engl. J. Med 387, 1099–1110. https://doi.org/10.1056/NEJMoa2204705.

Mueller, M., Vafaie, M., Biener, M., Giannitsis, E., Katus, H.A., 2013. Cardiac troponin T: – from diagnosis of myocardial infarction to cardiovascular risk prediction. Circ. J. 77, 1653–1661. https://doi.org/10.1253/circj.CJ-13-0706.

Neumann, M., Sampathu, D.M., Kwong, L.K., Truax, A.C., Micsenyi, M.C., Chou, T.T., Bruce, J., Schuck, T., Grossman, M., Clark, C.M., McCluskey, L.F., Miller, B.L., Masliah, E., Mackenzie, I.R., Feldman, H., Feiden, W., Kretzschmar, H.A., Trojanowski, J.Q., Lee, V.M.Y., 2006. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 314, 130–133. https://doi.org/10.1126/science.1134108.

Oeckl, P., Steinacker, P., Otto, M., 2018. Comparison of internal standard approaches for SRM analysis of alpha-synuclein in cerebrospinal fluid. J. Proteome Res 17, 516–523. https://doi.org/10.1021/acs.jproteome.7b00660.

Oeckl, P., Weydt, P., Steinacker, P., Anderl-Straub, S., Nordin, F., Volk, A.E., Diehl-Schmid, J., Andersen, P.M., Kornhuber, J., Danek, A., Fassbender, K., Fliessbach, K., German Consortium for Frontotemporal Lobar Degeneration, Jahn, H., Lauer, M., Müller, K., Knehr, A., Prudlo, J., Schneider, A., Thal, D.R., Yilmazer-Hanke, D., Weishaupt, J.H., Ludolph, A.C., Otto, M., 2019. Different neuroinflammatory profile in amyotrophic lateral sclerosis and frontotemporal dementia is linked to the clinical phase. J. Neurol. Neurosurg. Psychiatry 90, 4–10. https://doi.org/10.1136/jnnp-2018-318868.

Oeckl, P., Jardel, C., Salachas, F., Lamari, F., Andersen, PM., Bowser, R., de Carvalho, M., Costa, J., van Damme, P., Gray, E., Grosskreutz, J., Hernández-Barral, M., Herukka, SK., Huss, A., Jeromin, A., Kirby, J., Kuzma-Kozakiewicz, M., Amador Mdel, M., Mora, JS., Morelli, C., Muckova, P., Petri, S., Poesen, K., Rhode, H., Rikardsson, AK., Robberecht, W., Rodríguez Mahillo, AI, Shaw, P., Silani, V., Steinacker, P., Turner, MR., Tüzün, E., Yetimler, B., Ludolph, AC., Otto, M.M., 2016. Multicenter validation of CSF neurofilaments as diagnostic biomarkers for ALS. Amyotroph Lateral Scler Frontotemporal Degener 17 (5-6), 404–413. https://doi.org/10.3109/21678421.2016.1167913. Epub 2016 Apr 11. PMID: 27415180.

Olesen, M.N., Wuolikainen, A., Nilsson, A.C., Wirenfeldt, M., Forsberg, K., Madsen, J.S., Lillevang, S.T., Brandslund, I., Andersen, P.M., Asgari, N., 2020. Inflammatory profiles relate to survival in subtypes of amyotrophic lateral sclerosis. Neurol. Neuroimmunol. Neuroinflamm. 7, e697 https://doi.org/10.1212/NXI.0000000000000697.

Otake, K., Kamiguchi, H., Hirozane, Y., 2019. Identification of biomarkers for amyotrophic lateral sclerosis by comprehensive analysis of exosomal mRNAs in human cerebrospinal fluid. BMC Med Genom. 12, 7. https://doi.org/10.1186/s12920-019-0473-z.

