Blood GFAP as an emerging biomarker in brain and spinal cord disorders

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Abstract | Blood-derived biomarkers for brain and spinal cord diseases are urgently needed. The introduction of highly sensitive immunoassays led to a rapid increase in the number of potential blood-derived biomarkers for diagnosis and monitoring of neurological disorders. In 2018, the FDA authorized a blood test for clinical use in the evaluation of mild traumatic brain injury (TBI). The test measures levels of the astrocytic intermediate filament glial fibrillary acidic protein (GFAP) and neuroaxonal marker ubiquitin carboxy-terminal hydrolase L1. In TBI, blood GFAP levels are correlated with clinical severity and extent of intracranial pathology. Evidence also indicates that blood GFAP levels hold the potential to reflect, and might enable prediction of, worsening of disability in individuals with progressive multiple sclerosis. A growing body of evidence suggests that blood GFAP levels can be used to detect even subtle injury to the CNS. Most importantly, the successful completion of the ongoing validation of point-of-care platforms for blood GFAP might ameliorate the decision algorithms for acute neurological diseases, such as TBI and stroke, with important economic implications. In this Review, we provide a systematic overview of the evidence regarding the utility of blood GFAP as a biomarker in neurological diseases. We propose a model for GFAP concentration dynamics in different conditions and discuss the limitations that hamper the widespread use of GFAP in the clinical setting. In our opinion, the clinical use of blood GFAP measurements has the potential to contribute to accelerated diagnosis and improved prognostication, and represents an important step forward in the era of precision medicine.

In 2018, the FDA authorized the use of a blood test for glial fibrillary acidic protein (GFAP) and ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) in mild traumatic brain injury (mTBI), crowning a long success story of CNS-driven blood biomarker development[1–3]. Initial efforts to identify fluid biomarkers for neurological diseases focused on the cerebrospinal fluid (CSF) as, compared with blood, CSF is closer to the brain extracellular space and contains higher concentrations of CNS-derived proteins[4]. The establishment of fourth-generation immune assays in the last decade[3,5] brought the possibility of quickly obtaining rapid and robust protein biomarker measurements from blood samples, opening up new perspectives in the field of CNS-derived markers. For example, levels of classic CSF biomarkers of neuroaxonal damage, such as neurofilament light chain (NfL)[5], phosphorylated tau 217 (REF.[6]), and UCH-L1 (REF.[7]) can now be readily quantified in blood, indicating that these markers hold potential for use in diagnosis and monitoring of disease activity, and as surrogate end points for treatment trials. The literature on the utility of blood GFAP as a biomarker is also growing, reinforcing the large body of published data on CSF GFAP[3,8–14]. The evaluation of blood levels of GFAP has the potential to enable the in vivo longitudinal evaluation of different aspects of the astrocytic response in several neurological disorders. Here, we provide an up-to-date review of the analytical aspects, current evidence, perspectives, and limitations of blood GFAP as a biomarker, with the purpose of outlining how to refine its application in the diagnosis and monitoring of neurological diseases.

GFAP biology and analysis

Astrocytes represent around 30–40% of the cells in the CNS[15], form an integral part of the blood–brain barrier (BBB) and establish numerous interactions with other cells in the nervous system, including neurons.

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https://doi.org/10.1038/s41582-021-00616-3

Key points

- Glial fibrillary acidic protein (GFAP) levels reflect the clinical severity and extent of intracranial pathology after traumatic brain injury (TBI).
- In 2018, the FDA authorized the marketing of a blood test for GFAP and ubiquitin carboxy-terminal hydrolase L1 for clinical use in mild TBI.
- Growing evidence supports the potential clinical use of blood GFAP levels in numerous neuroinflammatory and neurodegenerative diseases, and in the context of CNS involvement in systemic diseases.
- Successful validation of the GFAP point-of-care analysis platform might ameliorate the decision algorithms for acute neurological diseases with important economic implications.

Hook effect
An excess of the analyte of interest overwhelms the capture antibodies in immunoassays, resulting in a falsely low reading.

Astrocytes are central to the normal function of synapses and contribute to axonal metabolic maintenance through the regulation of ion homeostasis[16] (FIG. 1). GFAP is the signature intermediate filament of astrocytes[17]. GFAP is a type-III intermediate filament and human GFAP comprises 432 amino acids, which are encoded by a gene on chromosome 17q21.1-q25. The filament is expressed in mature astrocytes in the grey and white matter, the cerebellum, the subventricular and subgranular zones, and Mueller cells in the retina[18]. GFAP is also expressed in the periphery by Schwann cells, mature glial cells in the gut, hepatic stellate cells, and other non-neural cells[18,19]. To date, evidence indicates that ten splice-isoforms of GFAP — α, β, δ, ζ, κ, Δ135, Δ164, Δexon6 and Δexon7 — are expressed in the nervous system[20]. The isoform that is most abundantly expressed and most often analysed in the literature is GFAPα[21].

Several sensitive enzyme-linked immunosorbent assays (ELISA), electrochemiluminescence (ECL), and fluorescence-based methods for the detection of GFAP in body fluids (for example, CSF, vitreous fluid and amniotic fluid) are commercially available[3,22]. However, detection of GFAP in the blood has historically been a challenge, as the available ELISA tests cannot reliably detect such low concentrations of GFAP. When high levels of GFAP in the CSF are observed (for example, in individuals with TBI[23] or neuromyelitis optica exacerbation[24]), detection of GFAP in the blood is usually possible with ELISA[3]. The development of highly sensitive assays, such as single-molecule arrays (Simoa), has enabled the detection of GFAP in the blood of healthy individuals and individuals with different neurological diseases[25]. Furthermore, the detection of blood GFAP is now possible with a portable, point-of-care platform that can deliver results within 15 min[26].

The mechanisms underlying drainage of GFAP and its breakdown products into the blood under pathological conditions seem to be complex and are a matter of continuing debate. Evidence indicates that drainage is likely to result from a combination of bulk flow into the blood via arachnoid villi, flow along the glymphatic system and the cervical lymph nodes, and continuous bidirectional fluid exchange at the barriers of the CNS (that is, the BBB and blood–CSF barrier)[27–29]. According to the available data, GFAP is stable in the blood (for at least five freeze–thaw cycles)[30]; however, a thorough characterization of pre-analytical confounders and an aggregation-related 'hook effect' remains to be completed. The hook effect is partially caused by the formation of protein aggregates that contribute to the extraordinary long-term stability of GFAP. These aggregates can last for millennia at ambient temperature, as exemplified by the Heslington brain[31]. The formation of pathological GFAP aggregates in vivo can accompany lethal neurological disorders such as Alexander disease[2].

Acute CNS injury

Traumatic brain injury

TBI is a common cause of disability worldwide, mostly among young adults[32]. The current standard of care requires the prompt evaluation of TBI severity; however, this evaluation relies on physical (for example, the Glasgow Coma Scale (GCS)) and radiological (head CT) tools that have several limitations. For example, GCS scores cannot be used to assess severity of TBI in patients who are sedated for intubation. Moreover, head CT, which has long been the clinical standard for the radiographic detection of TBI in the emergency department, can result in unnecessary exposure to ionizing radiation if used indiscriminately, especially in young individuals with mTBI[33]. These limitations have led to the investigation of a range of astroglial and neuronal biomarkers, including S100β calcium-binding protein (S100B), GFAP and UCH-L1, with the aim of improving the accuracy of TBI diagnosis and the associated decision-making process[34].

Diagnosis. The results of key studies of blood GFAP levels in TBI are summarized in TABLE 1. In a study in 584 participants with mild-to-moderate TBI, elevated levels of serum GFAP were detected within 1 h of injury, compared with levels in participants with non-TBI general trauma. GFAP levels in the group of participants with TBI peaked at 20 h after injury, and finally declined slowly until 72 h

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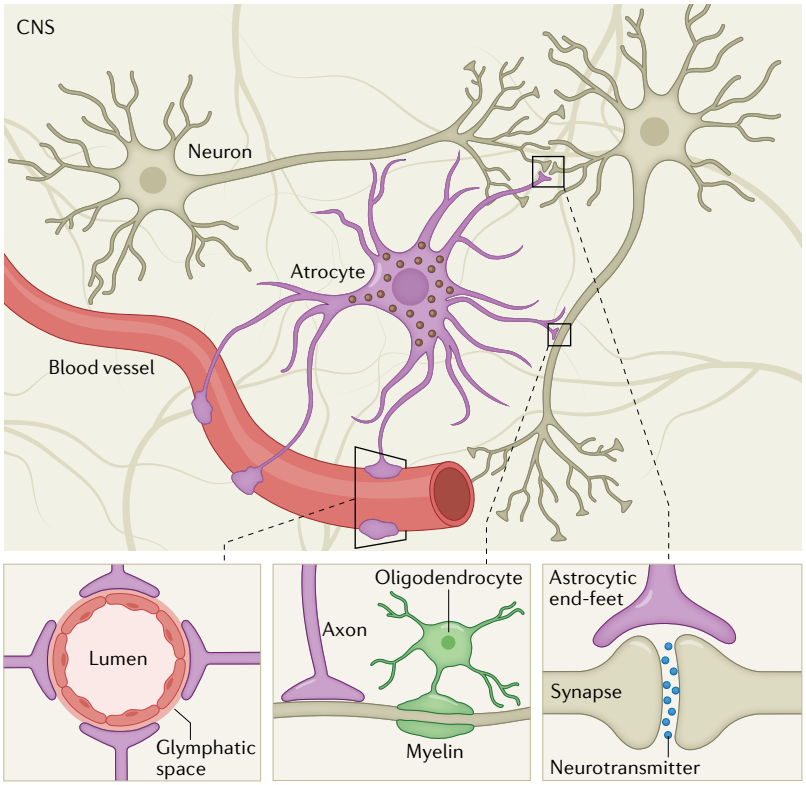

Fig. 1 | **Astrocytes have multiple physiological roles in the CNS.** Astrocytic end-feet containing glial fibrillary acidic protein (brown circles) are an essential component of the blood–brain barrier and the glymphatic system[157]. Astrocytes are critical in maintaining axonal metabolic homeostasis[158] and contribute to tripartite synapses[159].

after injury[35]. Bazarian et al. performed a large multicentre observational study in more than 1,900 participants with mild-to-moderate TBI (the ALERT-TBI study), and found that a prespecified cut-off value of 22 pg/ml for serum GFAP, in addition to serum UCH-L1 levels above 327 pg/ml, was able to predict the presence of intracranial injuries on head CT with an area under the receiving operator characteristic curve (AUC) of 0.98 (REF.[36]). This finding contributed to the FDA authorization in 2018 to market the first blood-based test for the avoidance of unnecessary exposure to radiation from CT in individuals with suspected TBI[1]. In a more recent study, serum GFAP levels were used to discriminate participants with mTBI from age-matched participants without intracranial traumatic pathology (AUC 0.69). This study used a higher GFAP cut-off value (0.23 ng/ml) than the study by Bazarian et al.[36], and involved concomitant assessment of serum S100B (AUC 0.84) and neurogranin (AUC 0.77)[37]. Another study compared the ability of serum and plasma GFAP to discriminate between participants with mTBI with and without acute abnormalities on head CT[38]. The AUC values were similar for serum (AUC 0.81) and plasma (AUC 0.79) GFAP although neither plasma nor serum levels were able to adequately predict functional outcomes at 1 week.

Some studies have found that, in comparison with other serum biomarkers (for example, UCH-L1, S100B and NfL), GFAP is the best marker for discriminating individuals with TBI and abnormalities on head CT from individuals with TBI and normal head CT scans[35,39–41]. For example, the large prospective multicentre Collaborative European NeuroTrauma Effectiveness Research (CENTRE-TBI) study (with more than 2,800 participants) found an AUC of 0.89 for GFAP[40]; the second highest AUC (0.83) was for UCH-L1. Moreover, levels of GFAP scaled with clinical severity and care path intensity (emergency department < ward admission < intensive care unit) especially in individuals with mTBI[40]. In another example, blood GFAP levels were better than blood NfL levels at discriminating between participants with normal and abnormal head CT scans; GFAP had an AUC of 0.77, compared with 0.65 for NfL[42]. Similarly, blood GFAP outperformed blood S100B in discriminating between participants with and without lesions on CT, both at 0–8 h and at 12–32 h after injury (AUC 0.89 and 0.63 for GFAP and S100B, respectively, at 0–8 h; AUC 0.94 and 0.72 for GFAP and S100B, respectively, at 12–32 h)[41].

