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

Ahmed Abdelhak[1,2], Matteo Foschi[3,4], Samir Abu-Rumeileh[2,5], John K. Yue[6], Lucio D'Anna[7,8], Andre Huss[2], Patrick Oeckl[2,9], Albert C. Ludolph[2,9], Jens Kuhle[10,11,12,13], Axel Petzold[14,15], Geoffrey T. Manley[6], Ari J. Green[1], Markus Otto[2,5] and Hayrettin Tumani[2,16,†]

[1] Department of Neurology, University of California San Francisco (UCSF), San Francisco, CA, USA.

[2] Department of Neurology, University Hospital of Ulm, Ulm, Germany.

[3] Department of Neuroscience, Neurology Unit, S. Maria delle Croci Hospital – AUSL Romagna, ambito di Ravenna, Ravenna, Italy.

[4] Department of Medical and Surgical Sciences, Alma Mater Studiorum, University of Bologna, Bologna, Italy.

[5] Department of Neurology, Martin-Luther-University of Halle-Wittenberg, Halle (Saale), Germany

[6] Department of Neurosurgery, University of California San Francisco (UCSF), San Francisco, CA, USA.

[7] Department of Stroke and Neuroscience, Charing Cross Hospital, Imperial College London NHS Healthcare Trust, London, UK.

[8] Department of Brain Sciences, Imperial College London, London, UK.

[9] German Center for Neurodegenerative Diseases (DZNE e.V.) Ulm, Germany

[10] Multiple Sclerosis Centre, Neurologic Clinic and Policlinic, Departments of Head, Spine and Neuromedicine, University Hospital Basel and University of Basel, Basel, Switzerland

[11] Research Center for Clinical Neuroimmunology and Neuroscience (RC2NB), University Hospital and University of Basel, Basel, Switzerland.

[12] Departments of Biomedicine and Clinical Research, University Hospital Basel, University of Basel, Basel, Switzerland.

[13] Department of Clinical Research, University Hospital Basel, University of Basel, Basel, Switzerland.

[14] The National Hospital for Neurology and Neurosurgery, Queen Square, UCL Institute of Neurology, Moorfields Eye Hospital, London, UK.

[15] Neuro-ophthalmology Expert Center, Amsterdam UMC, Amsterdam, The Netherlands.

[16] Fachklinik für Neurologie Dietenbronn, Schwendi, Germany.

[†]**e-mail:** Hayrettin.tumani@uni-ulm.de

Abstract

Blood-derived biomarkers for brain and spinal cord diseases are urgently needed. The introduction of highly sensitive immunoassays induced a rapid increase in the number of potential blood-derived biomarkers for diagnosis and monitoring of neurological disorders In 2018, the FDA authorised 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.

[H1] Introduction

In 2018, the FDA authorised 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 (TBI), 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[3], 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[6], and UCH-L1[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 endpoints 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.

[H1] 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. 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 zone, 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), electrochemiluninescence (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 minutes[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 characterisation of pre-analytical confounders and an aggregation-related 'hook effect' **[G]** 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].

[H1] Acute CNS injury

[H2] 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 ionising radiation if used indiscriminately, especially in young individuals with mild TBI (mTBI)[33]. These limitations have led to the investigation of a range of astroglial and neuronal biomarkers, including S100-beta 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].

[H3] Diagnosis

The results of key studies of blood GFAP levels in TBI are summarised in Table 1. In a study of 584 participants with mild-to-moderate TBI, elevated levels of serum GFAP were detected within 1 hour of injury, compared with participants with non-TBI general trauma. GFAP levels in the group of participants with TBI peaked at 20 hours post-injury, and finally declined slowly until 72 hours post-injury[35]. Bazarian et al. performed a large multicentre observational study of more than 1,900 participants with mild-to-moderate TBI (the ALERT-TBI study), and found that a pre-specified 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[36]. This finding contributed to the FDA authorization to market the first blood-based biomarker for the prevention of unnecessary CT radiation in individuals with suspected TBI in 2018[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 head CT abnormalities[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 1-week functional outcomes.

Some studies have found that, in head-to-head comparisons with other serum biomarkers (for example, UCH-L1, S100B and NfL), GFAP is the best marker for discriminating individuals with TBI and head CT abnormalities from individuals with TBI and normal head CT scans[35,39-41]. For example, the large (more than 2,800 participants) prospective multicentre Collaborative European NeuroTrauma Effectiveness Research (CENTRE-TBI) study

reported 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 hours and 12–32 hours post-injury (0–8h: GFAP AUC = 0.89 and S100B AUC = 0.63. 12–32h: GFAP AUC = 0.94 and S100B AUC = 0.72)[41].