Pasetto, L., Grassano, M., Pozzi, S., Luotti, S., Sammali, E., Migazzi, A., Basso, M., Spagnolli, G., Biasini, E., Micotti, E., Cerovic, M., Carli, M., Forloni, G., De Marco, G., Manera, U., Moglia, C., Mora, G., Traynor, B.J., Chiò, A., Calvo, A., Bonetto, V., 2021. Defective cyclophilin A induces TDP-43 proteinopathy: implications for amyotrophic lateral sclerosis and frontotemporal dementia. Brain: J. Neurol. 144, 3710–3726. https://doi.org/10.1093/BRAIN/AWAB333.

Petrozziello, T., Amaral, A.C., Dujardin, S., Farhan, S.M.K., Chan, J., Trombetta, B.A., Kivisäkk, P., Mills, A.N., Bordt, E.A., Kim, S.E., Dooley, P.M., Commins, C.,

Connors, T.R., Oakley, D.H., Ghosal, A., Gomez-Isla, T., Hyman, B.T., Arnold, S.E., Spires-Jones, T., Cudkowicz, M.E., Berry, J.D., Sadri-Vakili, G., 2022. Novel genetic variants in *MAPT* and alterations in tau phosphorylation in amyotrophic lateral sclerosis post-mortem motor cortex and cerebrospinal fluid. Brain Pathol. 32. https://doi.org/10.1111/bpa.13035.

Poesen, K., Van Damme, P., 2019. Diagnostic and prognostic performance of neurofilaments in ALS. Front. Neurol. 9, 1167. https://doi.org/10.3389/fneur.2018.01167.

Poesen, K., De Schaepdryver, M., Stubendorff, B., Gille, B., Muckova, P., Wendler, S., Prell, T., Ringer, TM., Rhode, H., Stevens, O., Claeys, KG., Couwelier, G., D'Hondt, A., Lamaire, N., Tilkin, P., Van Reijen, D., Gourmaud, S., Fedtke, N., Heiling, B., Rumpel, M., Rödiger, A., Gunkel, A., Witte, OW., Paquet, C., Vandenberghe, R., Grosskreutz, J., Van Damme, P., 2017. Neurofilament markers for ALS correlate with extent of upper and lower motor neuron disease. Neurology 88 (24), 2302–2309. https://doi.org/10.1212/WNL.0000000000004029. Epub 2017 May 12. PMID: 28500227.

Poesen, K., De Schaepdryver, M., Stubendorff, B., Gille, B., Muckova, P., Wendler, S., Prell, T., Ringer, T.M., Rhode, H., Stevens, O., Claeys, K.G., Couwelier, G., D'Hondt, A., Lamaire, N., Tilkin, P., Van Reijen, D., Gourmaud, S., Fedtke, N., Heiling, B., Rumpel, M., Rödiger, A., Gunkel, A., Witte, O.W., Paquet, C., Vandenberghe, R., Grosskreutz, J., Van Damme, P., 2017. Neurofilament markers for ALS correlate with extent of upper and lower motor neuron disease. Neurology 88, 2302–2309. https://doi.org/10.1212/WNL.0000000000004029.

Rao, M.V., Darji, S., Stavrides, P.H., Goulbourne, C.N., Kumar, A., Yang, D.-S., Yoo, L., Peddy, J., Lee, J.-H., Yuan, A., Nixon, R.A., 2023. Autophagy is a novel pathway for neurofilament protein degradation in vivo. Autophagy 19, 1277–1292. https://doi.org/10.1080/15548627.2022.2124500.

Ravits, J.M., La Spada, A.R., 2009. ALS motor phenotype heterogeneity, focality, and spread: Deconstructing motor neuron degeneration. Neurology 73, 805–811. https://doi.org/10.1212/WNL.0b013e3181b6bbbd.

Sankorrakul, K., Qian, L., Thangnipon, W., Coulson, E.J., 2021. Is there a role for the p75 neurotrophin receptor in mediating degeneration during oxidative stress and after hypoxia? J. Neurochem 158, 1292–1306. https://doi.org/10.1111/jnc.15451.