Importantly, evidence suggests that blood GFAP levels are sensitive to subclinical intracranial pathologies that are not visible on head CT scans. Indeed, in the 18-centre Transforming Research and Clinical Knowledge in TBI (TRACK-TBI) study (study years 2014–2018), plasma GFAP was used to identify participants with MRI abnormalities from a prospective cohort of 450 participants with normal head CT scans after mTBI[26]. Plasma GFAP concentrations measured within 24 h of injury were significantly higher among the 120 participants with positive MRI scans than among the 330 participants with negative MRI scans, with an AUC of 0.78. In this cohort, participants with diffuse axonal injuries detected on MRI had higher levels of plasma GFAP than participants with other lesion types on MRI, suggesting that changes in GFAP levels are specific to some cellular pathologies. Moreover, participants with mTBI, and negative CT and MRI scans had higher median plasma GFAP concentrations than healthy control participants (74.0 pg/ml versus 8.0 pg/ml), suggesting the presence of subtle and/or microscopic glial damage not detectable with MRI[26]. These findings provide the impetus for future translational and clinical applications of GFAP to bridge the gap between molecular changes and the structural injury that is visible with neuroimaging tools. Furthermore, they suggest that GFAP could be used as a triage tool to obtain short-interval scans and much-needed follow-up care in patients with negative initial imaging results, as the subtle and/or subclinical effects of TBI are often missed in a field without formal guidelines for clinical follow-up.

In one study, blood GFAP levels were higher in 73 participants with acute orthopaedic trauma than in 93 participants with mTBI and a negative head CT scan ($P = 0.026$) on arrival; however, no differences between the two groups were observed during the following days[43]. In a large prospective study, initial (4 h after injury) blood GFAP levels were also able to discriminate participants (both children and adults) with body and head trauma with concussion from participants with non-concussive trauma with AUCs of 0.80 and 0.76, respectively; the highest GFAP levels were observed in participants with concussive head trauma[44]. An early increase in blood GFAP levels after head

Table 1 | **Key studies of blood GFAP levels in traumatic brain injury**

Study	Assay	Participants	Methods	Main results[a]
Metting et al. (2012)[50]	ELISA	94 with TBI	Prospective cohort study: serum samples and head CT obtained after admission, MRI 3 months after injury, GOSE and RTW 6 months after injury	GFAP levels higher in participants with abnormal CT than in those with normal CT; GFAP levels higher in participants with axonal injury on MRI than in those without axonal injury on MRI; GFAP levels higher in participants with incomplete RTW than in those with complete RTW
Papa et al. (2012)[128]	ELISA	108 with TBI (97 with GCS score 13–15, and 11 with GCS score 9–12), 199 controls	Prospective cohort study: serum samples and head CT <4 h after injury	GFAP breakdown product levels distinguished participants with TBI from controls (AUC 0.90), identified TBI with a GCS score of 15 (AUC 0.88), identified participants with CT lesions (AUC 0.79), and identified participants with neurosurgical intervention (AUC 0.87)
Papa et al. (2014)[139]	ELISA	209 with mmTBI, 188 without mmTBI	Prospective cohort study: serum collected <4 h after injury; head CT in 262 participants	AUC for predicting presence of intracranial lesions on CT: 0.84 for GFAP and 0.78 for S100B
Papa et al. (2016)[35]	ELISA	584 with mmTBI, 259 without mmTBI	Prospective cohort study: blood samples obtained <4 h after injury; repeated sampling at 4 h, 8 h, 12 h and then each 12 h up to 180 h after injury	GFAP was detectable <1 h after injury, peaked at 20 h, and declined until 72 h; over the course of 1 week, GFAP identified participants with mmTBI (AUC 0.73–0.94), intracranial lesions on CT (AUC 0.80–0.97), and neurosurgical intervention (AUC 0.91–1.00)
Posti et al. (2016)[140]	CLIA	324 with acute TBI, 81 controls	Prospective cohort study: blood sample, GCS and head CT at admission; blood sample on days 1, 2, 3 and 7 after admission	Strong correlation between GFAP levels and GCS at admission ($r = 0.43$, $P < 0.001$); GFAP distinguished mass lesions from diffuse injuries on CT (AUC 0.64–0.82)
Posti et al. (2017)[43]	CLIA	73 with acute orthopaedic injury, 93 with CT-negative mild TBI	Prospective cohort study: blood sample and head CT on arrival; blood sample on days 1, 2, 3 and 7 after arrival; follow-up at 3–10 months; head MRI in 71% of participants	Higher GFAP levels in participants with orthopaedic trauma than in participants with CT-negative mild TBI ($P = 0.026$) on arrival, and no differences on the following days
Bogoslovsky et al. (2017)[47]	Simoa (kits used not specifically mentioned)	34 with TBI, 69 controls	Prospective multicentre cohort study: blood samples <24 h, and 30 and 90 days after TBI, head CT and GCS at admission; GOSE 6 months after injury	GFAP levels were highest on day 0 and distinguished participants with complicated mild TBI from controls (AUC 0.936); elevated GFAP levels up to 90 days after injury compared with levels in controls
Bazarian et al. (2018)[36]	CLIA	2,011 with non-penetrating TBI and GCS 9–15, of whom 1,959 had analysable data	Prospective multicentre observational study: serum samples and head CT <12 h after injury	66% of participants had GFAP >22 pg/ml; for the detection of intracranial injury, the test had a sensitivity of 0.976 and a negative predictive value of 0.996
Frankel et al. (2019)[51]	ELISA	566 with TBI	Prospective multicentre observational study: blood samples were obtained <4 h after injury; GOSE at 1–4 and 6 months after injury	Inclusion of GFAP improved ($P \leq 0.05$) prognostic capacity of GOSE scores compared with a model containing only baseline patient variables and characteristics; the best prognostic capability (AUC 0.85) was achieved by also incorporating blood S100B levels
Mahan et al. (2019)[41]	Not reported in detail	118 with TBI, 37 controls	Prospective observational cohort study: blood samples collected 0–8 h and 12–32 h after injury, head CT in the emergency department	GFAP levels higher in CT-positive participants than in CT-negative participants; higher median GFAP levels at 12–32 h than at 0–8 h. GFAP was a predictor of pathological head CT results (0.89 sensitivity and 0.62 specificity at 0–8 h; 0.94 sensitivity and 0.67 specificity at 12–32 h); GFAP alone outperformed all possible combinations of tested biomarkers (UCH-L1, SB100)
Yue et al. (2019)[26]	Prototype immunoassay assay on a point-of-care platform	450 with mild TBI, GCS 13–15 and normal head CT, of whom 330 had negative MRI; 122 orthopaedic trauma controls; 209 healthy controls	Prospective multicentre cohort study: blood samples obtained <24 h after injury, brain MRI 7–18 days after injury	GFAP identified participants with positive CT and MRI scan with an AUC of 0.777 over 24 h; median GFAP concentration was highest in participants with CT-negative and MRI-positive findings

Table 1 (cont.) | **Key studies of blood GFAP levels in traumatic brain injury**

Study	Assay	Participants	Methods	Main results[a]
Anderson et al. (2020)[52]	ELISA	243 with TBI (pre-hospital GCS score 3–12, SBP >90 mmHg)	Prospective observational cohort study: blood samples and head CT at baseline (median 84 min after injury), GOSE and DRS at 6 months	In the majority of predictive models, the inclusion of GFAP significantly improved AUC compared with models passed on pre-hospital variables alone
Huebschmann et al. (2020)[38]	Simoa single and multiplex kit for plasma and serum, respectively	121 (≥50 years old) with head trauma	Prospective observational cohort study: mean time between injury and blood sampling 3.4 h (s.d. 2.1; range 0.5–11.7); head CT scans at the emergency department; GOSE 1 week after injury	Higher GFAP levels in participants with abnormal CT scans than in those with normal head CT scans, and in those with poor compared with good functional outcome; similar serum (AUC 0.814) and plasma (AUC 0.778) levels, GFAP identified participants with head CT abnormalities
Czeiter et al. (2020)[40]	Simoa (multiplex kit)	2,867 with TBI	Prospective multicentre cohort study: serum samples and head CT <24 h after injury	GFAP predicted CT abnormalities with higher AUC (0.89) than S100B, NSE, UCH-L1, NfL and t-tau
Peltz et al. (2020)[54]	Simoa (kits used not specifically mentioned)	65 with TBI history[a] (35 with CI, 30 without CI), 90 controls (30 with CI, 60 without CI)	Cross-sectional	Higher concentration of exosomal GFAP in participants with TBI with cognitive impairment than in those with TBI without cognitive impairment ($P = 0.06$)
Shahim et al. (2020)[53]	Simoa (multiplex kit)	162 with TBI, 68 controls	Prospective cohort study: blood samples obtained at baseline, 30, 90 and 180 days and annually from 1 to 5 years after TBI in 102 out of 162 participants	Higher GFAP levels at baseline in participants with TBI than in controls ($P < 0.001$); GFAP levels decreased during the first 6 months after TBI, then increased; highest AUC (0.89) for distinguishing participants with moderate and severe TBI from controls was at 30 days

AUC, area under the receiver operating characteristics curve; CI, cognitive impairment; CLIA, chemiluminescent immunoassay; DRS, Disability Rating Scale; ELISA, enzyme-linked immunosorbent assay; GCS, Glasgow Coma Scale; GOSE, Glasgow Outcome Scale Extended; GFAP, glial fibrillary acidic protein; mmTBI, mild or moderate traumatic brain injury; NfL, neurofilament light chain; NSE, neuron-specific enolase; RTW, return to work; S100B, calcium-binding protein S100β; SBP, systolic blood pressure; Simoa, single molecular array; TBI, traumatic brain injury; t-tau, total tau; UCH-L1, ubiquitin carboxy-terminal hydrolase L1. [a]Average time from most recent TBI 37 years.

concussive trauma could reflect a mechanical disruption of the BBB that occurs in parallel with the observed transient and chronic GFAP over-expression following single and multiple concussions, respectively[45,46]. The temporal cascade of plasma GFAP following TBI was investigated in a small subset of 34 participants from the Citicoline Brain Injury Treatment Trial (COBRIT): plasma GFAP was maximal on day 0, remained elevated 30 and 90 days after TBI, and was excellent in discriminating participants with complicated (for example, CT-positive) mTBI from healthy control participants (AUC 0.94)[47].

Age-related differences in the ability of GFAP to detect TBI should be considered when interpreting these findings. The ability of GFAP to identify participants with intracranial trauma on CT from a group of participants with mTBI declined with increasing age in a subset of 169 participants from the three-centre TRACK-TBI pilot cohort (2010–2014; AUC 0.73 in participants aged >60 years; AUC 0.93 in participants aged <40 years)[48]. The study also examined the ability of plasma levels of phosphorylated tau and total tau to identify participants with intracranial trauma on CT; however the AUC for these markers did not differ greatly according to age. These observations support evidence that other glial biomarkers (for example, S100B) have reduced specificity in older individuals with TBI compared with specificity in younger individuals with TBI[49]. This effect of age could result from incipient neurodegeneration, different anatomical locations and types of injury in older individuals, or differences in the sensitivities of the assays or imaging methods used[48].

Prognosis. In one study, the prognostic utility of serum GFAP in mTBI was studied by recording return to work status and Glasgow Outcome Scale Extended (GOSE) scores at 6 months after injury[50]. Participants with incomplete return to work had higher GFAP levels at hospital admission than participants with complete return to work. However, in multivariate analysis, GFAP was not predictive of outcome determined by GOSE or complete return to work. The BIO-ProTECT study found an association between serum levels of GFAP and S100B and poor outcomes, as defined by GOSE scores at 1, 4 and 6 months after injury[51]. The prognostic capacity of a model containing participant variables (age, sex, GCS score) and CT score was consistently improved by the incorporation of biomarker data, which were available for 566 out of 882 participants[51]. In another study of 243 participants with moderate to severe TBI, the addition of blood GFAP, UCH-L1 and microtubule-associated protein 2 measurements to known clinical predictors (age, sex, GCS score) improved the prediction of a favourable outcome at 6 months compared with the known clinical predictors alone[52]. GFAP was the most promising of the blood markers: the AUC for GFAP and clinical predictors was 0.78 and the AUC for clinical predictors alone was 0.69. A comprehensive longitudinal assessment identified a biphasic profile of serum GFAP in participants with moderate and severe TBI. At enrolment, GFAP levels were elevated in participants with TBI compared with levels in control participants. This initial increase was followed by a reduction over the following 6 months after injury, but an increase over

the subsequent years[53]. A study in 155 veterans with a mean age of 79 years also demonstrated the reliability of GFAP combined with other biomarkers (phosphorylated tau, NfL, IL-6 and TNFα) for the differentiation of participants with a past medical history of TBI (up to decades before blood sampling) and cognitive impairment from those with a history of previous TBI but no cognitive impairment (AUC 0.85)[54]. The same panel of biomarkers was able to differentiate participants with cognitive impairment and TBI from those with cognitive impairment and no TBI (AUC 0.88).

