



Review article

The role of Neurofilament light (NfL) and glial fibrillary acidic protein (GFAP) in MS and AQP4-NMOSD: Advancing clinical applications.

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ABSTRACT

Fluid biomarkers such as Glial Fibrillary Acidic Protein (GFAP) and Neurofilament Light (NfL) play important roles in the diagnosis, monitoring, and evaluation of therapeutic responses in conditions such as Multiple Sclerosis (MS) and Aquaporin-4 Neuromyelitis Optica Spectrum Disorder (AQP4-NMOSD). These biomarkers offer key insights into the underlying pathophysiological mechanisms of these diseases, enabling effective follow-up and personalized treatment approaches, which are essential for improving patient outcomes. Herein, we synthesize the structural attributes, functional roles, and clinical significance of GFAP and NfL in the context of MS and AQP4-NMOSD. We explore the critical implications of these biomarkers in disease manifestation and progression, emphasizing the necessity to develop standardized methodologies and multicentric studies to confirm their clinical applicability.

1. Introduction

Fluid biomarkers are integral to the management of neurological disorders [1,2], serving as indicators of diagnosis, prognostication, and therapeutic monitoring [3]. These molecular markers are particularly valuable in conditions such as Multiple Sclerosis (MS) and Aquaporin-4 Neuromyelitis Optica Spectrum Disorder (AQP4-NMOSD) [4].

MS is a complex, chronic inflammatory and degenerative demyelinating disease of the central nervous system (CNS), influenced by both genetic and environmental factors. It manifests with symptoms that vary based on the location of lesions and is a major cause of neurological disability among young adults [5]. The diagnosis of MS has evolved with newer criteria, combining clinical evaluations with advanced magnetic resonance imaging (MRI) techniques and other supportive diagnostic tools. Despite these advances, there remains a pressing need for reliable and accessible biomarkers that accurately reflect disease severity, progression, and treatment response [6]. A suitable blood biomarker for MS should correlate with clinical severity, disease activity, increasing disability, and treatment efficacy, which would significantly enhance

clinical management and therapeutic strategies [7,8].

Similarly, AQP4-NMOSD is an autoimmune inflammatory disorder targeting astrocytes, primarily characterized by the presence of Aquaporin-4 antibodies that lead to astrocytic damage and subsequent neuroaxonal deterioration [9]. AQP4-NMOSD presents unique diagnostic and prognostic challenges. Although anti-Aquaporin-4 antibodies serve as a critical diagnostic biomarker for AQP4-NMOSD, their ability to reflect ongoing disease activity and predict outcomes is limited [10,11]. This limitation underscores a significant deficiency in the current approach and emphasizes the urgent need to develop and incorporate more effective biomarkers to better manage the complexities of AQP4-NMOSD.

In the context of MS and AQP4-NMOSD, two proteins, glial fibrillary acidic protein (GFAP) and neurofilament light chain (NfL) have been identified as key biomarkers [12,13]. GFAP is a marker of astrocytic damage and is particularly useful in AQP4-NMOSD, a condition marked by severe astrocyte involvement due to autoimmunity against AQP4 water channels on astrocytes [9]. Elevated levels of GFAP in AQP4-NMOSD indicate a high degree of astrocytic destruction and gliosis,

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which are hallmarks of the acute phase of the disease. Conversely, NfL is indicative of neuronal and axonal damage and is valuable in both MS and AQP4-NMOSD [14]. Increases in NfL levels in the cerebrospinal fluid (CSF), or blood, correlate with disease activity and progression. High NfL levels during relapses or in the progressive stages of these diseases signal active neurodegeneration, helping clinicians adjust therapeutic interventions accordingly [15–18]. This review explores the structural characteristics and clinical applications of the biomarkers GFAP and NfL in MS and AQP4-NMOSD.

2. Structure

2.1. Neurofilament light chain protein (NfL)

Intermediate filaments (IFs) are essential components of the cellular cytoskeleton, characterized by their intermediate diameter (~10 nm) compared to microfilaments (actin filaments, ~7 nm) and microtubules (~25 nm). They belong to a large and diverse family of over 70 human proteins, divided into six major classes: Class I and II keratins in epithelial cells; Class III proteins, including vimentin, peripherin, desmin, and GFAP, found in mesenchymal and glial cells; Class IV neurofilaments such as NfL, Neurofilament Medium (NfM), and Neurofilament Heavy (NfH), predominantly expressed in neurons; Class V nuclear lamins, which form a meshwork beneath the inner nuclear membrane; and Class VI proteins like nestin [19].

Neurofilaments, a type of intermediate filament, consist of five primary isoforms: NfL, NfM, NfH, α -internexin, and peripherin. These proteins are crucial as they form obligate heteropolymers essential for neuronal structure and function. Mutations in neurofilament genes have been linked to various neurological disorders, such as Amyotrophic Lateral Sclerosis (ALS), Charcot-Marie-Tooth disease, and spinal muscular atrophy [20].

NfL, also known as neurofilament light polypeptide (abbreviated as NF-L or NFL), has a structure defined by several key domains. The N-terminal or head domain regulates filament assembly and diameter and includes phosphorylation sites that modulate interactions with other cytoskeletal components. The rod domain, composed of coiled-coil alpha-helices, facilitates dimerization and further polymerization into stable filaments. The staggered arrangement of this domain within filaments is crucial for their flexibility and strength. The C-terminal, or tail, domain is involved in cross-bridging with other neurofilaments and, interacting with other proteins, plays a vital role in the dynamics of the neuronal cytoskeleton [20,21].

In terms of functional role and assembly, NfL partners with other neurofilament subunits—NfM, NfH, and α -internexin—to form heteropolymers. These heteropolymers assemble into 10 nm neurofilaments that are integral for maintaining neuronal shape and internal organization. Neurofilaments are particularly abundant in large-caliber axons, where they are essential for maintaining axonal diameter, which in turn influences nerve conduction velocity. This structural and functional setup underscores the critical role of NfL in neuron architecture and function [20,22].

The primary structure of NfL is a linear sequence of amino acids with a high concentration of acidic and basic residues, vital for its functional roles. Its secondary structure primarily features alpha-helices within the central rod domain, crucial for forming coiled-coil dimers with other neurofilament subunits. The tertiary structure consists of these alpha helices coiling into a more compact form, facilitating the formation of stable, elongated dimers that enhance filament integrity. At the quaternary level, NfL interacts with other neurofilament proteins (NfM, NfH, and α -internexin), forming heteropolymeric filaments that are essential to maintaining the structural integrity and functionality of neurons, particularly influencing axon diameter and nerve impulse conductivity [23].

These neurofilament proteins undergo various post-translational modifications such as phosphorylation, which is the most extensive

and affects neurofilament spacing and axonal transport. Hyperphosphorylation of neurofilament proteins can lead to the formation of pathological aggregates, a feature common in diseases such as Alzheimer's disease (AD), and ALS. These aggregates, further stabilized by amyloidogenic elements, become remarkably resistant to decay. Other modifications include citrullination, glycosylation, and glycation, each playing roles in neurodegeneration and autoimmune pathology [24].

2.2. Glial fibrillary acidic protein (GFAP)

GFAP, the signature intermediate filament of astrocytes, plays a crucial role within the CNS. As a type-III intermediate filament, it is part of a class that also includes vimentin, desmin, and peripherin, all essential to the structure and function of the cell's cytoskeleton. GFAP, encoded by gene on chromosome 17q21, consists of 432 amino acids. While this class is primarily associated with cells of mesenchymal origin, GFAP is specifically expressed in astrocytes within the CNS. It is prominently expressed in mature astrocytes located in various regions such as the gray and white matter, cerebellum, subventricular and subgranular zones, and Müller cells in the retina. Beyond the CNS, GFAP is also expressed in peripheral locations, including hepatic stellate cells, enteric glial cells, and other non-neural cells, underscoring its widespread relevance in both central and peripheral cellular structures [25–27].