Saucier, D., Wajnberg, G., Roy, J., Beauregard, A.-P., Chacko, S., Crapoulet, N., Fournier, S., Ghosh, A., Lewis, S.M., Marrero, A., O'Connell, C., Ouellette, R.J., Morin, P.J., 2019. Identification of a circulating miRNA signature in extracellular vesicles collected from amyotrophic lateral sclerosis patients. Brain Res. 1708, 100–108. https://doi.org/10.1016/j.brainres.2018.12.016.

Scarafino, A., D'Errico, E., Introna, A., Fraddosio, A., Distaso, E., Tempesta, I., Morea, A., Mastronardi, A., Leante, R., Ruggieri, M., Mastrapasqua, M., Simone, I.L., 2018. Diagnostic and prognostic power of CSF Tau in amyotrophic lateral sclerosis. J. Neurol. 265, 2353–2362. https://doi.org/10.1007/s00415-018-9008-3.

Schmid, J., Liesinger, L., Birner-Gruenberger, R., Stojakovic, T., Scharnagl, H., Dieplinger, B., Asslaber, M., Radl, R., Beer, M., Polacin, M., Mair, J., Szolar, D., Berghold, A., Quasthoff, S., Binder, J.S., Rainer, P.P., 2018. Elevated cardiac troponin T in patients with skeletal myopathies. J. Am. Coll. Cardiol. 71, 1540–1549. https://doi.org/10.1016/j.jacc.2018.01.070.

Shefner, J.M., Al-Chalabi, A., Baker, M.R., Cui, L.-Y., de Carvalho, M., Eisen, A., Grosskreutz, J., Hardiman, O., Henderson, R., Matamala, J.M., Mitsumoto, H., Paulus, W., Simon, N., Swash, M., Talbot, K., Turner, M.R., Ugawa, Y., van den Berg, L.H., Verdugo, R., Vucic, S., Kaji, R., Burke, D., Kiernan, M.C., 2020. A proposal for new diagnostic criteria for ALS. Clin. Neurophysiol. 131, 1975–1978. https://doi.org/10.1016/j.clinph.2020.04.005.

Shepheard, S.R., Chataway, T., Schultz, D.W., Rush, R.A., Rogers, M.-L., 2014. The extracellular domain of neurotrophin receptor p75 as a candidate biomarker for amyotrophic lateral sclerosis. PLoS ONE 9, e87398. https://doi.org/10.1371/journal.pone.0087398.

Shepheard, S.R., Wuu, J., Cardoso, M., Wiklendt, L., Dinning, P.G., Chataway, T., Schultz, D., Benatar, M., Rogers, M.-L., 2017. Urinary p75 ^{ECD}: a prognostic, disease progression, and pharmacodynamic biomarker in ALS. Neurology 88, 1137–1143. https://doi.org/10.1212/WNL.0000000000003741.

Shepheard, S.R., Karnaros, V., Benyamin, B., Schultz, D.W., Dubowsky, M., Wuu, J., Chataway, T., Malaspina, A., Benatar, M., Rogers, M., 2022. Urinary neopterin: a novel biomarker of disease progression in amyotrophic lateral sclerosis. Eur. J. Neurol. 29, 990–999. https://doi.org/10.1111/ene.15237.

Simrén, J., Andreasson, U., Gobom, J., Suarez Calvet, M., Borroni, B., Gillberg, C., Nyberg, L., Ghidoni, R., Fernell, E., Johnson, M., Depypere, H., Hansson, C., Jonsdottir, I.H., Zetterberg, H., Blennow, K., 2022. Establishment of reference values for plasma neurofilament light based on healthy individuals aged 5–90 years. Brain Commun. 4. https://doi.org/10.1093/braincomms/fcac174.

Sproviero, D., La Salvia, S., Colombo, F., Zucca, S., Pansarasa, O., Diamanti, L., Costa, A., Lova, L., Giannini, M., Gagliardi, S., Lauranzano, E., Matteoli, M., Ceroni, M., Malaspina, A., Cereda, C., 2019. Leukocyte derived microvesicles as disease progression biomarkers in slow progressing amyotrophic lateral sclerosis patients. Front. Neurosci. 13, 344. https://doi.org/10.3389/fnins.2019.00344.