In summary, GFAP might represent a reliable proxy for small and diffuse structural damage that is not easily assessed with CT, and even MRI, and therefore, it could be employed as a surrogate marker of intracranial pathology. We postulate that adding measurement of blood GFAP (among other biomarkers) to the diagnostic process might provide a more accurate definition of 'mild', 'moderate', and 'severe' TBI than clinical classification alone, which is frequently hampered by the caveat of impaired consciousness. A point-of-care analysis platform for serum GFAP[55] in ambulances might guide the triage of patients with TBI.

Traumatic spinal cord injury

A few studies have tested serum GFAP as a marker of the presence and severity of traumatic spinal cord injury (SCI) in patients who have had a traumatic injury[56,57]. GFAP levels seem to correlate with the severity of SCI, suggesting that the use of GFAP as a biomarker of neurological outcome (segmental motor recovery) is clinically feasible. Specifically, a biochemical model that included both CSF and serum S100B, GFAP and IL-8, measured 24 h after injury, was able to correctly predict the American Spinal Injury Association (ASIA) grade — an accurate predictor of neurological outcome — in a consistent proportion (89%) of 27 patients with SCI[57]. Remarkably, the combined evaluation of these three biomarkers outperformed the ASIA grade in predicting segmental motor recovery at 6 months. Following these results, another cohort study found significantly higher serum levels of GFAP in individuals with severe (ASIA grade A) SCI than in individuals with moderate SCI (grade B; $P < 0.05$), mild SCI (grade C; $P < 0.01$) or controls (individuals with vertebral fractures but without neurological symptoms; $P < 0.01$)[56]. In addition, individuals with SCI who died postoperatively had significantly higher serum GFAP levels in the first 24 h after injury than individuals with SCI who survived ($P < 0.05$)[56]. Last, following complex surgery of the thoracic aorta, serum GFAP levels were considerably higher in participants with SCI than in participants without SCI; however, these comparisons failed to reach statistical significance after adjusting for multiple testing, probably owing to the limited number ($n = 3$) of participants with SCI included in the study[58]. Although evidence is so far very limited, the measurement of serum GFAP levels could provide an avenue to determine the 'biological' severity of injury and predict neurological outcome in patients with SCI, thereby supporting clinical decision-making regarding the identification of patients who are likely to benefit from surgery.

Cerebrovascular accidents

Diagnosis. Biomarkers reflecting the underlying pathophysiological changes associated with cerebrovascular brain injury could improve the management and prognostic assessment of patients with acute stroke[59]. The results of previous studies suggest that serum GFAP could be employed as a biomarker of glial injury indicative of intracerebral haemorrhage in patients presenting with acute stroke symptoms[60–65]. As a result of sudden BBB disruption and subsequent brain injury, GFAP becomes rapidly detectable in blood during the hyperacute phase of intracerebral haemorrhage. Accordingly, studies have found serum levels of GFAP to be substantially higher in patients with intracerebral haemorrhage than in patients with ischaemic stroke[60,65]. In a multicentre cohort study, the analysis of plasma GFAP levels in 205 participants using electrochemiluminometric immunoassays within 4.5 h of symptom onset differentiated participants with intracerebral haemorrhage from participants with ischaemic stroke and stroke mimics[66]. Specifically, the use of a GFAP cut-off value of 0.29 μg/l enabled intracerebral haemorrhage from acute ischaemic stroke to be differentiated from stroke mimics with a sensitivity of 84.2% and a specificity of 96.3% (AUC 0.92). Interestingly, in the BE FAST II study, serum levels of GFAP obtained upon hospital admission were about 16 times higher in participants with intracerebral haemorrhage than in participants with acute ischaemic stroke. In the same study, participants with a large lobar intracerebral haemorrhage had a higher median serum GFAP concentration than participants with a small, deep intracerebral haemorrhage[61].

Prognosis. A number of studies investigated the role of GFAP as a predictor of functional outcomes after acute ischaemic stroke[67,68]. In one such study, serum GFAP levels were measured using ELISA in 286 participants with ischaemic stroke on the first day of admission and participants were followed up for a year[68]. After adjusting for all the established predictors (for example, stroke severity and infarct volume), multivariate analysis showed that elevated GFAP levels on the first day of admission independently predicted poor functional outcomes during the 1-year follow-up. Furthermore, a robust body of evidence suggests that GFAP is a sensitive indicator of injury and a predictor of outcome in patients with subarachnoid haemorrhage. In one study, GFAP levels in 67 participants with subarachnoid haemorrhage were measured at hospital admission[69]. The mean GFAP serum concentration in these participants was 1.8-fold higher than the upper limit of the normal laboratory reference range. In addition, participants in a coma at the time of hospital admission had higher serum GFAP levels than conscious participants. In another study, serum GFAP levels remained high from day 1 to day 6 after subarachnoid haemorrhage[70]. Similar to ischaemic stroke, blood GFAP concentration at admission could significantly predict poor outcomes after subarachnoid haemorrhage, as observed by Zheng et al. at 6 months after the event[71]. In another study, a secondary rise in CSF GFAP levels on about day 7 after subarachnoid haemorrhage was related to complications, including the development of

hydrocephalus and cerebral vasospasm[72]. Nevertheless, longitudinal data regarding blood GFAP dynamics following subarachnoid haemorrhage are still lacking.

Overall, these findings indicate a valuable prognostic role for blood GFAP in patients with stroke, although an important limitation of the diagnostic use of blood GFAP could be a low specificity for differentiating among stroke subtypes. In particular, in the setting of acute stroke symptoms, distinguishing between ischaemic stroke and intracerebral haemorrhage and between stroke and stroke mimics is essential, especially for the correct identification of patients who could be eligible for time-dependent reperfusion therapies (intravenous thrombolysis, mechanical thrombectomy for large-vessel occlusion). In this diagnostic context, the available data do not strongly support an imminent application of serum GFAP.

Inflammatory CNS diseases

Multiple sclerosis

Multiple sclerosis (MS) is the disease that led to the discovery of GFAP by Eng et al. in 1971 (REF.[73]). MS is a complex inflammatory and neurodegenerative disorder that affects more than two million people worldwide[74]. Therefore, efforts to identify a reliable and readily available biomarker that reflects disease severity and progression in MS are paramount in improving the clinical work-up and guiding the therapeutic approach. A reliable blood biomarker for use in MS will need to show an association with clinical severity, disease activity, worsening disability and treatment effectiveness.

Studies using ELISA or ECL assays to detect GFAP failed to identify significant differences between blood GFAP levels in participants with MS and participants with non-inflammatory neurological diseases[75,76]; these studies included a relatively small number of participants. However, subsequent studies using the more sensitive Simoa assay found evidence of higher serum GFAP levels in participants with MS than in healthy control participants and participants with non-inflammatory neurological diseases[25,77,78]. In particular, higher serum GFAP levels than in controls were consistently reported in participants with progressive MS (PMS), whereas the results for the relapsing–remitting MS (RRMS) phenotype differed between studies[25,78]. In one study, samples collected after a recent clinical relapse (RRMS+) had a higher concentration of GFAP than samples from healthy control participants, but no significant difference in GFAP levels was observed between participants with stable MS (RRMS−) and healthy control participants[77]. The same study reported higher levels of GFAP in participants with RRMS+ than in participants with RRMS− (129.8 pg/ml and 112.9 pg/ml, respectively; $P < 0.012$), but with a substantial overlap between the two groups.

In agreement with the proposed pathological role of astrocytes in MS[79–82], multiple studies have found a correlation between blood GFAP concentration and severity of disability, as assessed by the expanded disability status scale (EDSS)[8,25,77,78,83–86] (TABLE 2). Only a single study found a positive correlation between blood GFAP concentration and disease duration[78]. Notably, higher GFAP levels were associated with a greater lesion load on MRI in most of the reported studies[25,78,86]; blood GFAP levels also correlated with other markers of neurodegeneration (for example, NfL) and brain atrophy[8,25,77,78,84]. One study found an association between disease-modifying treatment (DMT) and reduced levels of GFAP[78], whereas all other studies found no change in GFAP levels associated with such treatment[8,25,78,86]. Another study assessed blood GFAP levels in patients receiving autologous haematopoietic stem cell transplantation, and identified a paradoxical increase compared with baseline after the initiation of treatment[85]. A possible explanation for this finding could be the transient worsening of CNS inflammation following the administration of the chemotherapeutic agent busulfan, which constitutes part of the haematopoietic stem cell transplantation procedure, and might cause intrinsic neurotoxicity[85]. A similar increase in GFAP levels was observed in the context of neurotoxicity following immune effector cell-associated neurotoxicity syndrome after chimeric antigen receptor T cell therapy[87]. The potential value of GFAP as a predictor of future relapses and disability progression over time has scarcely been explored in individuals with MS and in populations with heterogeneous characteristics. Indeed, although a study including fewer than 50 participants with MS[77] failed to identify a prognostic value of blood GFAP levels, preliminary results from a larger trial (EXPAND) identified a higher risk (HR 1.96) of reaching an EDSS of 7.0 in participants with secondary PMS who had higher GFAP levels (>80th percentile) at baseline[88].

Neuromyelitis optica spectrum disorder

Neuromyelitis optica spectrum disorder (NMOSD) is a classic autoimmune inflammatory astrocytopathy[89]. Aquaporin-4 antibodies, among other mechanisms, induce astrocytic damage in NMOSD lesions and subsequently cause neuroaxonal damage[90]. Data regarding GFAP concentrations in NMOSD are limited but promising[3]. Even using the standard ELISA, which is less sensitive than the ECL or Simoa assays, higher CSF and serum GFAP levels were reported in participants with NMOSD than in healthy control participants or participants with MS[76]. These findings are supported by more recent results obtained using more sensitive assays[3,77,84] and suggest that blood GFAP could be used to distinguish between NMOSD and MS.

Furthermore, in one study, GFAP levels were higher in 33 participants with NMOSD than in 16 participants with myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD), two diseases with overlapping clinical and radiological findings[83]. Similar to the findings in MS, evidence indicates that serum GFAP levels in patients with NMOSD are higher shortly before (within 1 week) and during acute clinical relapses than during stable disease[77,91]. Data also indicate that serum GFAP levels correlate with EDSS score, most notably in younger patients[77,84]. The GFAP to NfL ratio increased during NMOSD relapses and decreased during MS relapses (AUC = 0.78)[77], suggesting that this combination of markers could be used to distinguish between the two diseases. In contrast to MS and NMOSD, a correlation between GFAP levels and clinical severity was not observed in participants with MOGAD[83].