The structure of GFAP, a type III intermediate filament, comprises three key domains: head, rod, and tail. The central rod domain is crucial, as it coils around another filament, aligning the N-terminal and C-terminal ends in parallel to form a dimer. GFAP can form both homodimers and heterodimers with other type III proteins but does not interact with type I and II keratins, leading to separate intermediate filament networks in cells expressing both types. These filaments begin as GFAP dimers that link to create staggered tetramers, the basic building blocks of the filament. Filament formation relies on both the rod domain and the non-helical head and tail domains, which provide structural variability and essential sites for assembly, including two conserved arginines and an aromatic residue in the head domain required for proper filament assembly [28].

The primary structure of GFAP includes a sequence of amino acids featuring specific motifs typical of intermediate filament proteins, such as stutter interruptions which are crucial for the protein's assembly and alignment [29]. The secondary structure is dominated by alpha-helical regions within its rod domain, essential for forming coiled-coil dimer interactions. The tertiary structure of GFAP involves these helical rod domains flanked by non-helical head and tail domains, allowing both parallel and antiparallel alignments crucial for overall filament architecture. At the quaternary level, GFAP can form both homopolymeric filaments from GFAP alone and heteropolymeric filaments with other type III intermediate filaments like vimentin. This dimer and tetramer formation flexibility is key to GFAP's role in maintaining cellular architecture and mechanical stability, especially within astrocytes [30].

3. Function

3.1. Neurofilament light chain protein (NfL)

NfL's primary function is to support the structural framework of the neuron by integrating into the neurofilament network. This network is essential for maintaining the axon's diameter, which directly influences the speed and efficiency of nerve signal conduction. NfL also has a significant role in axonal transport, which involves the bidirectional movement of molecular cargos such as nutrients, organelles, and signaling molecules between the neuronal soma and synaptic terminals. This function is critical for neuronal survival and functionality [31,32].

In terms of clinical significance, elevated levels of NfL in the CSF and blood are associated with neurodegeneration, making it a valuable biomarker for various neurological diseases such as MS, AD, and ALS. The increase in NfL levels typically reflects the extent of neuronal

damage and can be used to monitor disease progression and response to therapy [33].

Furthermore, the stoichiometry of neurofilament proteins shifts in disease states, typically showing an increase in NfL relative to decreases in NfM and NfH. This alteration may represent an adaptive mechanism to conserve energy in neurons during progressive neurodegeneration, explaining why NfL is particularly promising as a biomarker. The release of stable, pathology-specific NfL cleavage products into body fluids enhances its effectiveness as a clinical biomarker, improving the sensitivity of measurements due to its abundance [23].

Neurofilament proteins and their cleavage products are released from neurons in response to axonal damage, with *in vitro* studies confirming a direct correlation between the number of degenerating neurons and neurofilament levels [34]. These proteins have been detected in CSF, blood, amniotic fluid, and ocular fluids, providing vital tools for the diagnosis and monitoring of neurological conditions [35]. The kinetics of NfL, governed by both release from injured neural tissue and clearance from the blood, exhibit an extended peak time and a decay rate that mirrors ongoing pathology, making NfL a valuable indicator of disease state and progression [20,36].

Advancements in detection technologies, such as the ultra-sensitive Single Molecule Array (SIMOA) technique, have significantly improved the ability to measure NfL levels in blood, offering over 25 times higher sensitivity compared to traditional assays like enzyme-linked immunosorbent assay (ELISA) and electrochemiluminescence (ECL). NfL's inherent stability further enhances the robustness of these detection methods [37,38].

3.2. Glial fibrillary acidic protein (GFAP)

GFAP provides mechanical strength and structural support to astrocytes, forming the main component of the intermediate filament network within these cells. This network is critical for maintaining the shape and mechanical integrity of astrocytes, particularly during the CNS development and repair processes. GFAP expression significantly increases in response to CNS injuries and diseases, a phenomenon known as reactive gliosis. During this process, astrocytes proliferate and become hypertrophic, with GFAP playing a significant role in the formation of a glial scar. This scar is essential for containing wounds and preventing further damage to CNS tissue [39].

GFAP influences synaptic function by modulating the ability of the astrocyte to regulate neurotransmitter levels within the synaptic cleft. Through its involvement in the intermediate filament network, GFAP indirectly contributes to the uptake and release of neurotransmitters, thus affecting neuronal excitability and synaptic plasticity [40].

With elevated levels in the CSF or blood indicating astrocytic damage, GFAP is a valuable biomarker for CNS disorders. Conditions such as traumatic brain injury, stroke, and neurodegenerative diseases show increased GFAP levels, which can be crucial for assessing the extent of glial injury and neuroinflammation, thus providing valuable insights into disease severity and progression [41].

Indeed, the role of GFAP extends to various diagnostic applications, where sensitive detection methods like ELISA, ECL, and fluorescence-based assays are used to measure its levels in body fluids such as CSF, vitreous fluid, and amniotic fluid. The development of SIMOA has enabled the detection of GFAP even in the blood of healthy individuals and those with neurological diseases, substantially improving diagnostic capabilities [40].

Furthermore, the mechanisms underlying the drainage of GFAP and its breakdown products into the blood under pathological conditions involve a combination of bulk flow through arachnoid villi, the glymphatic system, and continuous fluid exchange at the CNS barriers. These pathways highlight the complex nature of GFAP dynamics in health and disease [42].

4. Clinical application

4.1. Neurofilament light chain protein (NfL)

Neurofilament proteins, specifically NfL and NfH, are emerging as important biomarkers across a range of neurological diseases, reflecting disease activity, progression, and therapeutic responses [43,44] (Table 1). Elevated NfL levels in CSF and blood correlate with cognitive decline and brain atrophy in AD and frontotemporal dementia (FTD), aiding early diagnosis and monitoring [45–47]. In stroke and traumatic brain injury, increased neurofilaments signify acute neuroaxonal damage and help assess injury extent, essential for clinical decisions like safe return to sports [48]. In ALS, high neurofilament levels are linked to disease progression and prognosis, facilitating tracking and therapy evaluation [49]. Elevated neurofilament levels have also been observed in atypical parkinsonian disorders such as progressive supranuclear palsy and multiple system atrophy, as well as in Huntington's disease. Their potential utility is also being investigated in conditions like epilepsy, encephalitis, and bipolar disorder, though their clinical relevance in these areas remains less well-established. [50,51].

NfL is increasingly recognized as a vital biomarker in MS, indicative of active inflammation and ongoing neuroaxonal damage associated with greater disability, heightened disease activity, and increased relapse risk [52–61]. Elevated NfL levels in CSF and blood effectively distinguish MS patients from healthy individuals, although they do not differentiate between MS subtypes. As a nonspecific biomarker, NfL levels are also elevated in other neurological conditions as previously mentioned, limiting its diagnostic specificity for MS except in cases requiring differentiation from other disorders [23,62]. In MS clinical practice, NfL serves primarily as a prognostic indicator, with high serum NfL levels predicting future relapse risk, disability progression, and the development of new MRI lesions, including gadolinium-enhancing and T2 lesions [61,63–72]. Longitudinal studies have demonstrated that baseline and changes in NfL levels correlate with disease progression, brain atrophy, and cognitive decline, reinforcing its utility in monitoring disease trajectory [64,65,73–75]. In clinical trials, disease-modifying therapies (DMTs) consistently reduce NfL levels, reflecting decreased neuroaxonal degeneration and improved treatment efficacy [76–83]. For example, the RADIANCE trial demonstrated that Ozanimod significantly lowered MRI lesion activity and NfL levels over 24 weeks by sequestering lymphocytes, thereby reducing neuroinflammation [84]. Similarly, Evobrutinib, a Bruton tyrosine kinase (BTK) inhibitor, markedly decreased NfL levels over 2.5 years compared to placebo, highlighting its therapeutic potential [85]. Additionally, Ofatumumab, an anti-CD20 monoclonal antibody, has shown superior efficacy in reducing relapse rates, MRI lesions, and NfL concentrations compared to teriflunomide, with early initiation linked to better long-term outcomes [86–90]. These findings underscore NfL's potential in monitoring MS progression and assessing therapeutic responses [63].