Sproviero, D., Gagliardi, S., Zucca, S., Arigoni, M., Giannini, M., Garofalo, M., Fantini, V., Pansarasa, O., Avenali, M., Ramusino, M.C., Diamanti, L., Minafra, B., Perini, G., Zangaglia, R., Costa, A., Ceroni, M., Calogero, R.A., Cereda, C., 2022. Extracellular vesicles derived from plasma of patients with neurodegenerative disease have common transcriptomic profiling. Front. Aging Neurosci. 14. https://doi.org/10.3389/FNAGI.2022.785741.

Staats, K.A., Borchelt, D.R., Tansey, M.G., Wymer, J., 2022. Blood-based biomarkers of inflammation in amyotrophic lateral sclerosis. Mol. Neurodegener. 17, 11. https://doi.org/10.1186/s13024-022-00515-1.

Steinacker, P., Verde, F., Fang, L., Feneberg, E., Oeckl, P., Roeber, S., Anderl-Straub, S., Danek, A., Diehl-Schmid, J., Fassbender, K., Fliessbach, K., Foerstl, H., Giese, A., Jahn, H., Kassubek, J., Kornhuber, J., Landwehrmeyer, G.B., Lauer, M., Pinkhardt, E. H., Prudlo, J., Rosenbohm, A., Schneider, A., Schroeter, M.L., Tumani, H., von Arnim, C.A.F., Weishaupt, J., Weydt, P., Ludolph, A.C., Yilmazer Hanke, D., Otto, M., 2018. Chitotriosidase (CHIT1) is increased in microglia and macrophages in spinal cord of amyotrophic lateral sclerosis and cerebrospinal fluid levels correlate with disease severity and progression. J. Neurol. Neurosurg. Psychiatry 89, 239–247. https://doi.org/10.1136/jnnp-2017-317138.

Steinacker, P., Feneberg, E., Halbgebauer, S., Witzel, S., Verde, F., Oeckl, P., Van Damme, P., Gaur, N., Gray, E., Grosskreutz, J., Jardel, C.G., Kachanov, M., Kuhle, J., Lamari, F., Maceski, A., Del Mar Amador, M., Mayer, B., Morelli, C., Petri, S., Poesen, K., Raaphorst, J., Salachas, F., Silani, V., Turner, M.R., Verbeek, M.M., Volk, A.E., Weishaupt, J.H., Weydt, P., Ludolph, A.C., Otto, M., 2021. Chitotriosidase as biomarker for early stage amyotrophic lateral sclerosis: a multicenter study. Amyotroph. Lateral Scler. Front. Degener. 22, 276–286. https://doi.org/10.1080/21678421.2020.1861023.

Sucher, R., Schroecksnadel, K., Weiss, G., Margreiter, R., Fuchs, D., Brandacher, G., 2010. Neopterin, a prognostic marker in human malignancies. Cancer Lett. 287, 13–22. https://doi.org/10.1016/j.canlet.2009.05.008.

Tai, H., Cui, L., Liu, M., Guan, Y., Li, X., Shen, D., Zhang, K., Liu, S., Wu, S., Ding, Q., Hu, Y., 2018. Creatine kinase level and its relationship with quantitative electromyographic characteristics in amyotrophic lateral sclerosis. Clin. Neurophysiol. 129, 926–930. https://doi.org/10.1016/j.clinph.2018.01.071.