Table 2 | **Key studies of blood glial fibrillary acidic protein levels in multiple sclerosis**

Study	Assay	Disease course (number of participants)	GFAP level compared with levels in healthy control participants	GFAP level in PMS compared with RRMS	Treatment effect (% participants treated)	Correlation with other markers (correlation coefficient or β value)		
						Clinical disease severity (EDSS)	MRI T2 lesion load	Blood NfL levels
Abdelhak et al. (2018)[25]	Simoa (singleplex)	RRMS+ (18)	ns[a]	Higher in PMS than in RRMS ($P<0.05$)	ns (8.8%)	ns	Higher GFAP levels in participants with more than nine lesions than in participants with two to nine lesions ($P<0.05$)	$r=0.4$ ($P<0.01$)
		RRMS– (24)				ns		
		SPMS (13)	Higher[a] ($P<0.05$)			$r=0.5$ ($P<0.001$)		
		PPMS (25)						
Högel et al. (2018)[78]	Simoa (singleplex)	RRMS (46)	ns	Higher in SPMS than in RRMS ($P<0.001$)	Lower in participants receiving treatment (64.6%) than in untreated participants ($P<0.01$)	$r=0.5$ ($P<0.001$)	$r=0.3$ ($P<0.05$)	$r=0.5$ ($P<0.01$)
		SPMS (33)	Higher ($P<0.01$)					
Abdelhak et al. (2019)[8]	Simoa (singleplex)	PPMS (71)	NA	NA	ns (40.9%)	$\beta=0.3$ ($P<0.01$)	NA	NA
Watanabe et al. (2019)[77]	Simoa (singleplex)	RRMS (38)	Higher in RRMS+ ($P<0.01$)	ns	ns (55.1%)	$\beta=1.1$ ($P<0.05$)	ns	NA
		PMS (11)	Higher ($P<0.01$)					
Lee et al. (2020)[84]	Simoa (singleplex)	MS (112)	NA	NA	NA	$r=0.3$ ($P=0.001$)	NA	$r=0.6$ ($P<0.001$)
Thebault et al. (2020)[85]	Simoa (multiplex)	RRMS (12)	Higher ($P<0.001$)	NA	Higher after treatment with IAHSCT compared with baseline ($P<0.01$)	NA	NA	NA
		SPMS (10)						
Ayrignac et al. (2020)[86]	Simoa (singleplex)	PPMS (18)	NA	Higher in PPMS than in RRMS ($P<0.01$)	ns (48.7% of RRMS group, 0% of PPMS group)	ns	$r=0.4$ ($P<0.01$)	$r=0.7$ ($P<0.001$)
		RRMS (111)						

EDSS, expanded disability status scale; GFAP, glial fibrillary acidic protein; IAHSCT, immunoablation and autologous haematopoietic stem cell transplantation; MS, multiple sclerosis; NA, not assessed or not reported; NfL, neurofilament light chain; ns, not significant; PMS, progressive MS; PPMS, primary progressive MS; *r*, correlation coefficient (Pearson/Spearman); RRMS, relapsing–remitting MS; RRMS+, RRMS during clinical relapse; RRMS–, RRMS without evidence of clinical relapse; Simoa, single molecular array; SPMS, secondary progressive MS; *β*, regression estimates. [a]Compared with participants with non-inflammatory neurological diseases.

Finally, immunomodulatory therapies (corticosteroids, azathioprine, tacrolimus, methotrexate, cyclophosphamide, cyclosporine) did not seem to influence serum GFAP in patients with NMOSD[77], most probably owing to the timing and relative ineffectiveness of the DMT used in this study. Additionally, evidence indicates that the main extent of astrocytic loss occurs during acute inflammatory exacerbation[3,24,90]. Nevertheless, the N-MOmentum study[91] demonstrated that GFAP levels between NMOSD attacks were associated with risk of relapse and, therefore, could still be informative. Additionally, serum GFAP levels decreased by 12.9% from baseline in inebilizumab-treated participants with NMOSD, who did not show relapse over the follow-up period of the study[91]. The potential of serum GFAP as a possible treatment marker in NMOSD, including AQP4-seronegative disease, remains to be addressed in further studies.

In summary, GFAP might not be the most suitable marker for the differentiation of disease phenotypes in MS, or the monitoring of disease activity or treatment effectiveness, as blood levels of the marker in different subgroups seem to overlap substantially. However, several studies have found an association between high GFAP concentrations and PMS[25,78,86]. The consistent correlation between GFAP concentrations and clinical severity metrics suggest promising applications of the marker for exploring and monitoring relapse-independent progression in RRMS and PMS. However, in astrocytopathies, GFAP levels could be useful for the identification of patients with the highest relapse risk. Nevertheless, sufficiently powered prospective multicentre trials that aim to identify clear cut-off values are warranted to clarify some of these open questions.

Neurodegenerative diseases

Several studies have found increased levels of CSF GFAP in the most common neurodegenerative diseases, including Alzheimer disease (AD), prion diseases, frontotemporal lobar degeneration (FTLD), Parkinson disease (PD), PD dementia (PDD), and dementia with Lewy bodies (DLB)[9,12,13,30]. In contrast, only a few studies have explored levels of blood GFAP in these proteinopathies[92–95], supporting the notion that more extensive investigations are needed to address this topic in detail.

Alzheimer disease

In a study by Oeckl et al, blood GFAP levels were higher in participants with AD and in participants with DLB or PDD than in control participants, participants with

behavioural variant frontotemporal dementia (bvFTD) or participants with PD[30], whereas blood biomarker levels did not differ between control participants, participants with PD and participants with bvFTD[30]. Interestingly, CSF levels of GFAP were similar across all participants with neurodegenerative disease; the presence of higher blood GFAP levels (that is, higher CSF to serum ratio) in participants with AD only was attributed to the heterogeneous topographical involvement of neuroinflammation and/or distinct types and patterns of astrogliosis occurring among neurodegenerative diseases[13,30,92,95]. Another study also found increased blood GFAP levels in individuals with AD compared with levels in cognitively healthy individuals[96]. Most interestingly, one study reported a correlation between plasma GFAP levels and cortical Aβ deposition in individuals with symptomatic AD[97]. Linear, positive associations were observed early in disease and diverged during more severe disease stages. These findings suggest that astrocytic damage or activation begins in the presymptomatic phase of AD and is associated with brain Aβ load[98].

The FTLD spectrum

Compared with data in AD, the data on GFAP in FTLD spectrum diseases are inconsistent[30,92,93,95]. In the large, multicentre Genetic FTD Initiative (GENFI) study, including 469 participants with genetic FTD, plasma GFAP levels were elevated in symptomatic carriers of *GRN* mutations, but not in carriers of other FTD mutations, compared with levels in controls[93]. Moreover, biomarker changes were associated with the appearance of clinical symptoms and were not detectable in presymptomatic mutation carriers[93]. In support of these findings, two other studies found no changes in serum GFAP levels in participants with sporadic[30] and genetic bvFTD[95] compared with levels in control participants without neurodegenerative diseases. However, in a large Italian cohort, serum GFAP levels were elevated in participants with all FTLD clinical syndromes (sporadic and genetic) compared with those in healthy control participants[92]. The one exception was the group of participants with progressive supranuclear palsy, who had similar serum GFAP levels to healthy control participants. In this study, the two FTLD groups with the most elevated GFAP were those with bvFTD and those with agrammatic variant PPA; these groups included an unusually high percentage of participants with *GRN* mutations (13% and 25%, respectively). However, no significant difference in serum GFAP levels was observed between these two groups of participants and participants with sporadic FTLD. Several studies are ongoing in this field, the results of which might help clarify the discrepancies between the studies discussed here.

Alexander disease

Blood GFAP levels are of particular interest in specific genetic neurodegenerative diseases, such as Alexander disease. Alexander disease is caused by various dominant heterozygous mutations in the gene encoding GFAP[99]. The pathological hallmark of the disease is the formation of cytoplasmic aggregations in astrocytes[100]. These aggregates contain mainly GFAP, along with other cytoplasmic proteins. In a mouse model, the degree of GFAP expression in the brain showed a clear, negative correlation with survival[100]. Owing to the rarity of the disease, studies investigating GFAP levels in the blood of individuals with Alexander disease are limited. One study found a modest, elevation of GFAP levels in the serum of participants with infantile and juvenile Alexander disease, but not in adult participants with the disease, compared with levels in healthy controls[101]. This finding contrasts with the high concentrations of GFAP found in the CSF of participants with Alexander disease[101–103]. A possible explanation for this divergence is the hook effect mentioned above, whereby GFAP aggregate formation might limit its detection in the blood[3]. Blood GFAP might still serve as a promising treatment outcome parameter for future trials in Alexander disease (for example, in trials of antisense oligonucleotide therapies), but further studies are necessary.

Other neurodegenerative diseases

Data on blood GFAP in other neurodegenerative diseases are scarce. In one study, blood GFAP concentrations were not significantly elevated in participants with genetic or sporadic amyotrophic lateral sclerosis compared with levels in healthy control participants[95]. Another study found higher blood GFAP levels in participants with PD than in healthy control participants[104]. Blood GFAP levels were also elevated in participants with neurological manifestations of Wilson disease compared with levels in healthy controls and participants with pure hepatic manifestations of Wilson disease[105]. We found only one study that assessed blood GFAP levels in individuals with vascular cognitive impairment — no significant difference between healthy control participants and participants with vascular cognitive impairment was observed[106]. Notably, the studies discussed in this section used a range of analytical methods, including immunoassays with relatively low sensitivity (that is, standard ELISA).

Diagnosis and prognosis

Regarding the potential for diagnostic use, serum GFAP has shown promising performance in neurodegenerative diseases. In the study by Oeckl et al., mentioned above, serum GFAP allowed a better distinction between participants with AD and control participants than CSF $A\beta_{1-42}$ (AUC 0.91 and 0.87, respectively). In the same study, serum GFAP distinguished between participants with AD and participants with bvFTD with an AUC of 0.85 (REF.[30]). Moreover, blood GFAP was able to discriminate participants with PDD or DLB from control participants (AUC 0.87), participants with PD (AUC 0.88) and participants with bvFTD (AUC 0.79)[30]. In two other studies, plasma GFAP seemed to perform similarly to plasma $A\beta_{1-42}$ to $A\beta_{1-40}$ ratio for the identification of amyloid PET positivity in participants with AD[107,108]. Plasma GFAP level predicted amyloid PET positivity with an accuracy of 88% (when combined with $A\beta_{1-42}$ to $A\beta_{1-40}$ ratio, age and *APOE* genotype), and AD CSF biomarker profile with an accuracy of 79–80%. These findings might be relevant to the early identification of candidates for clinical trials.

Remarkably, blood GFAP levels correlated negatively with Mini-mental State Examination (MMSE) score and performance in the major cognitive domains in participants with AD or FTD[30,92,107]. Accordingly, in participants with presymptomatic *GRN*-related FTD, higher plasma GFAP levels were associated with lower MMSE scores and brain volumes[93]. Higher GFAP concentrations correlated with faster rates of atrophy in the temporal lobes of participants with symptomatic *GRN*-related FTD. Therefore, elevated GFAP levels might be a characteristic of the late presymptomatic phase and relate to disease severity[93]. Even in cognitively healthy older adults at risk of cognitive impairment, blood GFAP levels were higher than in control participants and were associated with a higher risk of dementia[98,107,109], conversion to AD[108,110], a faster rate of cognitive decline[109], and decline in hippocampal volume[110]. However, the prognostic value of GFAP levels in other neurodegenerative diseases has been poorly analysed; we could only find a cohort of participants with sporadic CJD[94] and a cohort with FTD[92]. In both studies, blood GFAP levels failed to predict survival.

In summary, the implementation of blood GFAP as a biomarker in neurodegenerative diseases, especially in combination with other markers, is a promising approach for improving the precision of differential diagnosis. The association of higher blood GFAP concentrations with faster cognitive decline, higher incidence of dementia and a greater likelihood of conversion to symptomatic cognitive impairment in the presence of amyloid pathology and in carriers of *GRN* mutations indicates potential prognostic applications. Nevertheless, blood GFAP levels might be affected by the heterogeneity of a disorder, the stage of disease and abnormal GFAP aggregation formation. This raises concerns about the practical usefulness of the marker and must be considered during data interpretation. More research is needed to clarify the effects of these possible confounders. Furthermore, in older individuals with neurodegenerative diseases, the coexistence of large and small cerebral vessel comorbidities might further complicate inferences based on measurements of brain-derived proteins in the blood.

Table 3 | **Other CNS and systemic diseases associated with changes in blood concentration of GFAP**

Disease	Changes in GFAP levels	Refs
Epilepsy	Transient elevation of GFAP levels (up to 100%) following epileptic seizures compared with those in controls and participants with psychogenic non-epileptic seizures	141–143
Delirium	In ICU patients with delirium following COVID-19 infection, GFAP increased and was correlated with delirium severity; GFAP might increase in association with postoperative delirium, but the evidence is conflicting	58,144–148
Sepsis-related encephalopathy	Elevated serum GFAP levels compared with levels in participants with sepsis without encephalopathy	149
Cardiac arrest	GFAP levels and proteolytic fragments were elevated after cardiac arrest	150–153
SARS-CoV-2 infection	Serum levels of GFAP, but not of other markers such as NfL, were increased in participants with moderate and severe COVID-19 infection, who were admitted to the ICU	144,154
West Nile virus infection	CSF and serum levels of GFAP were significantly higher in individuals with West Nile virus infection than in controls; these findings correlated with the severity of post-mortem histopathology	155
Atrial fibrillation	Elevated circulating levels of GFAP in individuals with atrial fibrillation compared with levels in control participants	156

GFAP, glial fibrillary acidic protein; ICU, intensive care unit; NfL, neurofilament light chain; COVID-19, coronavirus disease 2019.