Standard ELISA assays are used to measure NfL in CSF, while the SIMOA Quanterix NF-Light assay is preferred for blood measurements; both CSF and serum NfL are valuable, with CSF preferred for initial diagnosis and serum ideal for ongoing monitoring, and factors such as age, body mass index (BMI), renal function, diabetes, and neurotoxic treatments can influence NfL levels and should be accounted for during interpretation [63]. Overall, neurofilaments hold significant potential as biomarkers for a broad range of neurological conditions, aiding in diagnosis, monitoring disease progression, and evaluating therapeutic efficacy. As such, the ongoing development of sensitive immunoassays for their detection in blood is likely to expand their clinical application [91], making them integral to the proper management of neurodegenerative and neuroinflammatory diseases.

4.2. Glial fibrillary acidic protein (GFAP)

GFAP is a vital biomarker for various CNS disorders, reflecting

Table 1**Key Points on Neurofilament Biomarkers in Multiple Sclerosis.**

- > NFL is the primary biomarker for MS, indicating neuronal degeneration and disease activity, while NfM and NfH require further investigation.
- > Elevated NfL levels in CSF and blood signal both inflammation and neurodegeneration, making it useful prognosis but limited for diagnosis due to its non-specificity. NfL is a nonspecific marker elevated in various neuronal damage conditions, limiting its diagnostic specificity for MS but useful in differentiating it from other diseases in specific cases.
- > Elevated NfL levels in serum, which peak during relapses, predict greater disease progression including more lesions, relapses, disability, brain atrophy, and cognitive decline when measured at baseline and longitudinally.
- > Disease-modifying therapies (DMTs) consistently reduce NfL levels, indicating decreased neuroaxonal damage and improved clinical outcomes.
- > Standard ELISA assays are used for measuring NfL in CSF, and the Single-Molecule Array (SIMOA) Quanterix NF-Light assay is preferred for blood measurements. Both CSF and serum NfL measurements are valuable, with CSF preferred for initial diagnosis and serum ideal for ongoing monitoring, following specific resampling guidelines.
- > Age, BMI, renal function, diabetes, and neurotoxic treatments can influence NfL levels and should be accounted for during interpretation.

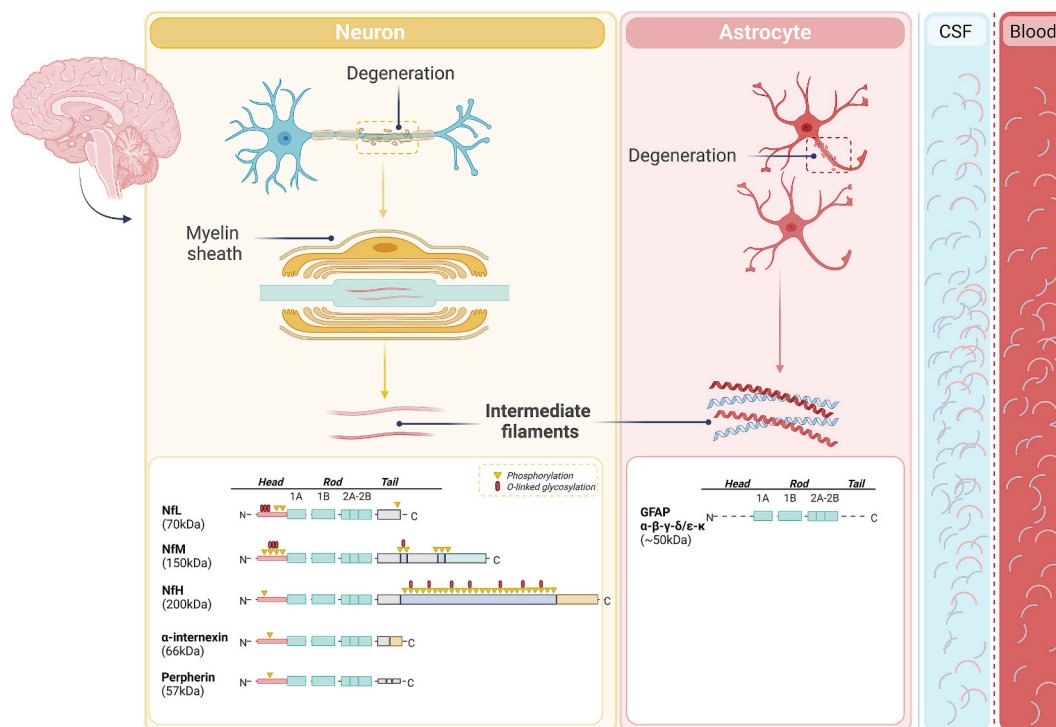


Fig. 1. Neurofilament Structure and Degeneration in Neurons and Astrocytes. In neurons, neurofilaments (NFL, NfM, NfH, α -internexin, peripherin) are crucial for maintaining structure and function. In astrocytes, GFAP, with its head, rod, and tail domains essential for structural integrity, plays a similar role. Neurofilament subunits are depicted with conserved α -helical rod domains and variable head and tail domains, showing phosphorylation and O-linked glycosylation, especially in the tails of NfM and NfH. Neurofilament assembly begins with monomers forming coiled-coil heterodimers, which align into tetramers and then assemble into mature filaments (~10 nm in diameter). GFAP in astrocytes similarly contributes to cellular structure with its distinct domains. Upon axonal and astrocytic damage, neurofilament and GFAP proteins are released into the extracellular space, cerebrospinal fluid (CSF), and blood. These cleavage products can be detected by highly sensitive immunoassays, making them valuable biomarkers for diagnosing neurological disorders.

neuronal damage and disease activity. In traumatic brain injury and spinal cord injuries, elevated GFAP levels assess injury severity and guide clinical decisions, leading to FDA-approved blood tests that reduce unnecessary CT scans [25,92–94]. In neurodegenerative diseases such as AD and FTD, increased GFAP correlates with disease progression and astrocytic damage, serving as both diagnostic and prognostic tools, while in Alexander disease, GFAP levels align with disease severity and inform therapeutic approaches [95]. In oncology, GFAP levels in glioblastoma and other brain tumors correlate with tumor volume and postoperative outcomes, though diagnostic accuracy requires further research [96–98]. Additionally, GFAP indicates disease presence and severity in conditions such as epilepsy, delirium, sepsis-related encephalopathy, and infections such as COVID-19 and West Nile virus [26,99]. This broad applicability makes GFAP a versatile biomarker in neurology, enhancing differential diagnosis precision, aiding management decisions, and tracking disease progression across diverse neurological contexts [100].

In MS and AQP4-NMOSD, GFAP serves as a critical biomarker reflecting astrocytic activation and neuroinflammation. Elevated GFAP

levels are significantly higher in MS and AQP4-NMOSD patients compared to healthy controls, with primary progressive MS patients exhibiting higher GFAP levels than those with relapsing-remitting MS, indicating more severe disease states [26,41,101–107]. Additionally, studies have shown higher GFAP levels in NMOSD compared to MS, aiding in differential diagnosis. This is particularly relevant in the presence of AQP4 autoantibodies, which contribute to astrocyte destruction during acute relapses—an essential factor for guiding distinct treatment protocols [108].

GFAP levels correlate with various clinical characteristics, serological biomarkers, disability measures like Expanded Disability Status Scale (EDSS), disease duration, NFL levels, and imaging measures such as T2 lesion volume, underscoring its role in disease progression and severity [30,103,104,106,107,109–116]. The correlation of GFAPs with brain atrophy and cognitive decline further supports the role of the protein in monitoring disease progression and therapeutic efficacy [117].