Théry, C., Witwer, K.W., Aikawa, E., Alcaraz, M.J., Anderson, J.D., Andriantsitohaina, R., Antoniou, A., Arab, T., Archer, F., Atkin-Smith, G.K., Ayre, D.C., Bach, J.-M., Bachurski, D., Baharvand, H., Balaj, L., Baldacchino, S., Bauer, N.N., Baxter, A.A., Bebawy, M., Beckham, C., Bedina Zavec, A., Benmoussa, A., Berardi, A.C., Bergese, P., Bielska, E., Blenkiron, C., Bobis-Wozowicz, S., Boilard, E., Boireau, W., Bongiovanni, A., Borràs, F.E., Bosch, S., Boulanger, C.M., Breakefield, X., Breglio, A. M., Brennan, M.Á., Brigstock, D.R., Brisson, A., Broekman, M.L., Bromberg, J.F., Bryl-Górecka, P., Buch, S., Buck, A.H., Burger, D., Busatto, S., Buschmann, D., Bussolati, B., Buzás, E.I., Byrd, J.B., Camussi, G., Carter, M.J., Caruso, S., Chamley, L. W., Chang, Y.-T., Chen, C., Chen, S., Cheng, L., Chin, A.R., Clayton, A., Clerici, S.P., Cocks, A., Cocucci, E., Coffey, R.J., Cordeiro-da-Silva, A., Couch, Y., Coumans, F.A., Coyle, B., Crescitelli, R., Criado, M.F., D'Souza-Schorey, C., Das, S., Datta Chaudhuri, A., de Candia, P., De Santana, E.F., De Wever, O., del Portillo, H.A., Demaret, T., Deville, S., Devitt, A., Dhondt, B., Di Vizio, D., Dieterich, L.C., Dolo, V., Dominguez Rubio, A.P., Dominici, M., Dourado, M.R., Driedonks, T.A., Duarte, F.V., Duncan, H.M., Eichenberger, R.M., Ekström, K., Andaloussi, E.L., Elie-Caille, S., Erdbrügger, C., Falcón-Pérez, U., Fatima, J.M., Fish, F., Flores-Bellver, J.E., Försönits, M., Frelet-Barrand, A., Fricke, A., Fuhrmann, F., Gabrielsson, G., Gámez-Valero, S., Gardiner, A., Gärtner, C., Gaudin, K., Gho, R., Giebel, Y.S., Gilbert, B., Gimona, C., Giusti, M., Goberdhan, I., Görgens, D.C., Gorski, A., Greening, S.M., Gross, D.W., Gualerzi, J.C., Gupta, A., Gustafson, L.W., Handberg, D., Haraszti, A., Harrison, R.A., Hegyesi, P., Hendrix, H., Hill, A., Hochberg, A.F., Hoffmann, F.H., Holder, K.F., Holthofer, B., Hosseinkhani, H., Hu, B., Huang, G., Huber, Y., Hunt, V., Ibrahim, S., Ikezu, A.G.-E., Fricke, T., Isin, J.M., Ivanova, M., Jackson, A., Jacobsen, H.K., Jay, S., Jayachandran, S.M., Jenster, M., Jiang, G., Johnson, L., Jones, S.M., Jong, J.C., Jovanovic-Talisman, A., Jung, T., Kalluri, S., Kano, R., Kaur, S., Kawamura, S., Keller, Y., Khamari, E.T., Khomyakova, D., Khvorova, E., Kierulf, A., Kim, P., Kislinger, K.P., Klingeborn, T., Klinke, M., Kornek, D.J., Kosanović, M., Kovács, M.M., Krämer-Albers, Á.F., Krasemann, E.-M., Krause, S., Kurochkin, M., Kusuma, I.V., Kuypers, G.D., Laitinen, S., Langevin, S., Languino, S. M., Lannigan, L.R., Lässer, J., Laurent, C., Lavieu, L.C., Lázaro-Ibáñez, G., Le Lay, E., Lee, S., Lee, M.