Brain tumours

Similar to other structural neurological diseases, a large body of evidence indicates that blood GFAP levels are elevated in individuals with brain tumours. Some studies have found blood GFAP levels to be higher in participants with glioblastoma multiforme (GBM) than in healthy control participants, participants with other non-glial primary tumours and participants with brain metastasis[111–115], whereas, in other studies, a statistically significant difference in blood GFAP levels between participants with high-grade (that is, GBM) and low-grade brain tumours was not detected[116,117]. In participants with GBM, blood GFAP concentration correlated with preoperative tumour volume[111,112,114,118,119], volume of necrosis[111,119] and GFAP expression levels in tumour tissue[111,119]. In one study, individuals with systemic metastasis of myxopapillary ependymoma, a brain tumour with high GFAP expression, had very high blood GFAP concentrations compared with those in healthy controls[120].

Data regarding the prognostic value of blood GFAP levels in individuals with brain tumours seem to be inconsistent. Evidence indicates that blood GFAP levels rise shortly after operative treatment compared with preoperative levels[121], before ultimately decreasing[122]. One study found that blood GFAP levels did not correlate with the amount of malignant tissue that remained postoperatively[123]. In several studies, blood GFAP levels did not help predict postoperative tumour recurrence or overall survival[112,116,122]. However, two studies found an association between high blood GFAP levels and poor progression-free survival[113,114]. The major limitations of the studies discussed here are that they used immunoassays with lower sensitivity and lower readout resolution than highly sensitive bead-based assays such as Simoa, and that they included a relatively small number of participants. Overall, additional, sufficiently powered studies with newer immunoassays are a major unmet need for the evaluation of the diagnostic and prognostic application of GFAP in brain tumours.

In addition to the conditions discussed in this Review, changes in blood GFAP levels have been observed in various other neurological and systemic conditions; a summary of the available evidence is provided in TABLE 3.

Challenges facing clinical use of GFAP

In addition to the disease-specific limitations mentioned in each section of this Review, the accurate implementation of blood GFAP measurement and the correct interpretation of the results faces other challenges (BOX 1). Evidence indicates that the expression of GFAP by astrocytes increases with age in healthy individuals[124],

so the correlation between GFAP levels and age needs to be explored in more extensive studies to enable the definition of age-specific normal ranges. Sex-specific normal ranges might also be required. Additionally, the mechanisms that underlie the release of intermediate filaments such as GFAP following astrocytic activation remain unclear.

With the exception of the work on TBI, most of the studies discussed in this Review were single-centre, retrospective, or had methodological limitations such as small sample sizes. Furthermore, as different platforms and methods are available to detect GFAP in blood, it is essential to note that many of the studies are not directly comparable with each other and that a general agreement on a 'gold standard' detection method is currently lacking. Also, the epitopes targeted by GFAP antibodies are mostly unknown or proprietary, raising some concerns about the GFAP isoforms detected by different assays. Therefore, the identification of a reference method for detecting blood GFAP (for example, by mass spectrometry) is highly recommended. Furthermore, whether the antibody pairs used in existing assays detect the full-length GFAP protein or proteolytic fragments is unclear. GFAP also undergoes various post-translational modifications (for example, phosphorylation, citrullination and acetylation) and is vulnerable to the proteolytic activity of calpain and caspase 6 (REF.[17]). Site-specific phosphorylation can be disease-relevant and has been reported to be associated with disease severity — for example, in Alexander disease[125] and following hypoxic injury[126].

Box 1 | **Unmet needs on the way towards clinical utilization of GFAP**

Numerous limitations hamper the clinical applicability of blood GFAP in the field of neurology. Many aspects of the mechanisms and pathways of GFAP release into the blood are still not completely understood. In addition, blood GFAP half-life in health is still to be described. For a better definition of the clinical context of its use in various neurological conditions, coordinated multicentre trials using a predefined gold standard method to assess GFAP levels longitudinally are still needed. Here, we list the gaps in our knowledge that will need to be addressed before blood GFAP can be used as a clinical biomarker.

Preclinical and analytical aspects

- Effect of GFAP aggregation on analytical accuracy
- Identification of isoform(s) specificity for current assays
- Defining the mechanisms of GFAP release from various astrocyte subclasses
- Magnitude of GFAP release from astrocyte subclasses
- Contribution of GFAP breakdown products and vesicular GFAP to total blood concentration

In vivo physiological considerations

- Accurate determination of blood GFAP half-life
- Characterization of GFAP protein binding, metabolism and excretion
- Defining the effect of age and gender on blood GFAP concentrations

Clinical context of use

- Defining age-specific and gender-specific reference ranges in healthy individuals
- Identification of clinically useful cut-off values
- Agreement on a gold standard measurement technique
- Coordination of multicentre studies to address the clinical applicability of GFAP in difference diseases
- Longitudinal studies assessing GFAP dynamics in subacute and chronic conditions

The effects of post-translational modifications on the analytical performance and clinical utilization of different GFAP assays, which use different proprietary antibody pairs, has been poorly characterized.

Similarly, compared with levels in healthy participants, blood levels of GFAP breakdown products seem to be elevated following TBI and follow a diagnostic and prognostic pattern similar to that of the blood levels of the full GFAP protein[127–129]. If we consider GFAP breakdown products as a product of activated calpain proteolysis following TBI, these products might be a better marker of astrocyte damage, but not necessarily astrocyte activation, when compared with standard GFAP assays. However, the added value of assays that measure levels of GFAP breakdown products, compared with conventional GFAP assays, should be investigated further. One hint that some antibodies in GFAP assays do not recognize (proteolytic) protein fragments is the observation that subjecting blood or CSF samples to several freeze–thaw cycles results in a significant decrease in the detected GFAP concentration, especially in the CSF[8]. In addition, the effect of inhibitory matrix effects has not yet been completely clarified. For example, circulating GFAP autoantibodies have been reported in the blood of individuals with AD and following traumatic CNS injury[125,130–132]. The effect of these autoantibodies on the measurement of circulating GFAP levels with the different commercial platforms is poorly characterized.

Interpreting the meaning of elevated GFAP concentrations in CNS chronic diseases could be challenging. Indeed, GFAP expression accompanies astrocytic activation, which is a 'double-edged sword' in neurological diseases[133]. Although some subclasses of astrocytes (for example, neurotoxic astrocytes) are toxic to neurons and oligodendrocytes, other subclasses promote CNS repair[133,134]. Data suggest that harmful pan-activated astrocytes at the rim of MS lesions are GFAP-positive, whereas direct neurotoxic astrocyte subpopulations are not[79,82,135]. So far, the expression of GFAP over the spectrum of astrocyte subclasses remains poorly characterized, and more specific markers are needed to investigate the different subclasses of activated astrocytes and the different isoforms of GFAP[82] (BOX 2).

Furthermore, the dynamics of blood GFAP levels depend on the underlying pathology. In acute events without major astrogliosis and gliotic scar formation, such as mTBI[136], the half-life of GFAP in blood is around 24–72 h[35,137]. In this context, GFAP could be merely a marker of structural damage to the CNS. In less acute events, such as inflammatory relapses, GFAP remains elevated for weeks after clinical onset[138]. In such cases, blood GFAP levels are likely to reflect ongoing astrocytic activation in addition to the possible astrocytic damage, as has been shown in NMOSD[91]. In chronic neuroinflammatory (for example, PMS) and neurodegenerative diseases, the levels of GFAP in the blood are expected to increase with accumulating astrogliosis. However, whether GFAP levels continue to climb, become stable or even decrease over time remains unclear, as the counterbalance between GFAP release and clearance is still not well defined.

Box 2 | **The spectrum of astrocytic body fluid markers**

A broad panel of astrocytic proteins can be detected in the cerebrospinal fluid (CSF) and blood. In addition to glial fibrillary acidic protein (GFAP), calcium-binding protein B (S100B), glutamine synthetase and chitinase 3 like 1 (CHI3L1) are among the most studied biological markers for the definition of astrocyte involvement in health and diseases. These markers constitute a multifaceted profile of astrocyte activity in vivo and have the potential to reflect astrocyte integrity and cellular activation. Of note, no single biomarker reflects astrocyte damage or aberrant activity in its entirety, either in vitro or in vivo[160,161]. The expression and secretion of astrocyte biomarkers varies according to the age of the individual, cellular location of the marker and astrocyte subtypes[162]. Moreover, other cell types can contribute to the concentrations of these biomarkers circulating in the CSF and blood. For example, GFAP is secreted from renal tubular cells and enteric cells[17], S100B is secreted from skeletal muscles[163] and CHI3L1 can be secreted from microglia and macrophages[164]. In 2021, advanced single-cell sequencing techniques were used to unravel the complexity of astrocyte subpopulation heterogeneity[165]; however, the definition of a biomarker-based signature for these different subpopulations is still far from reach.

Conclusions

Unprecedentedly, the FDA recently authorized a panel test for blood-derived brain protein biomarkers, including GFAP, for clinical use in a neurological diseases. GFAP is a well-established marker of astrocyte injury and activation in CNS diseases and is a valuable addition to the expanding panel of CNS-based blood biomarkers. The potential for clinical application of blood GFAP is encouraging, especially in the field of TBI, where robust data show that the marker has discriminatory ability for CNS injuries evident on CT and MRI head scans. Importantly, historical data on the diagnostic performance of GFAP has been validated in multicentre prospective studies using point-of-care assays, which might facilitate the triage of patients with TBI in pre-hospital and acute hospital settings if integrated into standard care. In inflammatory neurological diseases, blood GFAP has promising applications in PMS, as the marker could reflect and predict long-term disability worsening and, therefore, contribute to the treatment decision algorithm. In the older population, GFAP seems to predict the rate of cognitive decline and conversion to overt dementia, which makes it an attractive marker to recognize individuals at risk and enable rapid initiation of future preventive, and eventually therapeutic measures. Finally, recent insights suggest that blood GFAP has the potential to track even subtle structural CNS involvement in various neurological and systemic diseases. Academic collaborations could significantly accelerate efforts to fill current knowledge gaps and facilitate the implementation of blood GFAP as a biomarker on a wide scale.