While initial ELISA and ECL assays did not find significant differences in GFAP levels between MS and non-inflammatory disorders,

sensitive SIMOA assays have detected higher serum GFAP in MS, particularly in progressive MS [103,118–120].

Autoimmune GFAP astrocytopathy is another condition where GFAP plays a central role. This steroid-responsive central nervous system disease can present as a monophasic or relapsing condition, characterized by symptoms and signs of meningoencephalomyelitis or its limited forms. The diagnosis is supported by the detection of GFAP-IgG in CSF along with paraclinical findings such as inflammatory CSF or characteristic T1 postgadolinium imaging features. Approximately 25 % of patients have an associated neoplasm, most commonly ovarian teratoma, while others develop the condition for unknown reasons. Recently, autoimmune GFAP astrocytopathy has been increasingly differentiated from other autoimmune CNS disorders, further highlighting the importance of accurate diagnostic markers such as GFAP-IgG and associated clinical and imaging features to guide appropriate treatment and management strategies [121,122].

Overall, GFAP's ability to differentiate CNS conditions, its association with disease severity, and its responsiveness to treatments make it an invaluable biomarker in neurology. However, further research is needed to refine its clinical application and fully integrate it into patient care strategies.

5. Conclusion and future perspectives

In summary, the clinical application of GFAP and neurofilament protein measurements holds transformative promise for enhancing the management of neurological disorders by providing a sensitive and minimally invasive measure of neuroaxonal damage. These biomarkers are poised to revolutionize our approach to diagnosing and monitoring diseases, such as MS, AQP4-NMOSD, and others. The ability to assess brain tissue damage with a simple blood sample marks a significant advancement in both research and clinical practice, facilitating early detection, more informed therapeutic decisions and improved patient outcomes.

However, several challenges must be addressed for widespread clinical adoption. For GFAP, further research is needed to understand its release mechanisms, effects of protein aggregation on analytical accuracy, and physiological dynamics, including protein binding and metabolism. The specificity of assays for different GFAP isoforms and the contributions of distinct astrocyte subclasses to circulating GFAP levels require clarification. For NfL, the establishment of normative data across all age groups, the impact of comorbidities, and the standardization of measurement techniques are critical. Additionally, defining disease-specific cutoff values and correlating NfL changes with clinical outcomes will enhance its clinical utility, particularly in MS [20,123,124].

Efforts should focus on developing robust, disease-specific databases for both GFAP and NfL to provide precise cutoff values and reference ranges. These resources, combined with multicenter trials and retrospective analyses of existing data, will validate the clinical use of these biomarkers and refine their role in monitoring disease progression, therapeutic efficacy, and patient outcomes.

As research advances, integrating GFAP and NfL into routine clinical practice has the potential to transform neurological care. By enabling more accurate diagnoses, better monitoring of disease activity, and tailored therapeutic strategies, these biomarkers could significantly improve patient outcomes and contribute to the development of new treatments.

CRedit authorship contribution statement

Sara Samadzadeh: Writing – original draft. **Roy D. Sleator:** Writing – review & editing, Supervision.

Declaration of competing interest

None.

References

- [1] T. Quirke, R.D. Sleator, A beginner's guide to genomics in complex neurological disorders, *Innovation Discovery* 1 (4) (2024).
- [2] C. Casey, J.F. Fullard, R.D. Sleator, Unravelling the genetic basis of schizophrenia, *Gene* 902 (2024) 148198.
- [3] E.D. Schilke, G. Remoli, E. Funelli, M. Galimberti, M.L. Fusco, D. Cereda, et al., Current use of fluid biomarkers as outcome measures in multiple sclerosis (MS): a review of ongoing pharmacological clinical trials, *Neurol. Sci.* 45 (5) (2024) 1931–1944.
- [4] N. Mikolajewicz, P.P. Yee, D. Bhanja, M. Trifoi, A.M. Miller, P. Metellus, et al., Systematic review of cerebrospinal fluid biomarker discovery in neuro-oncology: a roadmap to standardization and clinical application, *J. Clin. Oncol.* (2024) Jco2301621.
- [5] S. Krieger, K. Cook, C.M. Hersh, Understanding multiple sclerosis as a disease spectrum: above and below the clinical threshold, *Curr. Opin. Neurol.* 37 (3) (2024) 189–201.
- [6] A.J. Thompson, B.L. Banwell, F. Barkhof, W.M. Carroll, T. Coetzee, G. Comi, et al., Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria, *Lancet Neurol.* 17 (2) (2018) 162–173.
- [7] M. Aliyu, F.T. Zohora, A. Ceylan, F. Hossain, R. Yazdani, G. Azizi, Immunopathogenesis of multiple sclerosis: molecular and cellular mechanisms and new immunotherapeutic approaches, *Immunopharmacol. Immunotoxicol.* 1-23 (2024).
- [8] D. Ontaneda, T. Chitnis, K. Rammohan, A.Z. Obeidat, Identification and management of subclinical disease activity in early multiple sclerosis: a review, *J. Neurol.* 271 (4) (2024) 1497–1514.
- [9] L. Cacciaguerra, E.P. Flanagan, Updates in NMOSD and MOGAD diagnosis and treatment: a tale of two central nervous system autoimmune inflammatory disorders, *Neurol. Clin.* 42 (1) (2024) 77–114.
- [10] M.A. Rocca, L. Cacciaguerra, M. Filippi, Moving beyond anti-aquaporin-4 antibodies: emerging biomarkers in the spectrum of neuromyelitis optica, *Expert. Rev. Neurother.* 20 (6) (2020) 601–618.
- [11] J. Liu, G. Tan, B. Li, J. Zhang, Y. Gao, Y. Cao, et al., Serum aquaporin 4-immunoglobulin G titer and neuromyelitis optica spectrum disorder activity and severity: a systematic review and meta-analysis, *Front. Neurol.* 12 (2021) 746959.
- [12] L. Heimfarth, F.R.S. Passos, B.S. Monteiro, A.A.S. Araújo, L.J. Quintans Júnior, J. S.S. Quintans, Serum glial fibrillary acidic protein is a body fluid biomarker: a valuable prognostic for neurological disease - a systematic review, *Int. Immunopharmacol.* 107 (2022) 108624.
- [13] L. Ning, B. Wang, Neurofilament light chain in blood as a diagnostic and predictive biomarker for multiple sclerosis: a systematic review and meta-analysis, *PLoS One* 17 (9) (2022) e0274565.
- [14] C. Hagbohm, R. Ouellette, E.P. Flanagan, D.I. Jonsson, F. Piehl, B. Banwell, R. Wickström, E. Jacobaeus, T. Granberg, B.V. Ineichen, Clinical and neuroimaging phenotypes of autoimmune glial fibrillary acidic protein astrocytopathy: a systematic review and meta-analysis, *Eur. J. Neurol.* (2024), <https://doi.org/10.1111/ene.16284>. PMID: 38506182; PMCID: PMC11235751 e16284.
- [15] S. Kim, J.J. Lee, J.S. Park, M. Kang, H.Y. Seok, Neurofilament light chain as a biomarker in neuromyelitis optica spectrum disorder: a comprehensive review and integrated analysis with glial fibrillary acidic protein, *Neurol. Sci.* 45 (3) (2024) 1255–1261.
- [16] A. Ghezzi, R.F. Neuteboom, Neurofilament light chain in adult and pediatric multiple sclerosis: a promising biomarker to better characterize disease activity and personalize MS treatment, *Neurol. Ther.* 12 (6) (2023) 1867–1881.
- [17] C. Kessler, C. Ruschil, A. Abdelhak, C. Wilke, A. Maleska, J. Kuhle, et al., Serum neurofilament light chain and glial fibrillary acidic protein as biomarkers in primary progressive multiple sclerosis and hereditary spastic paraplegia type 4, *Int. J. Mol. Sci.* 23 (21) (2022).
- [18] A. Abdelhak, J. Kuhle, A.J. Green, Challenges and opportunities for the promising biomarker blood Neurofilament light Chain, *JAMA Neurol.* 80 (6) (2023) 542–543.
- [19] S. Narayanan, A. Shanker, T. Khera, B. Subramaniam, Neurofilament light: a narrative review on biomarker utility, *Fac. Rev.* 10 (2021) 46.
- [20] M. Khalil, C.E. Teunissen, S. Lehmann, M. Otto, F. Piehl, T. Ziemssen, S. Bittner, M.P. Sormani, T. Gatteringer, S. Abu-Rumeileh, S. Thebault, A. Abdelhak, A. Green, P. Benkert, L. Kappos, M. Comabella, H. Tuman, M.S. Freedman, A. Wickzold, K. Blennow, H. Zetterberg, D. Leppert, J. Kuhle, Neurofilaments as biomarkers in neurological disorders - towards clinical application, *Nat Rev Neurol* 20 (5) (2024) 269–287, <https://doi.org/10.1038/s41582-024-00955-x>.
- [21] T. Ziemssen, K. Akgün, W. Brück, Molecular biomarkers in multiple sclerosis, *J. Neuroinflammation* 16 (1) (2019) 272.
- [22] A. Yuan, M.V. Rao, Nixon R.A. Veeranna, Neurofilaments at a glance, *J. Cell Sci.* 125 (Pt 14) (2012) 3257–3263.
- [23] S. Thebault, R.A. Booth, M.S. Freedman, Blood neurofilament light chain: the neurologist's troponin? *Biomedicines* 8 (11) (2020).
- [24] S. Coppens, S. Lehmann, C. Hopley, C. Hirtz, Neurofilament-light, a promising biomarker: analytical, metrological and clinical challenges, *Int. J. Mol. Sci.* 24 (14) (2023).
- [25] X. Zheng, J. Yang, Y. Hou, X. Shi, K. Liu, Prediction of clinical progression in nervous system diseases: plasma glial fibrillary acidic protein (GFAP), *Eur. J. Med. Res.* 29 (1) (2024) 51.