-S., Lemos, Y.X.F., Lenassi, D.S., Leszczynska, M., Li, A., Liao, I.T., Libregts, K., Ligeti, S.F., Lim, E., Lim, R., Linē, S.K., Linnemannstöns, A., Llorente, K., Lombard, A., Lorenowicz, C.A., Lörincz, M.J., Lötvall, Á.M., Lovett, J., Lowry, J., Loyer, M.C., Lu, X., Lukomska, Q., Lunavat, B., Maas, T.R., Malhi, S.L., Marcilla, H., Mariani, J., Mariscal, J., Martens-Uzunova, J., Martin-Jaular, E.S., Martinez, L., Martins, M.C., Mathieu, V.R., Mathivanan, M., Maugeri, S., McGinnis, M., McVey, L. K., Meckes, M.J., Meehan, D.G., Mertens, K.L., Minciacchi, I., Möller, V.R., Møller Jørgensen, A., Morales-Kastresana, M., Morhayim, A., Mullier, J., Muraca, F., Musante, M., Mussack, L., Dolo, V., Myburgh, D.C., Najrana, K.H., Nawaz, T., Nazarenko, M., Nejsum, I., Neri, P., Neri, C., Nieuwland, T., Nimrichter, R., Nolan, L., Nolte-'t Hoen, J.P., Noren Hooten, E.N., O'Driscoll, N., O'Grady, L., O'Loghlen, T., Ochiya, A., Olivier, T., Ortiz, M., Ortiz, A., Osteikoetxea, L.A., Østergaard, X., Ostrowski, O., Park, M., Pegtel, J., Peinado, D.M., Perut, H., Pfaffl, F., Phinney, M.W., Pieters, D.G., Pink, B.C., Pisetsky, R.C., Pogge von Strandmann, D.S., Polakovicova, E., Poon, I., Powell, I.K., Prada, B.H., Pulliam, I., Quesenberry, L., Radeghieri, P., Raffai, A., Raimondo, R.L., Rak, S., Ramirez, J., Raposo, M.I., Rayyan, G., Regev-Rudzki, M.S., Ricklefs, N., Robbins, F.L., Roberts, P.D., Rodrigues, D.D., Rohde, S.C., Rome, E., Rouschop, S., Rughetti, K.M., Russell, A., Saá, A.E., Sahoo, P., Salas-Huenuleo, S., Sánchez, E., Saugstad, C., Saul, J.A., Schiffelers, M.J., Schneider, R.M., Schøyen, R., Scott, T.H., Shahaj, A., Kano, E., Shatnyeva, S., Shekari, O., Shelke, F., Shetty, G.V., Shiba, A.K., Siljander, K., Silva, P. R.-M., Skowronek, A.M., Snyder, A., Soares, O.L., Sódar, R.P., Soekmadji, B.W., Sotillo, C., Stahl, J., Stoorvogel, P.D., Stott, W., Strasser, S.L., Swift, E.F., Kosanović, S., Tewari, H., Timms, M., Tiwari, K., Tixeira, S., Tkach, R., Toh, M., Tomasini, W.S., Torrecilhas, R., Tosar, A.C., Toxavidis, J.P., Urbanelli, V., Vader, L., van Balkom, P., van der Grein, B.W., Van Deun, S.G., van Herwijnen, J., Van Keuren-Jensen, M.J., van Niel, K., van Royen, G., van Wijnen, M.E., Vasconcelos, A.J., Vechetti, M.H., Veit, I.J., Vella, T.D., Velot, L.J., Verweij, É., Vestad, F.J., Viñas, C., Visnovitz, J.L., Vukman, T., Wahlgren, K.V., Watson, J., Wauben, D.C., Weaver, M. H., Webber, A., Weber, J.P., Wehman, V., Weiss, A.M., Welsh, D.J., Wendt, J.A., Wheelock, S., Wiener, A.M., Witte, Z., Wolfram, L., Xagorari, J., Xander, A., Xu, P., Yan, J., Yáñez-Mó, X., Saugstad, M., Yuana, H., Zappulli, A., Zarubova, V., Žėkas, J., Zhang, V., Zhao, K.P., Zheng, Z., Zheutlin, L., Zickler, A.R., Zimmermann, A.M.,