Published online 3 February 2022

1. US Food and Drug Administration. FDA authorizes marketing of first blood test to aid in the evaluation of concussion in adults. https://www.fda.gov/news-events/press-announcements/fda-authorizes-marketing-first-blood-test-aid-evaluation-concussion-adults (2018)
2. Messing, A. & Brenner, M. GFAP at 50. *ASN Neuro* **12**, 1759091420949680 (2020).
3. Petzold, A. Glial fibrillary acidic protein is a body fluid biomarker for glial pathology in human disease. *Brain Res.* **1600**, 17–31 (2015).
4. Tumani, H. et al. Cerebrospinal fluid biomarkers of neurodegeneration in chronic neurological diseases. *Expert Rev. Mol. Diagn.* **8**, 479–494 (2008).
5. Khalil, M. et al. Neurofilaments as biomarkers in neurological disorders. *Nat. Rev. Neurol.* **14**, 577–589 (2018).
This review article highlights the potential of highly sensitive immunoassays in the field of neurology by discussing the application of neurofilament light chain measurements in different neurological conditions.
6. Palmqvist, S. et al. Discriminative accuracy of plasma phospho-tau217 for Alzheimer disease vs other neurodegenerative disorders. *JAMA* **324**, 772–781 (2020).
7. Mondello, S. et al. Clinical utility of serum levels of ubiquitin C-terminal hydrolase as a biomarker for severe traumatic brain injury. *Neurosurgery* **70**, 666–675 (2012).
8. Abdelhak, A. et al. Glial activation markers in CSF and serum from patients with primary progressive multiple sclerosis: potential of serum GFAP as disease severity marker? *Front. Neurol.* **10**, 280 (2019).
A multicentre study exploring a broad spectrum of glial markers in primary progressive multiple sclerosis, underpinning the emerging potential of GFAP in this population.
9. Ishiki, A. et al. Glial fibrillar acidic protein in the cerebrospinal fluid of Alzheimer's disease, dementia with Lewy bodies, and frontotemporal lobar degeneration. *J. Neurochem.* **136**, 258–261 (2016).
10. Martinez, M. A. et al. Glial and neuronal markers in cerebrospinal fluid predict progression in multiple sclerosis. *Mult. Scler.* **21**, 550–561 (2015).
11. Madeddu, R. et al. Cytoskeletal proteins in the cerebrospinal fluid as biomarker of multiple sclerosis. *Neurol. Sci.* **34**, 181–186 (2013).
12. Jesse, S. et al. Glial fibrillary acidic protein and protein S-100B: different concentration pattern of glial proteins in cerebrospinal fluid of patients with Alzheimer's disease and Creutzfeldt-Jakob disease. *J. Alzheimers Dis.* **17**, 541–551 (2009).
13. Abu-Rumeileh, S. et al. CSF biomarkers of neuroinflammation in distinct forms and subtypes of neurodegenerative dementia. *Alzheimers Res. Ther.* **12**, 2 (2019).
14. Petzold, A., Keir, G., Green, A. J., Giovannoni, G. & Thompson, E. J. An ELISA for glial fibrillary acidic protein. *J. Immunol. Methods* **287**, 169–177 (2004).
15. Verkhratsky, A. & Butt, A. *Glial Physiology and Pathophysiology* 93–96 (Wiley, 2013).
16. Sofroniew, M. V. & Vinters, H. V. Astrocytes: biology and pathology. *Acta Neuropathol.* **119**, 7–35 (2010).
A review article that provides key insights into the role of astrocytes in health and disease.
17. Yang, Z. & Wang, K. K. Glial fibrillary acidic protein: from intermediate filament assembly and gliosis to neurobiomarker. *Trends Neurosci.* **38**, 364–374 (2015).
18. Middeldorp, J. & Hol, E. M. GFAP in health and disease. *Prog. Neurobiol.* **93**, 421–443 (2011).
19. Clairembault, T. et al. Enteric GFAP expression and phosphorylation in Parkinson's disease. *J. Neurochem.* **130**, 805–815 (2014).
20. Kamphuis, W. et al. GFAP isoforms in adult mouse brain with a focus on neurogenic astrocytes and reactive astrogliosis in mouse models of Alzheimer disease. *PLoS ONE* **7**, e42823 (2012).
21. Hol, E. M. & Capetanaki, Y. Type III intermediate filaments desmin, glial fibrillary acidic protein (GFAP), vimentin, and peripherin. *Cold Spring Harb. Perspect. Biol.* **9**, a021642 (2017).
22. Junemann, A. G. et al. Elevated vitreous body glial fibrillary acidic protein in retinal diseases. *Graefes Arch. Clin. Exp. Ophthalmol.* **253**, 2181–2186 (2015).
23. Lei, J. et al. Glial fibrillary acidic protein as a biomarker in severe traumatic brain injury patients: a prospective cohort study. *Crit. Care* **19**, 362 (2015).
24. Takano, R. et al. Astrocytic damage is far more severe than demyelination in NMO: a clinical CSF biomarker study. *Neurology* **75**, 208–216 (2010).
25. Abdelhak, A., Huss, A., Kassubek, J., Tumani, H. & Otto, M. Serum GFAP as a biomarker for disease severity in multiple sclerosis. *Sci. Rep.* **8**, 14798 (2018).
26. Yue, J. K. et al. Association between plasma GFAP concentrations and MRI abnormalities in patients with CT-negative traumatic brain injury in the TRACK-TBI cohort: a prospective multicentre study. *Lancet Neurol.* **18**, 953–961 (2019).
This study extensively investigates the association between blood GFAP, measured using a prototype assay on a point-of-care platform, and different neuroimaging abnormalities following TBI in a deeply curated prospective multicentre population.
27. Brinker, T., Stopa, E., Morrison, J. & Klinge, P. A new look at cerebrospinal fluid circulation. *Fluids Barriers CNS* **11**, 10 (2014).
28. Tumani, H., Huss, A. & Bachhuber, F. The cerebrospinal fluid and barriers–anatomic and physiologic considerations. *Handb. Clin. Neurol.* **146**, 21–32 (2017).
29. Plog, B. A. et al. Biomarkers of traumatic injury are transported from brain to blood via the glymphatic system. *J. Neurosci.* **35**, 518–526 (2015).
30. Oeckl, P. et al. Glial fibrillary acidic protein in serum is increased in Alzheimer's Disease and correlates with cognitive impairment. *J. Alzheimers Dis.* **67**, 481–488 (2019).
31. Petzold, A. et al. Protein aggregate formation permits millennium-old brain preservation. *J. R. Soc. Interface* **17**, 20190775 (2020).
32. Maas, A. I. R. et al. Traumatic brain injury: integrated approaches to improve prevention, clinical care, and research. *Lancet Neurol.* **16**, 987–1048 (2017).
33. Lingsma, H. F. & Cnossen, M. C. Identification of patients at risk for poor outcome after mTBI. *Lancet Neurol.* **16**, 494–495 (2017).
34. Bouvier, D., Oris, C., Brailova, M., Durif, J. & Sapin, V. Interest of blood biomarkers to predict lesions in medical imaging in the context of mild traumatic brain injury. *Clin. Biochem.* **85**, 5–11 (2020).
35. Papa, L. et al. Time course and diagnostic accuracy of glial and neuronal blood biomarkers GFAP and UCH-L1 in a large cohort of trauma patients with and without mild traumatic brain injury. *JAMA Neurol.* **73**, 551–560 (2016).
A prospective study reporting the dynamics of GFAP in the acute phase following TBI.
36. Bazarian, J. J. et al. Serum GFAP and UCH-L1 for prediction of absence of intracranial injuries on head CT (ALERT-TBI): a multicentre observational study. *Lancet Neurol.* **17**, 782–789 (2018).
37. Cevik, S. et al. NRGN, S100B and GFAP levels are significantly increased in patients with structural lesions resulting from mild traumatic brain injuries. *Clin. Neurol. Neurosurg.* **183**, 105380 (2019).