- [26] A. Abdelhak, M. Foschi, S. Abu-Rumeileh, J.K. Yue, L. D'Anna, A. Huss, et al., Blood GFAP as an emerging biomarker in brain and spinal cord disorders, *Nat. Rev. Neurol.* 18 (3) (2022) 158–172.
- [27] N.J. Laping, B. Teter, N.R. Nichols, I. Rozovsky, C.E. Finch, Glial fibrillary acidic protein: regulation by hormones, cytokines, and growth factors, *Brain Pathol.* 4 (3) (1994) 259–275.
- [28] E.G. Sukhorukova, D. Kruzhevskii, O.S. Alekseeva, Glial fibrillary acidic protein: the component of intermediate filaments in the vertebrate brain astrocytes, *Zh. Evol. Biokhim. Fiziol.* 51 (1) (2015) 3–10.
- [29] L.F. Eng, R.S. Ghirnikar, GFAP and astrogliosis, *Brain Pathol.* 4 (3) (1994) 229–237.
- [30] L.F. Eng, R.S. Ghirnikar, Y.L. Lee, Glial fibrillary acidic protein: GFAP-thirty-one years (1969–2000), *Neurochem. Res.* 25 (9–10) (2000) 1439–1451.
- [31] C. Ferreira-Atuesta, S. Reyes, G. Giovanonni, S. Gnanapavan, The evolution of Neurofilament light Chain in multiple sclerosis, *Front. Neurosci.* 15 (2021) 642384.
- [32] E.P. Culligan, J.R. Marchesi, C. Hill, R.D. Slesator, Combined metagenomic and phenomic approaches identify a novel salt tolerance gene from the human gut microbiome, *Front. Microbiol.* 5 (2014) 189.
- [33] B. Arslan, H. Zetterberg, Neurofilament light chain as neuronal injury marker - what is needed to facilitate implementation in clinical laboratory practice? *Clin. Chem. Lab. Med.* 61 (7) (2023) 1140–1149.
- [34] A.R. Gafoin, N.R. Barthélemy, P. Bomont, R.O. Carare, H.D. Durham, J.P. Julien, et al., Neurofilaments: neurobiological foundations for biomarker applications, *Brain* 143 (7) (2020) 1975–1998.
- [35] G. Mak, S. Menon, J.Q. Lu, Neurofilaments in neurologic disorders and beyond, *J. Neurol. Sci.* 441 (2022) 120380.
- [36] G. Palermo, S. Mazzucchi, A. Della Vecchia, G. Siciliano, U. Bonuccelli, C. Azuar, et al., Different clinical contexts of use of blood Neurofilament light Chain protein in the Spectrum of neurodegenerative diseases, *Mol. Neurobiol.* 57 (11) (2020) 4667–4691.
- [37] D.M. Rissin, C.W. Kan, T.G. Campbell, S.C. Howes, D.R. Fournier, L. Song, et al., Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations, *Nat. Biotechnol.* 28 (6) (2010) 595–599.
- [38] R. Dong, N. Yi, D. Jiang, Advances in single molecule arrays (SIMOA) for ultra-sensitive detection of biomolecules, *Talanta* 270 (2024) 125529.
- [39] J. Huang, W. Huang, R. Zhou, W. Lin, T. Chen, Y. Long, Detection and significance of glial fibrillary acidic protein antibody in autoimmune astocytopathy and related diseases, *Ann. Transl. Med.* 11 (7) (2023) 288.
- [40] E.M. Hol, M. Pekny, Glial fibrillary acidic protein (GFAP) and the astrocyte intermediate filament system in diseases of the central nervous system, *Curr. Opin. Cell Biol.* 32 (2015) 121–130.
- [41] H. Kim, E.J. Lee, Y.M. Lim, K.K. Kim, Glial fibrillary acidic protein in blood as a disease biomarker of Neuromyelitis Optica Spectrum disorders, *Front. Neurol.* 13 (2022) 865730.
- [42] A. Petzold, Glial fibrillary acidic protein is a body fluid biomarker for glial pathology in human disease, *Brain Res.* 1600 (2015) 17–31.
- [43] S. Abu-Rumeileh, A. Abdelhak, M. Foschi, L. D'Anna, M. Russo, P. Steinacker, et al., The multifaceted role of neurofilament light chain protein in non-primary neurological diseases, *Brain* 146 (2) (2023) 421–437.
- [44] C. Barro, H. Zetterberg, Neurological symptoms and blood neurofilament light levels, *Acta Neurol. Scand.* 144 (1) (2021) 13–20.
- [45] R. Zanardini, C. Saraceno, L. Benussi, R. Squitti, R. Ghidoni, Exploring Neurofilament light chain and exosomes in the genetic forms of frontotemporal dementia, *Front. Neurosci.* 16 (2022) 758182.
- [46] Y. Jung, J.S. Damoiseaux, The potential of blood neurofilament light as a marker of neurodegeneration for Alzheimer's disease, *Brain* 147 (1) (2024) 12–25.
- [47] E. Karantali, D. Kazis, S. Chatzikonstantinou, F. Petridis, I. Mavroudis, The role of neurofilament light chain in frontotemporal dementia: a meta-analysis, *Aging Clin. Exp. Res.* 33 (4) (2021) 869–881.
- [48] X. Zhang, H. Wang, L. Li, X. Deng, L. Bo, Neurofilament light Chain: a candidate biomarker of perioperative stroke, *Front. Aging Neurosci.* 14 (2022) 921809.
- [49] M. Benatar, L.W. Ostrow, J.W. Lewcock, F. Bennett, J. Shefner, R. Bowser, et al., Biomarker qualification for Neurofilament light Chain in amyotrophic lateral sclerosis: theory and practice, *Ann. Neurol.* 95 (2) (2024) 211–216.
- [50] S. Bayoumy, I.M.W. Verberk, L. Vermunt, E. Willemse, B. den Dulk, A.T. van der Ploeg, D. Pajkrt, E. Nitz, J.M.P. van den Hout, J. van der Post, N.I. Wolf, S. Beerepoot, E.J.N. Groen, V. Tüngler, C.E. Teunissen, Neurofilament light protein as a biomarker for spinal muscular atrophy: a review and reference ranges, *Clin Chem Lab Med* 62 (7) (2024) 1252–1265, <https://doi.org/10.1515/ccim-2023-1311>. PMID: 38215341.
- [51] C. Barro, T. Chitnis, H.L. Weiner, Blood neurofilament light: a critical review of its application to neurologic disease, *Ann. Clin. Transl. Neurol.* 7 (12) (2020) 2508–2523.
- [52] M.K. Sen, M.J. Hossain, D.A. Mahns, B.J. Brew, Validity of serum neurofilament light chain as a prognostic biomarker of disease activity in multiple sclerosis, *J. Neurol.* 270 (4) (2023) 1908–1930.
- [53] H.L. Desu, K.M. Sawicka, E. Wuerch, V. Kitchin, J.A. Quandt, A rapid review of differences in cerebrospinal neurofilament light levels in clinical subtypes of progressive multiple sclerosis, *Front. Neurosci.* 15 (2024) 1382468.
- [54] R. Kapoor, K.E. Smith, M. Allegretta, D.L. Arnold, W. Carroll, M. Comabella, et al., Serum neurofilament light as a biomarker in progressive multiple sclerosis, *Neurology* 95 (10) (2020) 436–444.
- [55] P. Arroyo Pereira, A. Muñoz-Vendrell, I. León Moreno, L. Bau, E. Matas, L. Romero-Pinel, et al., Baseline serum neurofilament light chain levels differentiate aggressive from benign forms of relapsing-remitting multiple sclerosis: a 20-year follow-up cohort, *J. Neurol.* 271 (4) (2024) 1599–1609.