Zivkovic, P., Zocco, A.M., Zuba-Surma, E.K, D., 2018. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the international society for extracellular vesicles and update of the MISEV2014 guidelines. J. Extracell. Vesicles 7, 1535750. https://doi.org/10.1080/20013078.2018.1535750.

Thompson, A.G., Turner, M.R., 2019. Untangling neuroinflammation in amyotrophic lateral sclerosis. J. Neurol. Neurosurg. Psychiatry jnnp 2019–321242. https://doi.org/10.1136/jnnp-2019-321242.

Thompson, A.G., Gray, E., Thézénas, M.-L., Charles, P.D., Evetts, S., Hu, M.T., Talbot, K., Fischer, R., Kessler, B.M., Turner, M.R., 2018. Cerebrospinal fluid macrophage biomarkers in amyotrophic lateral sclerosis: CSF macrophage biomarkers in ALS. Ann. Neurol. 83, 258–268. https://doi.org/10.1002/ana.25143.

Thompson, A.G., Gray, E., Mäger, I., Thézénas, M.-L., Charles, P.D., Talbot, K., Fischer, R., Kessler, B.M., Wood, M., Turner, M.R., 2020. CSF extracellular vesicle proteomics demonstrates altered protein homeostasis in amyotrophic lateral sclerosis. Clin. Proteom. 17. https://doi.org/10.1186/s12014-020-09294-7.

Thompson, A.G., Gray, E., Verber, N., Bobeva, Y., Lombardi, V., Shepheard, S.R., Yildiz, O., Feneberg, E., Farrimond, L., Dharmadasa, T., Gray, P., Edmond, E.C., Scaber, J., Gagliardi, D., Kirby, J., Jenkins, T.M., Fratta, P., McDermott, C.J., Manohar, S.G., Talbot, K., Malaspina, A., Shaw, P.J., Turner, M.R., 2022. Multicentre appraisal of amyotrophic lateral sclerosis biofluid biomarkers shows primacy of blood neurofilament light chain. Brain Commun. 4. https://doi.org/10.1093/braincomms/fcac029.

Thouvenot, E., Demattei, C., Lehmann, S., Maceski-Maleska, A., Hirtz, C., Juntas-Morales, R., Pageot, N., Esselin, F., Alphandéry, S., Vincent, T., Camu, W., 2020. Serum neurofilament light chain at time of diagnosis is an independent prognostic factor of survival in amyotrophic lateral sclerosis. Eur. J. Neurol. 27, 251–257. https://doi.org/10.1111/ene.14063.

Vacchiano, V., Mastrangelo, A., Zenesini, C., Baiardi, S., Avoni, P., Polischi, B., Capellari, S., Salvi, F., Liguori, R., Parchi, P., 2023. Elevated plasma p-tau181 levels unrelated to Alzheimer's disease pathology in amyotrophic lateral sclerosis. J. Neurol. Neurosurg. Psychiatry 94, 428–435. https://doi.org/10.1136/jnnp-2022-330709.

Vacchiano, V., Mastrangelo, A., Zenesini, C., Masullo, M., Quadalti, C., Avoni, P., Polischi, B., Cherici, A., Capellari, S., Salvi, F., Liguori, R., Parchi, P., 2021. Plasma and CSF Neurofilament Light Chain in Amyotrophic Lateral Sclerosis: A Cross-Sectional and Longitudinal Study. Front Aging Neurosci. 13, 753242 https://doi.org/10.3389/fnagi.2021.753242. PMID: 34744694; PMCID: PMC8569186.

Vargas, M.R., Johnson, J.A., 2010. Astrogliosis in amyotrophic lateral sclerosis: role and therapeutic potential of astrocytes. Neurotherapeutics 7, 471–481. https://doi.org/10.1016/j.nurt.2010.05.012.

Varghese, A.M., Ghosh, M., Bhagat, S.K., Vijayalakshmi, K., Preethish-Kumar, V., Vengalil, S., Chevula, P.-C.-R., Nashi, S., Polavarapu, K., Sharma, M., Dhaliwal, R.S., Philip, M., Nalini, A., Alladi, P.A., Sathyaprabha, T.N., Raju, T.R., 2020. Chitotriosidase, a biomarker of amyotrophic lateral sclerosis, accentuates neurodegeneration in spinal motor neurons through neuroinflammation. J. Neuroinflamm. 17, 232. https://doi.org/10.1186/s12974-020-01909-y.