38. Huebschmann, N. A. et al. Comparing glial fibrillary acidic protein (GFAP) in serum and plasma following mild traumatic brain injury in older adults. *Front. Neurol.* **11**, 1054 (2020).
39. Diaz-Arrastia, R. et al. Acute biomarkers of traumatic brain injury: relationship between plasma levels of ubiquitin C-terminal hydrolase-L1 and glial fibrillary acidic protein. *J. Neurotrauma* **31**, 19–25 (2014).
40. Czeiter, E. et al. Blood biomarkers on admission in acute traumatic brain injury: relations to severity, CT findings and care path in the CENTER-TBI study. *EBioMedicine* **56**, 102785 (2020).
41. Mahan, M. Y. et al. Glial fibrillary acidic protein (GFAP) outperforms S100 calcium-binding protein B (S100B) and ubiquitin C-terminal hydrolase L1 (UCH-L1) as predictor for positive computed tomography of the head in trauma subjects. *World Neurosurg.* **128**, e434–e444 (2019).
42. Gill, J. et al. Glial fibrillary acidic protein elevations relate to neuroimaging abnormalities after mild TBI. *Neurology* **91**, e1385–e1389 (2018).
43. Posti, J. P. et al. Glial fibrillary acidic protein and ubiquitin C-terminal hydrolase-L1 are not specific biomarkers for mild CT-negative traumatic brain injury. *J. Neurotrauma* **34**, 1427–1438 (2017).
44. Papa, L. et al. Evaluating glial and neuronal blood biomarkers GFAP and UCH-L1 as gradients of brain injury in concussive, subconcussive and non-concussive trauma: a prospective cohort study. *BMJ Paediatr. Open* **3**, e000473 (2019).
45. Mountney, A. et al. Functional and molecular correlates after single and repeated rat closed-head concussion: indices of vulnerability after brain injury. *J. Neurotrauma* **34**, 2768–2789 (2017).
46. Johnson, V. E. et al. Mechanical disruption of the blood-brain barrier following experimental concussion. *Acta Neuropathol.* **135**, 711–726 (2018).
47. Bogoslovsky, T. et al. Increases of plasma levels of glial fibrillary acidic protein, tau, and amyloid β up to 90 days after traumatic brain injury. *J. Neurotrauma* **34**, 66–73 (2017).
48. Gardner, R. C. et al. Age-related differences in diagnostic accuracy of plasma glial fibrillary acidic protein and tau for identifying acute intracranial trauma on computed tomography: a TRACK-TBI study. *J. Neurotrauma* **35**, 2341–2350 (2018).
49. Calcagnile, O., Holmen, A., Chew, M. & Unden, J. S100B levels are affected by older age but not by alcohol intoxication following mild traumatic brain injury. *Scand. J. Trauma. Resusc. Emerg. Med.* **21**, 52 (2013).
50. Metting, Z., Wilczak, N., Rodiger, L. A., Schaaf, J. M. & van der Naalt, J. GFAP and S100B in the acute phase of mild traumatic brain injury. *Neurology* **78**, 1428–1433 (2012).
51. Frankel, M. et al. Association of very early serum levels of S100B, glial fibrillary acidic protein, ubiquitin C-terminal hydrolase-L1, and spectrin breakdown product with outcome in ProTECT III. *J. Neurotrauma* **36**, 2863–2871 (2019).
52. Anderson, T. N. et al. Blood-based biomarkers for prediction of intracranial hemorrhage and outcome in patients with moderate or severe traumatic brain injury. *J. Trauma. Acute Care Surg.* **89**, 80–86 (2020).
53. Shahim, P. et al. Time course and diagnostic utility of NfL, tau, GFAP, and UCH-L1 in subacute and chronic TBI. *Neurology* **95**, e623–e636 (2020).
54. Peltz, C. B. et al. Blood biomarkers of traumatic brain injury and cognitive impairment in older veterans. *Neurology* **95**, e1126–e1133 (2020).
55. Okonkwo, D. O. et al. Point-of-care platform blood biomarker testing of glial fibrillary acidic protein versus S100 calcium-binding protein B for prediction of traumatic brain injuries: a Transforming Research and Clinical Knowledge in Traumatic Brain Injury study. *J. Neurotrauma* **37**, 2460–2467 (2020).
56. Ahadi, R. et al. Diagnostic value of serum levels of GFAP, pNF-H, and NSE compared with clinical findings in severity assessment of human traumatic spinal cord injury. *Spine* **40**, e823–e830 (2015).
57. kwon, B. K. et al. Cerebrospinal fluid inflammatory cytokines and biomarkers of injury severity in acute human spinal cord injury. *J. Neurotrauma* **27**, 669–682 (2010).
58. Lindblom, R. P. F. et al. Protein profiling in serum and cerebrospinal fluid following complex surgery on the thoracic aorta identifies biological markers of neurologic injury. *J. Cardiovasc. Transl. Res.* **11**, 503–516 (2018).
59. Powers, W. J. et al. Guidelines for the early management of patients with acute ischemic stroke: 2019 update to the 2018 guidelines for the early management of acute ischemic stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke* **50**, e344–e418 (2019).
60. Foerch, C. et al. Serum glial fibrillary acidic protein as a biomarker for intracerebral haemorrhage in patients with acute stroke. *J. Neurol. Neurosurg. Psychiatry* **77**, 181–184 (2006).
61. Luger, S. et al. Glial fibrillary acidic protein serum levels distinguish between intracerebral hemorrhage and cerebral ischemia in the early phase of stroke. *Clin. Chem.* **63**, 377–385 (2017).
62. Dvorak, F., Haberer, I., Sitzer, M. & Foerch, C. Characterisation of the diagnostic window of serum glial fibrillary acidic protein for the differentiation of intracerebral haemorrhage and ischaemic stroke. *Cerebrovasc. Dis.* **27**, 37–41 (2009).
63. Foerch, C., Pfeilschifter, W., Zeiner, P. & Brunkhorst, R. Glial fibrillary acidic protein in patients with symptoms of acute stroke: diagnostic marker of cerebral hemorrhage [German]. *Nervenarzt* **85**, 982–989 (2014).
64. Brunkhorst, R., Pfeilschifter, W. & Foerch, C. Astroglial proteins as diagnostic markers of acute intracerebral hemorrhage–pathophysiological background and clinical findings. *Transl. Stroke Res.* **1**, 246–251 (2010).
65. Unden, J. et al. Explorative investigation of biomarkers of brain damage and coagulation system activation in clinical stroke differentiation. *J. Neurol.* **256**, 72–77 (2009).
66. Foerch, C. et al. Diagnostic accuracy of plasma glial fibrillary acidic protein for differentiating intracerebral hemorrhage and cerebral ischemia in patients with symptoms of acute stroke. *Clin. Chem.* **58**, 237–245 (2012).
67. Puspitasari, V., Gunawan, P. Y., Wiradarma, H. D. & Hartoyo, V. Glial fibrillary acidic protein serum level as a predictor of clinical outcome in ischemic stroke. *Open. Access. Maced. J. Med. Sci.* **7**, 1471–1474 (2019).
68. Liu, G. & Geng, J. Glial fibrillary acidic protein as a prognostic marker of acute ischemic stroke. *Hum. Exp. Toxicol.* **37**, 1048–1053 (2018).
69. Vos, P. E., van Gils, M., Beems, T., Zimmerman, C. & Verbeek, M. M. Increased GFAP and S100β but not NSE serum levels after subarachnoid haemorrhage are associated with clinical severity. *Eur. J. Neurol.* **13**, 632–638 (2006).
70. Kedziora, J. et al. Biomarkers of neurological outcome after aneurysmal subarachnoid hemorrhage as early predictors at discharge from an intensive care unit. *Neurocrit Care* **34**, 856–866 (2020).
71. Zheng, Y. K. et al. Comparison of plasma copeptin and multiple biomarkers for assessing prognosis of patients with aneurysmal subarachnoid hemorrhage. *Clin. Chim. Acta* **475**, 64–69 (2017).
72. Petzold, A. et al. Early identification of secondary brain damage in subarachnoid hemorrhage: a role for glial fibrillary acidic protein. *J. Neurotrauma* **23**, 1179–1184 (2006).
73. Eng, L. F., Vanderhaeghen, J. J., Bignami, A. & Gerstl, B. An acidic protein isolated from fibrous astrocytes. *Brain Res.* **28**, 351–354 (1971).
74. GBD 2016 Multiple Sclerosis Collaborators.Global, regional, and national burden of multiple sclerosis 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol.* **18**, 269–285 (2019).
75. Mayer, C. A. et al. Blood levels of glial fibrillary acidic protein (GFAP) in patients with neurological diseases. *PLoS ONE* **8**, e62101 (2013).
76. Storoni, M. et al. Serum GFAP levels in optic neuropathies. *J. Neurol. Sci.* **317**, 117–122 (2012).
77. Watanabe, M. et al. Serum GFAP and neurofilament light as biomarkers of disease activity and disability in NMOSD. *Neurology* **93**, e1299–e1311 (2019).
78. Högel, H. et al. Serum glial fibrillary acidic protein correlates with multiple sclerosis disease severity. *Mult. Scler.* **26**, 210–219 (2018).
79. Park, C. et al. The landscape of myeloid and astrocyte phenotypes in acute multiple sclerosis lesions. *Acta Neuropathol. Commun.* **7**, 130 (2019).
80. Pitt, D. & Ponath, G. Astrocytes play a crucial role in the formation and evolution of MS lesions–Yes. *Mult. Scler.* **25**, 15–17 (2019).
81. Abdelhak, A., Weber, M. S. & Tumani, H. Primary progressive multiple sclerosis: putting together the puzzle. *Front. Neurol.* **8**, 234 (2017).
82. Liddelow, S. A. et al. Neurotoxic reactive astrocytes are induced by activated microglia. *Nature* **541**, 481–487 (2017).
A key study that characterized the neurotoxic effect of a particular astrocyte subpopulation: the so-called A1 astrocytes.
83. Kim, H. et al. Serum biomarkers in myelin oligodendrocyte glycoprotein antibody-associated disease. *Neurol. Neuroimmunol. Neuroinflamm* **7**, e708 (2020).
84. Lee, E. J. et al. Clinical implication of serum biomarkers and patient age in inflammatory demyelinating diseases. *Ann. Clin. Transl. Neurol.* **7**, 992–1001 (2020).
85. Thebault, S. et al. Neurotoxicity after hematopoietic stem cell transplant in multiple sclerosis. *Ann. Clin. Transl. Neurol.* **7**, 767–775 (2020).
86. Ayrignac, X. et al. Serum GFAP in multiple sclerosis: correlation with disease type and MRI markers of disease severity. *Sci. Rep.* **10**, 10923 (2020).
87. Gust, J. et al. Glial injury in neurotoxicity after pediatric CD19-directed chimeric antigen receptor T cell therapy. *Ann. Neurol.* **86**, 42–54 (2019).
88. Kuhle, J. et al. High plasma glial fibrillary acidic protein levels predict disability milestone EDSS 7 in non-active secondary progressive multiple sclerosis [abstract FC04.03]. *Mult. Scler. J.* **26**, 10 (2020).
89. Fujihara, K. Neuromyelitis optica spectrum disorders: still evolving and broadening. *Curr. Opin. Neurol.* **32**, 385–394 (2019).
90. Lucchinetti, C. F. et al. The pathology of an autoimmune astrocytopathy: lessons learned from neuromyelitis optica. *Brain Pathol.* **24**, 83–97 (2014).
91. Aktas, O. et al. Serum glial fibrillary acidic protein: a neuromyelitis optica spectrum disorder biomarker. *Ann. Neurol.* **89**, 895–910 (2021).
This study leveraged blood samples from a large multicentre cohort of participants with neuromyelitis optica spectrum disorder and described in detail the dynamics of blood GFAP levels following inflammatory-mediated astrocyte injury.
92. Benussi, A. et al. Serum glial fibrillary acidic protein (GFAP) is a marker of disease severity in frontotemporal lobar degeneration. *J. Alzheimers Dis.* **77**, 1129–1141 (2020).
93. Heller, C. et al. Plasma glial fibrillary acidic protein is raised in progranulin-associated frontotemporal dementia. *J. Neurol. Neurosurg. Psychiatry* **91**, 263–270 (2020).
94. Staffaroni, A. M. et al. Association of blood and cerebrospinal fluid tau level and other biomarkers with survival time in sporadic Creutzfeldt-Jakob disease. *JAMA Neurol.* **76**, 969–977 (2019).
95. Oeckl, P. et al. Different neuroinflammatory profile in amyotrophic lateral sclerosis and frontotemporal dementia is linked to the clinical phase. *J. Neurol. Neurosurg. Psychiatry* **90**, 4–10 (2019).
96. Elahi, F. M. et al. Plasma biomarkers of astrocytic and neuronal dysfunction in early- and late-onset Alzheimer's disease. *Alzheimers Dement.* **16**, 681–695 (2020).
97. Asken, B. M. et al. Plasma glial fibrillary acidic protein levels differ along the spectra of amyloid burden and clinical disease stage. *J. Alzheimers Dis.* **78**, 265–276 (2020).
98. Chatterjee, P. et al. Plasma glial fibrillary acidic protein is elevated in cognitively normal older adults at risk of Alzheimer's disease. *Transl. Psychiatry* **11**, 27 (2021).
99. Messing, A., Brenner, M., Feany, M. B., Nedergaard, M. & Goldman, J. E. Alexander disease. *J. Neurosci.* **32**, 5017–5023 (2012).
100. Messing, A. et al. Fatal encephalopathy with astrocyte inclusions in GFAP transgenic mice. *Am. J. Pathol.* **152**, 391–398 (1998).
101. Jany, P. L. et al. CSF and blood levels of GFAP in Alexander disease. *eNeuro* **2**, e0080-15.2015 (2015).
102. Kyllerman, M., Rosengren, L., Wiklund, L. M. & Holmberg, E. Increased levels of GFAP in the cerebrospinal fluid in three subtypes of genetically confirmed Alexander disease. *Neuropediatrics* **36**, 319–323 (2005).
103. Schmidt, H. et al. Acute onset of adult Alexander disease. *J. Neurol. Sci.* **331**, 152–154 (2013).
104. Su, W., Chen, H. B., Li, S. H. & Wu, D. Y. Correlational study of the serum levels of the glial fibrillary acidic protein and neurofilament proteins in Parkinson's disease patients. *Clin. Neurol. Neurosurg.* **114**, 372–375 (2012).