- [56] A. Ashkar, M.M.A. Baig, A. Arif, M.M. Ali, F. Yousof, R. Ashkar, Prognostic significance of neurofilament light in Fingolimod therapy for multiple sclerosis: a systematic review and meta-analysis based on randomized control trials, *Mult. Scler. Relat. Disord.* 69 (2023) 104416.
- [57] N. Liu, M. Sun, W. Zhang, J. Sun, P. Gong, H. Wang, et al., Prognostic value of neurofilament light chain in natalizumab therapy for different phases of multiple sclerosis: a systematic review and meta-analysis, *J. Clin. Neurosci.* 101 (2022) 198–203.
- [58] A. Abdelhak, C. Cordano, W.J. Boscardin, E. Caverzasi, J. Kuhle, B. Chan, J. M. Gelfand, H.H. Yiu, F.C. Oertel, A. Beaudry-Richard, Montes S. Condor, J. R. Oksenberg, A. Lario Lago, A. Boxer, J.C. Rojas-Martinez, F.M. Elahi, J.R. Chan, A.J. Green, Plasma neurofilament light chain levels suggest neuroaxonal stability following therapeutic remyelination in people with multiple sclerosis, *J Neurol Neurosurg Psychiatry* (2022), <https://doi.org/10.1136/jnnp-2022-329221>. Epub ahead of print. PMID: 35710320; PMCID: PMC9984688.
- [59] A. Huss, M. Senel, A. Abdelhak, B. Mayer, J. Kassubek, A.C. Ludolph, et al., Longitudinal serum neurofilament levels of multiple sclerosis patients before and after treatment with first-line immunomodulatory therapies, *Biomedicines* 8 (9) (2020).
- [60] R.J. Fox, B.A.C. Cree, J. de Sèze, R. Gold, H.P. Hartung, D. Jeffery, et al., Temporal relationship between serum neurofilament light chain and radiologic disease activity in patients with multiple sclerosis, *Neurology* 102 (9) (2024) e209357.
- [61] A. Abdelhak, P. Benkert, S. Schaedelin, W.J. Boscardin, C. Cordano, J. Oechtering, et al., Neurofilament light Chain elevation and disability progression in multiple sclerosis, *JAMA Neurol.* 80 (12) (2023) 1317–1325.
- [62] C. Bridel, W.N. van Wieringen, H. Zetterberg, B.M. Tijms, C.E. Teunissen, J. C. Alvarez-Cermeño, et al., Diagnostic value of cerebrospinal fluid Neurofilament light protein in neurology: a systematic review and Meta-analysis, *JAMA Neurol.* 76 (9) (2019) 1035–1048.
- [63] M.S. Freedman, S. Gnanapavan, R.A. Booth, P.A. Calabresi, M. Khalil, J. Kuhle, et al., Guidance for use of neurofilament light chain as a cerebrospinal fluid and blood biomarker in multiple sclerosis management, *eBioMedicine* (2024) 101.
- [64] T. Uphaus, F. Steffen, M. Muthuraman, N. Ripfel, V. Fleischer, S. Groppa, et al., NFL predicts relapse-free progression in a longitudinal multiple sclerosis cohort study, *eBioMedicine* 72 (2021) 103590.
- [65] S. Thebault, M. Reaume, R.A. Marrie, J.J. Marriott, R. Furlan, A. Laroni, et al., High or increasing serum NFL is predictive of impending multiple sclerosis relapses, *Mult. Scler. Relat. Disord.* 59 (2022) 103535.
- [66] I. Rosenstein, M. Axelsson, L. Novakova, K. Blennow, H. Zetterberg, J. Lycke, Exploring CSF neurofilament light as a biomarker for MS in clinical practice; a retrospective registry-based study, *Mult. Scler.* 28 (6) (2022) 872–884.
- [67] C. Malmeström, S. Haghighi, L. Rosengren, O. Andersen, J. Lycke, Neurofilament light protein and glial fibrillary acidic protein as biological markers in MS, *Neurology* 61 (12) (2003) 1720–1725.
- [68] G. Disanto, R. Adutori, R. Dobson, V. Martinelli, G. Dalla Costa, T. Runia, et al., Serum neurofilament light chain levels are increased in patients with a clinically isolated syndrome, *J. Neurol. Neurosurg. Psychiatry* 87 (2) (2016) 126–129.
- [69] S. Bittner, F. Steffen, T. Uphaus, M. Muthuraman, V. Fleischer, A. Salmen, et al., Clinical implications of serum neurofilament in newly diagnosed MS patients: a longitudinal multicentre cohort study, *eBioMedicine* 56 (2020) 102807.
- [70] J. Kuhle, H. Kropshofer, D.A. Haering, U. Kundu, R. Meinert, C. Barro, et al., Blood neurofilament light chain as a biomarker of MS disease activity and treatment response, *Neurology* 92 (1A) (2019) e1007–e15.
- [71] P. Benkert, S. Meier, S. Schaedelin, A. Manouchehrinia, Ö. Yaldizli, A. Maceski, et al., Serum neurofilament light chain for individual prognostication of disease activity in people with multiple sclerosis: a retrospective modelling and validation study, *Lancet Neurol.* 21 (3) (2022) 246–257.
- [72] G. Disanto, C. Barro, P. Benkert, Y. Naegelin, S. Schädelin, A. Giardiello, et al., Serum Neurofilament light: a biomarker of neuronal damage in multiple sclerosis, *Ann. Neurol.* 81 (6) (2017) 857–870.
- [73] T. Williams, C. Tur, A. Eshaghi, A. Doshi, D. Chan, S. Binks, et al., Serum neurofilament light and MRI predictors of cognitive decline in patients with secondary progressive multiple sclerosis: analysis from the MS-STAT randomised controlled trial, *Mult. Scler.* 28 (12) (2022) 1913–1926.
- [74] J. Kuhle, T. Plavina, C. Barro, G. Disanto, D. Sangurdekar, C.M. Singh, et al., Neurofilament light levels are associated with long-term outcomes in multiple sclerosis, *Mult. Scler.* 26 (13) (2020) 1691–1699.
- [75] C. Barro, P. Benkert, G. Disanto, C. Tsagkas, M. Amann, Y. Naegelin, et al., Serum neurofilament as a predictor of disease worsening and brain and spinal cord atrophy in multiple sclerosis, *Brain* 141 (8) (2018) 2382–2391.
- [76] A. Pedersen, T.M. Stanne, S. Nilsson, S. Klasson, L. Rosengren, L. Holmegaard, et al., Circulating neurofilament light in ischemic stroke: temporal profile and outcome prediction, *J. Neurol.* 266 (11) (2019) 2796–2806.
- [77] E. Tavazzi, D. Jakimovski, J. Kuhle, J. Hagemeyer, O. Ozel, M. Ramanathan, et al., Serum neurofilament light chain and optical coherence tomography measures in MS: a longitudinal study, *Neurol. Neuroimmunol. Neuroinflamm.* 7 (4) (2020).
- [78] P.A. Calabresi, D.L. Arnold, D. Sangurdekar, C.M. Singh, A. Altincatal, C. de Moor, et al., Temporal profile of serum neurofilament light in multiple sclerosis: implications for patient monitoring, *Mult. Scler.* 27 (10) (2021) 1497–1505.
- [79] J. Kuhle, N. Daizadeh, P. Benkert, A. Maceski, C. Barro, Z. Michalak, et al., Sustained reduction of serum neurofilament light chain over 7 years by alemtuzumab in early relapsing-remitting MS, *Mult. Scler.* 28 (4) (2022) 573–582.