Verde, F., Otto, M., Silani, V., 2021. Neurofilament light chain as biomarker for amyotrophic lateral sclerosis and frontotemporal dementia. Front. Neurosci. 15, 679199 https://doi.org/10.3389/fnins.2021.679199.

Verde, F., Steinacker, P., Weishaupt, JH., Kassubek, J., Oeckl, P., Halbgebauer, S., Tumani, H., von Arnim, CAF, Dorst, J., Feneberg, E., Mayer, B., Müller, HP., Gorges, M., Rosenbohm, A., Volk, AE., Silani, V., Ludolph, AC., Otto, M., 2019. Neurofilament light chain in serum for the diagnosis of amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 90 (2), 157–164. https://doi.org/10.1136/jnnp-2018-318704. Epub 2018 Oct 11. PMID: 30309882.

Verde, F., Milone, I., Maranzano, A., Colombo, E., Torre, S., Solca, F., Doretti, A., Gentile, F., Manini, A., Bonetti, R., Peverelli, S., Messina, S., Maderna, L., Morelli, C., Poletti, B., Ratti, A., Silani, V., Ticozzi, N., 2023. Serum levels of glial fibrillary acidic protein in patients with amyotrophic lateral sclerosis. Ann. Clin. Transl. Neurol. 10, 118–129. https://doi.org/10.1002/acn3.51708.

Vu, L., An, J., Kovalik, T., Gendron, T., Petrucelli, L., Bowser, R., 2020. Cross-sectional and longitudinal measures of chitinase proteins in amyotrophic lateral sclerosis and expression of CHI3L1 in activated astrocytes. J. Neurol. Neurosurg. Psychiatry 91, 350–358. https://doi.org/10.1136/jnnp-2019-321916.

Vucic, S., 2019. Differences in inflammatory profiles between ALS and FTD. J. Neurol. Neurosurg. Psychiatry 90. https://doi.org/10.1136/jnnp-2018-319377.

Vucic, S., Ferguson, T.A., Cummings, C., Hotchkin, M.T., Genge, A., Glanzman, R., Roet, K.C.D., Cudkowicz, M., Kiernan, M.C., 2021. Gold Coast diagnostic criteria: implications for ALS diagnosis and clinical trial enrollment. Muscle Nerve 64, 532–537. https://doi.org/10.1002/mus.27392.

Wang, Y., Mandelkow, E., 2016. Tau in physiology and pathology. Nat. Rev. Neurosci. 17, 22–35. https://doi.org/10.1038/nrn.2015.1.

Wilke, C., Pujol-Calderón, F., Barro, C., Stransky, E., Blennow, K., Michalak, Z., Deuschle, C., Jeromin, A., Zetterberg, H., Schüle, R., Höglund, K., Kuhle, J., Synofzik, M., 2019. Correlations between serum and CSF pNfH levels in ALS, FTD and controls: a comparison of three analytical approaches. Clin. Chem. Lab. Med. (CCLM) 57, 1556–1564. https://doi.org/10.1515/cclm-2019-0015.

Zhu, S., Wuolikainen, A., Wu, J., Öhman, A., Wingsle, G., Moritz, T., Andersen, P.M., Forsgren, L., Trupp, M., 2019. Targeted multiple reaction monitoring analysis of CSF identifies UCHL1 and GPNMB as candidate biomarkers for ALS. J. Mol. Neurosci. 69, 643–657. https://doi.org/10.1007/s12031-019-01411-y.

Zucchi, E., Bonetto, V., Sorarù, G., Martinelli, I., Parchi, P., Liguori, R., Mandrioli, J., 2020. Neurofilaments in motor neuron disorders: towards promising diagnostic and prognostic biomarkers. Mol. Neurodegener. 15, 58. https://doi.org/10.1186/s13024-020-00406-3.