Importantly, evidence suggests that blood GFAP levels are sensitive to subclinical intracranial pathologies that are not visible using 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 concentration measured within 24 hours of injury was 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 with MRI had higher levels of plasma GFAP than participants with other MRI lesion types, 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 for 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 on the following days[43]. In a large prospective study, initial (4 hours after injury) blood GFAP levels were also able to discriminate participants with body and head trauma with concussion from participants with non-concussive trauma in both children and adults with an AUC 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 concussive trauma could reflect a mechanical disruption of the BBB that occurs in parallel with the observed transient and chronic GFAP overexpression following single and multiple concussions, respectively[45,46]. The temporal cascade of plasma GFAP following TBI was investigated using a small subset of 34 participants from the Citicoline Brain Injury Treatment Trial (COBRIT): plasma GFAP was maximal at day 0, remained elevated at 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 majorly according to age. These observations support evidence that other glial biomarkers (for example, S100B) have reduced specificity in older individuals with TBI compared with younger individuals with TBI[49]. This effect of age could result from incipient neurodegeneration, different anatomical location and type of injury in older individuals, or differences in the sensitivities of the assays or imaging methods used[48].

[H3] 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 post-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 for 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 outcome, as defined by GOSE scores at 1, 4 and 6 months post-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 (MAP2) measurements to known clinical predictors (age, sex, GCS score) improved the prediction of 6-month favourable outcome 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 partcipants with TBI compared with 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 (pTAU, NfL, IL-6, and TNFα) for the differentiation of participants with past medical history of TBI (up to decades before blood sampling) and cognitive impairment from those with 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.

[H2] 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 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 at 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 reported 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 post-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 postoperative SCI participants 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 SCI, thereby supporting clinical decision-making regarding the identification of patients who are likely to benefit from surgery.

[H2] Cerebrovascular accidents

[H3] 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 ischemic stroke[60,65]. In a multicentre cohort study, the analysis of plasma GFAP levels in 205 participants using electrochemiluminometric immunoassays within 4.5 hours of symptom onset differentiated participants with intracerebral haemorrhage from participants with ischemic stroke and stroke mimics[66]. Specifically, use of a GFAP cut-off of 0.29 µg/L

enabled the distinguishment of intracerebral haemorrhage from acute ischaemic stroke and 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 large lobar intracerebral haemorrhages had a higher median serum GFAP concentration than participants with small, deep intracerebral haemorrhages[61].

[H3] 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 ischemic 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 found 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 value. 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 ischemic 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 (~ day 7) in 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 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 ischemic 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.

[H1] Inflammatory CNS diseases

[H2] MS

MS was the disease that led to the discovery of GFAP by Lawrence F. Eng in 1971[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 to improving the clinical workup 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 reported 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 controls were consistently reported in participants with progressive MS, 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 reported a correlation between blood GFAP concentration and severity of disability, as assessed by the expanded disability status score (EDSS)[8,77,78,83-87] (Table 2). Only a single study reported a positive correlation of blood GFAP concentration with disease duration[78]. Notably, higher GFAP levels were associated with a greater MRI lesion load in most of the reported studies[25,78,87]; blood GFAP levels also correlated with other markers of neurodegeneration (for example, NfL) and brain atrophy[8,25,77,78,85]. One study reported 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[8,25,78,87]. Another study assessed blood GFAP levels in patients receiving autologous hematopoietic stem cell transplantation and identified a paradoxical increase compared with baseline after the initiation of treatment[86]. 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 haematopoetic stem cell transplantation procedure and might cause intrinsic neurotoxicity[86]. A similar increase in GFAP concentration was observed in the context of neurotoxicity following immune effector

cell-associated neurotoxicity syndrome (ICANS) after chimeric antigen receptor (CAR)-T cell therapy[88]. 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 (hazard ratio of 1.96) of reaching an EDSS of 7.0 in participants with SPMS who had higher GFAP levels (>80th percentile) at baseline[89].

[H2] Neuromyelitis optica spectrum disorder

Neuromyelitis optica spectrum disorder (NMOSD) is a classical autoimmune inflammatory astrocytopathy[90]. Aquaporin 4 antibodies, among other mechanisms, induce astrocytic damage in NMOSD lesions and subsequently cause neuroaxonal damage[91]. Data regarding GFAP concentrations in NMOSD are limited but promising[3]. Even with 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[3,77,85] and suggest that blood GFAP could be used to distinguish between NMOSD and MS.

Furthermore, one study found that GFAP levels were higher in 33 participants with NMOSD than 16 participants with myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD), two diseases with similar clinical and radiological findings[84]. Similar to MS, evidence indicates that serum GFAP in NMOSD is higher shortly before (within 1 week) and during acute clinical relapses than during stable disease[77,92]. Data also indicate that serum GFAP levels correlate with EDSS score, most notably in younger patients[77,85]. The ratio between GFAP and NfL (GFAP:NfL) 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[84]. Finally,in a study that included 33 participants with NMOSD, immunomodulatory therapies (corticosteroids, azathioprine, tacrolimus, methotrexate, cyclophosphamide, cyclosporine) did not seem to influence serum GFAP concentrations in NMOSD[77], most probably owing to the timing and relative ineffectiveness of the disease-modifying treatment used in this study. Additionally, evidence indicates that the main extent of astrocytic loss occurs during acute inflammatory exacerbation[3,24,91]. Nevertheless, the N-MOmentum study[92] demonstrated that GFAP levels between the 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[92]. 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 reported an association between higher GFAP concentrations and PMS[25,78,87]. The consistent correlation of GFAP concentrations with 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.