- [80] D. Leppert, H. Kropshofer, D.A. Häring, F. Dahlke, A. Patil, R. Meinert, et al., Blood neurofilament light in progressive multiple sclerosis: post hoc analysis of 2 randomized controlled trials, *Neurology* 98 (21) (2022) e2120–e31.
- [81] D.A. Häring, H. Kropshofer, L. Kappos, J.A. Cohen, A. Shah, R. Meinert, et al., Long-term prognostic value of longitudinal measurements of blood neurofilament levels, *Neurol. Neuroimmunol. Neuroinflamm.* 7 (5) (2020).
- [82] K. Akgün, N. Kretschmann, R. Haase, U. Proschmann, H.H. Kitzler, H. Reichmann, et al., Profiling individual clinical responses by high-frequency serum neurofilament assessment in MS, *Neurol. Neuroimmunol. Neuroinflamm.* 6 (3) (2019) e555.
- [83] L. Novakova, M. Axelsson, C. Malmeström, H. Zetterberg, K. Blennow, A. Svenningsson, et al., NFL and CXCL13 may reveal disease activity in clinically and radiologically stable MS, *Mult. Scler. Relat. Disord.* 46 (2020) 102463.
- [84] F.L. Scott, B. Clemons, J. Brooks, E. Brahmachary, R. Powell, H. Dedman, et al., Ocanimod (RPC1063) is a potent sphingosine-1-phosphate receptor-1 (S1P1) and Receptor-5 (S1P5) agonist with autoimmune disease-modifying activity, *Br. J. Pharmacol.* 173 (11) (2016) 1778–1792.
- [85] O. Shulga, A. Chabanova, O. Kotsiuba, Bruton's tyrosine kinase inhibitors in the treatment of multiple sclerosis, *Postep. Psychiatr. Neurol.* 32 (1) (2023) 23–30.
- [86] S.L. Hauser, R. Zielman, A. Das Gupta, J. Xi, D. Stoneman, G. Karlsson, et al., Efficacy and safety of four-year ofatumumab treatment in relapsing multiple sclerosis: the ALITHIOS open-label extension, *Mult. Scler.* 29 (11–12) (2023) 1452–1464.
- [87] J. Gärtner, S.L. Hauser, A. Bar-Or, X. Montalban, J.A. Cohen, A.H. Cross, et al., Efficacy and safety of ofatumumab in recently diagnosed, treatment-naïve patients with multiple sclerosis: results from ASCLEPIOS I and II, *Mult. Scler.* 28 (10) (2022) 1562–1575.
- [88] K. Harding, O. Williams, M. Willis, J. Hrstelj, A. Rimmer, F. Joseph, et al., Clinical outcomes of escalation vs early intensive disease-modifying therapy in patients with multiple sclerosis, *JAMA Neurol.* 76 (5) (2019) 536–541.
- [89] A. He, B. Merkel, J.W.L. Brown, L. Zhovits Ryerson, I. Kister, C.B. Malpas, et al., Timing of high-efficacy therapy for multiple sclerosis: a retrospective observational cohort study, *Lancet Neurol.* 19 (4) (2020) 307–316.
- [90] P. Iaffaldano, G. Lucisano, F. Caputo, D. Paolicelli, F. Patti, M. Zaffaroni, et al., Long-term disability trajectories in relapsing multiple sclerosis patients treated with early intensive or escalation treatment strategies, *Ther. Adv. Neurol. Disord.* 14 (2021), 175628642111019574.
- [91] D. Vecchio, C. Puricelli, S. Malucchi, E. Virgilio, S. Martire, S. Perga, et al., Serum and cerebrospinal fluid neurofilament light chains measured by SIMOA™, Ella™, and Lumpulse™ in multiple sclerosis naïve patients, *Mult. Scler. Relat. Disord.* 82 (2024) 105412.
- [92] Y. Pei, X. Tang, E. Zhang, K. Lu, B. Xia, J. Zhang, et al., The diagnostic and prognostic value of glial fibrillary acidic protein in traumatic brain injury: a systematic review and meta-analysis, *Eur. J. Trauma Emerg. Surg.* 49 (3) (2023) 1235–1246.
- [93] L. Schiff, N. Hadker, S. Weiser, C. Rausch, A literature review of the feasibility of glial fibrillary acidic protein as a biomarker for stroke and traumatic brain injury, *Mol. Diagn. Ther.* 16 (2) (2012) 79–92.
- [94] FDA authorizes marketing of first blood test to aid in the evaluation of concussion in adults, US Food and Drug Administration, 2018. Available from: <https://www.fda.gov/news-events/press-announcements/fda-authorizes-marketing-first-blood-test-aid-evaluation-concussion-adults>.
- [95] Y. Zou, L. Li, L. Guan, C. Ma, S. Yu, X. Ma, et al., Research trends and hotspots of glial fibrillary acidic protein within the area of Alzheimer's disease: a bibliometric analysis, *Front. Aging Neurosci.* 15 (2023) 1196272.
- [96] J. Tichy, S. Spechtmeier, M. Mittelbronn, E. Hattingen, J. Rieger, C. Senft, et al., Prospective evaluation of serum glial fibrillary acidic protein (GFAP) as a diagnostic marker for glioblastoma, *J. Neuro-Oncol.* 126 (2) (2016) 361–369.
- [97] A. Ilhan-Mutlu, A.S. Berghoff, J. Furtner, K. Dieckmann, I. Slavc, T. Czech, et al., High plasma-GFAP levels in metastatic myxopapillary ependymoma, *J. Neuro-Oncol.* 113 (3) (2013) 359–363.
- [98] E.J. van Bodegraven, J.V. van Asperen, P.A.J. Robe, E.M. Hol, Importance of GFAP isoform-specific analyses in astrocytoma, *Glia* 67 (8) (2019) 1417–1433.
- [99] L. Bark, I.M. Larsson, E. Wallin, J. Simrén, H. Zetterberg, M. Lipcsey, et al., Central nervous system biomarkers GFAP and NFL associate with post-acute cognitive impairment and fatigue following critical COVID-19, *Sci. Rep.* 13 (1) (2023) 13144.
- [100] J.V. van Asperen, D.M. Fedorushkova, P. Robe, E.M. Hol, Investigation of glial fibrillary acidic protein (GFAP) in body fluids as a potential biomarker for glioma: a systematic review and meta-analysis, *Biomarkers* 27 (1) (2022) 1–12.
- [101] R. Kassubek, M. Gorges, M. Schocke, V.A.M. Hagenston, A. Huss, A.C. Ludolph, et al., GFAP in early multiple sclerosis: a biomarker for inflammation, *Neurosci. Lett.* 657 (2017) 166–170.