105. Lin, J. et al. Higher concentration of plasma glial fibrillary acidic protein in Wilson disease patients with neurological manifestations. *Mov. Disord.* **36**, 1446–1450 (2021).
106. Rosen, C. et al. Discriminatory analysis of biochip-derived protein patterns in CSF and plasma in neurodegenerative diseases. *Front. Aging Neurosci.* **3**, 1 (2011).
107. Verberk, I. M. W. et al. Combination of plasma amyloid beta(1-42/1-40) and glial fibrillary acidic protein strongly associates with cerebral amyloid pathology. *Alzheimers Res. Ther.* **12**, 118 (2020).
108. Cicognola, C. et al. Plasma glial fibrillary acidic protein detects Alzheimer pathology and predicts future conversion to Alzheimer dementia in patients with mild cognitive impairment. *Alzheimers Res. Ther.* **13**, 68 (2021).
109. Verberk, I. M. W. et al. Serum markers glial fibrillary acidic protein and neurofilament light for prognosis and monitoring in cognitively normal older people: a prospective memory clinic-based cohort study. *Lancet Healthy Longev.* **2**, e87–e95 (2021).
This study demonstrates that GFAP is a valuable marker in the context of prognostication of cognitive impairment in older people.
110. Rajan, K. B. et al. Remote blood biomarkers of longitudinal cognitive outcomes in a population study. *Ann. Neurol.* **88**, 1065–1076 (2020).
111. Jung, C. S. et al. Serum GFAP is a diagnostic marker for glioblastoma multiforme. *Brain* **130**, 3336–3341 (2007).
112. Gallego Perez-Larraya, J. et al. Diagnostic and prognostic value of preoperative combined GFAP, IGFBP-2, and YKL-40 plasma levels in patients with glioblastoma. *Cancer* **120**, 3972–3980 (2014).
113. Lyubimova, N. V. et al. Glial fibrillary acidic protein in the diagnosis and prognosis of malignant glial tumors. *Bull. Exp. Biol. Med.* **168**, 503–506 (2020).
114. Kiviniemi, A. et al. Serum levels of GFAP and EGFR in primary and recurrent high-grade gliomas: correlation to tumor volume, molecular markers, and progression-free survival. *J. Neurooncol* **124**, 237–245 (2015).
115. Ilhan-Mutlu, A. et al. Exploratory investigation of eight circulating plasma markers in brain tumor patients. *Neurosurg. Rev.* **36**, 45–55 (2013). discussion 55–46.
116. Shih, C. C. et al. Pretreatment serum lactate level as a prognostic biomarker in patients undergoing supratentorial primary brain tumor resection. *Oncotarget* **8**, 63715–63723 (2017).
117. Lange, R. P. et al. Evaluation of eight plasma proteins as candidate blood-based biomarkers for malignant gliomas. *Cancer Invest.* **32**, 423–429 (2014).
118. Brommeland, T., Rosengren, L., Fridlund, S., Hennig, R. & Isaksen, V. Serum levels of glial fibrillary acidic protein correlate to tumour volume of high-grade gliomas. *Acta Neurol. Scand.* **116**, 380–384 (2007).
119. Tichy, J. et al. Prospective evaluation of serum glial fibrillary acidic protein (GFAP) as a diagnostic marker for glioblastoma. *J. Neurooncol.* **126**, 361–369 (2016).
120. Ilhan-Mutlu, A. et al. High plasma-GFAP levels in metastatic myxopapillary ependymoma. *J. Neurooncol.* **113**, 359–363 (2013).
121. Husain, H. et al. Pre- and post-operative plasma glial fibrillary acidic protein levels in patients with newly diagnosed gliomas. *J. Neurooncol.* **109**, 123–127 (2012).
122. Vietheer, J. M. et al. Serum concentrations of glial fibrillary acidic protein (GFAP) do not indicate tumor recurrence in patients with glioblastoma. *J. Neurooncol.* **135**, 193–199 (2017).
123. Baumgarten, P. et al. Pre- and early postoperative GFAP serum levels in glioma and brain metastases. *J. Neurooncol.* **139**, 541–546 (2018).
124. Nichols, N. R., Day, J. R., Laping, N. J., Johnson, S. A. & Finch, C. E. GFAP mRNA increases with age in rat and human brain. *Neurobiol. Aging* **14**, 421–429 (1993).
125. Battaglia, R. A. et al. Site-specific phosphorylation and caspase cleavage of GFAP are new markers of Alexander disease severity. *eLife* **8**, e47789 (2019).
126. Sullivan, S. M. et al. Phosphorylation of GFAP is associated with injury in the neonatal pig hypoxic–ischemic brain. *Neurochem. Res.* **37**, 2364–2378 (2012).
127. McMahon, P. J. et al. Measurement of the glial fibrillary acidic protein and its breakdown products GFAP-BDP biomarker for the detection of traumatic brain injury compared to computed tomography and magnetic resonance imaging. *J. Neurotrauma* **32**, 527–533 (2015).
128. Papa, L. et al. Elevated levels of serum glial fibrillary acidic protein breakdown products in mild and moderate traumatic brain injury are associated with intracranial lesions and neurosurgical intervention. *Ann. Emerg. Med.* **59**, 471–483 (2012).
129. Okonkwo, D. O. et al. GFAP-BDP as an acute diagnostic marker in traumatic brain injury: results from the prospective Transforming Research and Clinical Knowledge in Traumatic Brain Injury study. *J. Neurotrauma* **30**, 1490–1497 (2013).
130. Shan, F., Long, Y. & Qiu, W. Autoimmune glial fibrillary acidic protein astrocytopathy: a review of the literature. *Front. Immunol.* **9**, 2802 (2018).
131. Zhang, Z. et al. Human traumatic brain injury induces autoantibody response against glial fibrillary acidic protein and its breakdown products. *PLoS ONE* **9**, e92698 (2014).
132. Wang, K. K. et al. Plasma anti-glial fibrillary acidic protein autoantibody levels during the acute and chronic phases of traumatic brain injury: a Transforming Research and Clinical Knowledge in Traumatic Brain Injury pilot study. *J. Neurotrauma* **33**, 1270–1277 (2016).
133. Liddelow, S. A. & Barres, B. A. Reactive astrocytes: production, function, and therapeutic potential. *Immunity* **46**, 957–967 (2017).
134. Li, T., Chen, X., Zhang, C., Zhang, Y. & Yao, W. An update on reactive astrocytes in chronic pain. *J. Neuroinflammation* **16**, 140 (2019).
135. Pitt, D. Contribution of astrocyte responses to MS pathogenesis [abstract PS14.02]. Presented at the 8th Joint ACTRIMS-ECTRIMS Meeting. https://cslide.ctimeetingtech.com/msdc2020/attendee/confcal/session/calendar?q=PS14.02 (2020).
136. Burda, J. E., Bernstein, A. M. & Sofroniew, M. V. Astrocyte roles in traumatic brain injury. *Exp. Neurol.* **275**, 305–315 (2016).
137. Thelin, E. P. et al. Serial sampling of serum protein biomarkers for monitoring human traumatic brain injury dynamics: a systematic review. *Front. Neurol.* **8**, 300 (2017).
138. Burman, J. et al. Assessing tissue damage in multiple sclerosis: a biomarker approach. *Acta Neurol. Scand.* **130**, 81–89 (2014).
139. Papa, L. et al. GFAP out-performs S100β in detecting traumatic intracranial lesions on computed tomography in trauma patients with mild traumatic brain injury and those with extracranial lesions. *J. Neurotrauma* **31**, 1815–1822 (2014).
140. Posti, J. P. et al. The levels of glial fibrillary acidic protein and ubiquitin C-terminal hydrolase-L1 during the first week after a traumatic brain injury: correlations with clinical and imaging findings. *Neurosurgery* **79**, 456–464 (2016).
141. Simani, L., Elmi, M. & Asadollahi, M. Serum GFAP level: a novel adjunctive diagnostic test in differentiate epileptic seizures from psychogenic attacks. *Seizure* **61**, 41–44 (2018).
142. Elhady, M. et al. Circulating glial fibrillary acidic protein and ubiquitin carboxy-terminal hydrolase-L1 as markers of neuronal damage in children with epileptic seizures. *Childs Nerv. Syst.* **37**, 879–884 (2021).
143. Nass, R. D. et al. Serum biomarkers of cerebral cellular stress after self-limiting tonic clonic seizures: an exploratory study. *Seizure* **85**, 1–5 (2021).
144. Cooper, J. et al. Quantification of neurological blood-based biomarkers in critically Ill patients with coronavirus disease 2019. *Crit. Care Explor.* **2**, e0238 (2020).
145. Ballweg, T. et al. Association between plasma tau and postoperative delirium incidence and severity: a prospective observational study. *Br. J. Anaesth.* **126**, 458–466 (2021).
146. Xin, X., Chen, J., Hua, W. & Wang, H. Intraoperative dexmedetomidine for prevention of postoperative delirium in elderly patients with mild cognitive impairment. *Int. J. Geriatr. Psychiatry* **36**, 143–151 (2021).
147. Anderson, B. J. et al. Incidence, risk factors, and clinical implications of post-operative delirium in lung transplant recipients. *J. Heart Lung Transpl.* **37**, 755–762 (2018).
148. Gailiusas, M. et al. Association between serum biomarkers and postoperative delirium after cardiac surgery. *Acta Med. Litu.* **26**, 8–10 (2019).
149. Wu, L. et al. Serum glial fibrillary acidic protein and ubiquitin C-terminal hydrolase-L1 for diagnosis of sepsis-associated encephalopathy and outcome prognostication. *J. Crit. Care* **52**, 172–179 (2019).
150. Jonesco, D. S. et al. A caspase-6-cleaved fragment of glial fibrillary acidic protein as a potential serological biomarker of CNS injury after cardiac arrest. *PLoS ONE* **14**, e0224633 (2019).
151. Kaneko, T. et al. Serum glial fibrillary acidic protein as a predictive biomarker of neurological outcome after cardiac arrest. *Resuscitation* **80**, 790–794 (2009).
152. Helwig, K. et al. Elevated serum glial fibrillary acidic protein (GFAP) is associated with poor functional outcome after cardiopulmonary resuscitation. *Neurocrit Care* **27**, 68–74 (2017).
153. Larsson, I. M. et al. Post-cardiac arrest serum levels of glial fibrillary acidic protein for predicting neurological outcome. *Resuscitation* **85**, 1654–1661 (2014).
154. Kanberg, N. et al. Neurochemical evidence of astrocytic and neuronal injury commonly found in COVID-19. *Neurology* **95**, e1754–e1759 (2020).
155. Petzold, A., Groves, M., Leis, A. A., Scaravilli, F. & Stokic, D. S. Neuronal and glial cerebrospinal fluid protein biomarkers are elevated after West Nile virus infection. *Muscle Nerve* **41**, 42–49 (2010).
156. Galenko, O. et al. Circulating levels of biomarkers of cerebral injury in patients with atrial fibrillation. *Am. J. Cardiol.* **124**, 1697–1700 (2019).
157. Jessen, N. A., Munk, A. S., Lundgaard, I. & Nedergaard, M. The glymphatic system: a beginner's guide. *Neurochem. Res.* **40**, 2583–2599 (2015).
158. Bouzier-Sore, A. K. & Pellerin, L. Unraveling the complex metabolic nature of astrocytes. *Front. Cell. Neurosci.* **7**, 179 (2013).
159. Perea, G., Navarrete, M. & Araque, A. Tripartite synapses: astrocytes process and control synaptic information. *Trends Neurosci.* **32**, 421–431 (2009).
160. Boesmans, W., Rocha, N. P., Reis, H. J., Holt, M. & Vanden Berghe, P. The astrocyte marker Aldh1L1 does not reliably label enteric glial cells. *Neurosci. Lett.* **566**, 102–105 (2014).
161. Guttenplan, K. A. & Liddelow, S. A. Astrocytes and microglia: models and tools. *J. Exp. Med.* **216**, 71–83 (2019).
162. Miller, S. J. Astrocyte heterogeneity in the adult central nervous system. *Front. Cell. Neurosci.* **12**, 401 (2018).
163. Zimmer, D. B., Chessher, J., Wilson, G. L. & Zimmer, W. E. S100A1 and S100B expression and target proteins in type I diabetes. *Endocrinology* **138**, 5176–5183 (1997).
164. Zhao, T., Su, Z., Li, Y., Zhang, X. & You, Q. Chitinase-3 like-protein-1 function and its role in diseases. *Signal. Transduct. Target. Ther.* **5**, 201 (2020).
165. Lo, C. H. et al. Astrocyte heterogeneity in multiple sclerosis: current understanding and technical challenges. *Front. Cell. Neurosci.* **15**, 726479 (2021).

Author contributions

A.A., M.F., S.A.-R., J.K.Y., L.D'A., A.H. and P.O. researched data for the article, made a substantial contribution to discussion of content, wrote the article, and reviewed and edited the manuscript before submission. H.T., A.C.L., A.P., J.K., G.T.M, A.J.G., and M.O. made a substantial contribution to discussion of content, wrote the article, and reviewed and edited the manuscript before submission.

Competing interests

A.A. received research grants from the German Multiple Sclerosis Society (DMSG). P.O. received research support from the Michael J. Fox Foundation for Parkinson´s Research (grant ID MJFF-010349) and Alzheimer Forschung Initiative e.V. (20059CB). J.K. received speaker fees, research support, travel support and/or served on advisory boards of the Swiss MS Society, Swiss National Research Foundation (320030_189140/1), University of Basel, Progressive MS Alliance, Bayer, Biogen, Celgene, Merck, Novartis, Roche and Sanofi. G.T.M. reports funding from the US Department of Defense – TBI Endpoints Development Initiative (grant #W81XWH-14-2-0176), TRACK-TBI Precision Medicine (grant #W81XWH-18-2-0042), TRACK-TBI NETWORK (grant #W81XWH-15-9-0001), NIH-NINDS – TRACK-TBI (grant #U01NS086090), National Football League (NFL) Scientific Advisory Board, with support from the NFL for the research efforts of TRACK-TBI NETWORK. In addition, the US Department of Energy supports G.T.M. for a precision medicine collaboration, Abbott Laboratories has provided funding for TRACK-TBI clinical studies, NeuroTrauma Sciences LLC has provided funding to support TRACK-TBI data curation efforts, One Mind has provided funding for TRACK-TBI patients stipends and support to clinical sites. J.K.Y. received funding from NIH-NINDS – TRACK-TBI (grant #U01NS086090). A.J.G. reports personal fees from Bionure, Mylan, Neurona and Viela Bio; other support from Pipeline

Therapeutics; and grants and other support from Inception Sciences outside the submitted work. M.O. gave scientific advice to Axon, Biogen Idec, Fujirebio and Roche. H.T. reports funding for research projects, lectures and travel from Bayer, Biogen, Genzyme, Merck Serono, Novartis, Roche and Teva, and received research support from DMSG and the German Ministry for Education and Research (BMBF). No other disclosures are reported.

Peer review information
Nature Reviews Neurology thanks C.A. Gonçalves, C. Foerch, S. Mondello, K. Wang and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Review criteria
For this Review, we screened the published literature in PubMed using the following terms in the title or abstract: 'GFAP' OR 'glial fibrillary acidic protein', 'blood' OR 'plasma' OR 'serum', and the disease of interest. Hence, we added the following terms: 'multiple sclerosis', 'MS', 'neuromyelitis optica', 'NMO', 'MOG antibody disease', 'MOG associated disease', 'traumatic brain injury', 'TBI', 'spinal trauma', 'spinal injury', 'stroke', 'cerebral ischemia', 'cerebral ischaemia', 'intracranial haemorrhage', 'intracranial hemorrhage, 'subarachnoid haemorrhage', 'subarachnoid hemorrhage', 'Alzheimer's', 'Parkinson', 'dementia', 'Creutzfeldt-Jakob disease', 'vascular cognitive impairment', 'vascular dementia', 'amyotrophic lateral sclerosis', 'motor neuron disease', 'ALS', 'MND', 'frontotemporal', 'prion', 'epilepsy', 'seizures', 'convulsions', 'encephalitis', 'encephalopathy', 'tumours', 'tumors', 'glioma', 'glioblastoma', 'COVID-19', 'SARS-CoV-2', 'cardiac arrest', 'hypoxic' and 'meningitis'. Animal studies, previous reviews, and studies reporting only GFAP values in CSF were considered beyond the scope of this article.