- [102] A. Abdelhak, A. Huss, J. Kassubek, H. Tumani, M. Otto, Serum GFAP as a biomarker for disease severity in multiple sclerosis, *Sci. Rep.* 8 (1) (2018) 14798.
- [103] O. Aktas, M.A. Smith, W.A. Rees, J.L. Bennett, D. She, E. Katz, et al., Serum glial fibrillary acidic protein: a Neuromyelitis Optica Spectrum disorder biomarker, *Ann. Neurol.* 89 (5) (2021) 895–910.
- [104] X. Aygnacq, E. Le Bars, C. Duflos, C. Hirtz, A. Maleska Maceski, C. Carra-Dallière, et al., Serum GFAP in multiple sclerosis: correlation with disease type and MRI markers of disease severity, *Sci. Rep.* 10 (1) (2020) 10923.
- [105] M. Watanabe, Y. Nakamura, Z. Michalak, N. Isobe, C. Barro, D. Leppert, et al., Serum GFAP and neurofilament light as biomarkers of disease activity and disability in NMOSD, *Neurology* 93 (13) (2019) e1299–e311.
- [106] S. Momtazmanesh, P. Shobeiri, A. Saghadzadeh, C.E. Teunissen, J. Burman, L. Szalardy, et al., Neuronal and glial CSF biomarkers in multiple sclerosis: a systematic review and meta-analysis, *Rev. Neurosci.* 32 (6) (2021) 573–595.
- [107] R.A. Linker, P. Brechlin, S. Jesse, P. Steinacker, D.H. Lee, A.R. Asif, et al., Proteome profiling in murine models of multiple sclerosis: identification of stage specific markers and culprits for tissue damage, *PLoS One* 4 (10) (2009) e7624.
- [108] P. Schindler, O. Aktas, M. Ringelstein, B. Wildemann, S. Jarius, F. Paul, et al., Glial fibrillary acidic protein as a biomarker in neuromyelitis optica spectrum disorder: a current review, *Expert Rev. Clin. Immunol.* 19 (1) (2023) 71–91.
- [109] F. Azzolini, L. Gilio, L. Pavone, E. Iezzi, E. Dolcetti, A. Bruno, et al., Neuroinflammation is associated with GFAP and sTREM2 levels in multiple sclerosis, *Biomolecules* 12 (2) (2022).
- [110] M. Saraste, S. Bezukladova, M. Matilainen, M. Sucksdorff, J. Kuhle, D. Leppert, et al., Increased serum glial fibrillary acidic protein associates with microstructural white matter damage in multiple sclerosis: GFAP and DTI, *Mult. Scler. Relat. Disord.* 50 (2021) 102810.
- [111] S. Brahmachari, Y.K. Fung, K. Pahan, Induction of glial fibrillary acidic protein expression in astrocytes by nitric oxide, *J. Neurosci.* 26 (18) (2006) 4930–4939.
- [112] Z. Yang, K.K. Wang, Glial fibrillary acidic protein: from intermediate filament assembly and gliosis to neurobiomarker, *Trends Neurosci.* 38 (6) (2015) 364–374.
- [113] B.D. Gulbransen, K.A. Sharkey, Novel functional roles for enteric glia in the gastrointestinal tract, *Nat. Rev. Gastroenterol. Hepatol.* 9 (11) (2012) 625–632.
- [114] J.N. Whitaker, Myelin encephalitogenic protein fragments in cerebrospinal fluid of persons with multiple sclerosis, *Neurology* 27 (10) (1977) 911–920.
- [115] C.F. Brosnan, C.S. Raine, The astrocyte in multiple sclerosis revisited, *Glia* 61 (4) (2013) 453–465.
- [116] R. Aharoni, R. Eilam, R. Arnon, Astrocytes in multiple sclerosis-essential constituents with diverse multifaceted functions, *Int. J. Mol. Sci.* 22 (11) (2021).
- [117] A. Shaygannejad, N. Rafiei, S. Vaheb, M. Yazdan Panah, V. Shaygannejad, O. Mirmosayyeb, The role of glial fibrillary acidic protein as a biomarker in multiple sclerosis and neuromyelitis optica spectrum disorder: a systematic review and meta-analysis, *Medicina (Kaunas)* 60 (7) (2024).
- [118] M.A. Schaller-Paule, M. Maiworm, J.H. Schäfer, L. Friedauer, E. Hattingen, K. J. Wenger, et al., Matching proposed clinical and MRI criteria of aggressive multiple sclerosis to serum and cerebrospinal fluid markers of neuroaxonal and glial injury, *J. Neurol.* 271 (6) (2024) 3512–3526.
- [119] S. Meier, E.A.J. Willemsse, S. Schaedelin, J. Oechtering, J. Lorscheider, L. Melie-Garcia, et al., Serum glial fibrillary acidic protein compared with Neurofilament light chain as a biomarker for disease progression in multiple sclerosis, *JAMA Neurol.* 80 (3) (2023) 287–297.
- [120] X. Aygnacq, E. Le Bars, C. Duflos, C. Hirtz, A. Maleska Maceski, C. Carra-Dallière, et al., Serum GFAP in multiple sclerosis: correlation with disease type and MRI markers of disease severity, *Sci. Rep.* 10 (1) (2020) 10923.
- [121] A. Kunchok, A. Zekeridou, A. McKeon, Autoimmune glial fibrillary acidic protein astrocytopathy, *Curr. Opin. Neurol.* 32 (3) (2019) 452–458.
- [122] A. Gravier-Dumonceau, R. Ameli, V. Rogemond, A. Ruiz, B. Joubert, S. Muñoz-Castrillo, et al., Glial fibrillary acidic protein autoimmunity, *Neurology* 98 (6) (2022) e653–e68.
- [123] M. Khalil, C.E. Teunissen, M. Otto, F. Piehl, M.P. Sormani, T. Gatteringer, et al., Neurofilaments as biomarkers in neurological disorders, *Nat. Rev. Neurol.* 14 (10) (2018) 577–589.
- [124] C.R. Zamecnik, G.M. Sowa, A. Abdelhak, R. Dandekar, R.D. Bair, K.J. Wade, C. M. Bartley, K. Kizer, D.G. Augusto, A. Tubati, R. Gomez, C. Fouassier, C. Gerungan, C.M. Caspar, J. Alexander, A.E. Wapniarski, R.P. Loudermilk, E. L. Eggers, K.C. Zorn, K. Ananth, N. Jabassini, S.A. Mann, N.R. Ragan, A. Santaniello, R.G. Henry, S.E. Baranzini, S.S. Zamvil, J.J. Sabatino Jr., R. M. Bove, C.Y. Guo, J.M. Gelfand, R. Cuneo, H.C. von Büdingen, J.R. Oksenberg, B. A.C. Cree, J.A. Hollenbach, A.J. Green, S.L. Hauser, M.T. Wallin, J.L. DeRisi, M. R. Wilson, An autoantibody signature predictive for multiple sclerosis, *Nat Med* 30 (5) (2024) 1300–1308, <https://doi.org/10.1038/s41591-024-02938-3>. Epub 2024 Apr 19. PMID: 38641750.