[H1] Neurodegenerative diseases

Several studies have reported increased levels of CSF GFAP in the most common neurodegenerative diseases, including Alzheimer disease (AD), prion diseases, frontotemporal lobar degeneration (FTLD), Parkinson disease (PD), Parkinson disease 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[93-96], supporting the notion that more extensive investigations are needed to address this topic in detail.

[H2] 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 FTD (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 rate) 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[13,30,93,96]. Another study also reported increased blood GFAP levels in individuals with AD compared with cognitively healthy individuals[97]. Most interestingly, one study reported a correlation between plasma GFAP levels and cortical Aβ deposition in individuals with symptomatic AD[98]. Linear, positive associations were observed early in disease and diverged at more severe disease stages. These findings suggest that astrocytic damage or activation begins in the pre-symptomatic phase of AD and is associated with brain Aβ load[99].

[H2] The FTLD spectrum

Compared with AD, the data on GFAP in the FTLD spectrum are inconsistent[30,93,94,96]. The analysis of plasma samples from the large, multicentric Genetic FTD Initiative (GENFI) cohort, including 469 participants with genetic FTD, revealed that GFAP levels were elevated compared with controls in symptomatic *GRN* mutation carriers but not in other FTD mutation carriers[94]. Moreover, biomarker changes were associated with the appearance of clinical symptoms and not detectable in presymptomatic mutation carriers[94]. In support of these findings, two other studies reported no changes in serum GFAP levels in participants with sporadic[30] and genetic bvFTD[96] compared with control participants without neurodegenerative diseases. However, in a large Italian cohort, serum GFAP was elevated in participants with all FTLD clinical syndromes (sporadic and genetic) compared with healthy control participants[93]. 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 bvFTD and agrammatic variant PPA; these groups comprised participants with an unusually high percentage of *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.

[H2] 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[100]. The pathological hallmark of the disease is the formation of cytoplasmic aggregations in astrocytes[101]. 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[101]. Owing to the

453 rarity of the disease, studies reporting GFAP levels in the blood of individuals with
454 Alexander disease are limited. One study reported a modest, elevation of GFAP in the serum
455 of participants with infantile and juvenile Alexander disease, but not in adult participants with
456 the disease, compared with healthy controls[102]. This finding contrasts with the high
457 concentrations of GFAP found in the CSF of participants with Alexander disease[102-104]. A
458 possible explanation for this divergence is the 'hook effect' mentioned above, whereby
459 GFAP aggregate formation might limit its detection in the blood[3]. Blood GFAP might still
460 serve as a promising treatment outcome parameter for future trials in Alexander disease (for
461 example, in trials of antisense oligonucleotide therapies), but further studies are necessary.
462
463 ***[H2] Other neurodegenerative diseases***

464 Data on blood GFAP in other neurodegenerative diseases is scarce. In one study, blood
465 GFAP concentrations were not significantly elevated in participants with genetic or sporadic
466 amyotrophic lateral sclerosis compared with healthy control participants[96]. Another study
467 reported higher blood GFAP levels in participants with PD than in healthy control
468 participants[105]. Blood GFAP was also elevated in participants with neurological
469 manifestations of Wilson disease compared with healthy control participants and participants
470 with pure hepatic manifestations of Wilson disease[106]. We found only one study that assessed
471 blood GFAP levels in individuals with vascular cognitive impairment — no significant
472 difference between healthy control participants and participants with vascular cognitive
473 impairment was observed[107]. Notably, the studies discussed in this section used a range of
474 analytical methods, including sensitive immunoassays with relatively low sensitivity (that is,
475 standard ELISA).
476 ***[H2] 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 $Aß_{1–42}$ (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[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 $Aß_{1–42}$:$Aβ_{1–40}$ for the identification of amyloid PET positivity in participants with AD[108,109]. Plasma GFAP predicted amyloid PET positivity with an accuracy of 88% (when combined with $Aß_{1–42}$:$Aβ_{1–40}$, 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[30,93,108]. Accordingly, in participants with presymptomatic *GRN*-related FTD, higher plasma GFAP levels were associated with lower MMSE scores and brain volumes[94]. 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[94]. 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[99,108,110], conversion to AD[109,111], a faster rate of cognitive decline[110], and decline in hippocampal volume[111]. However, the prognostic value of GFAP in other neurodegenerative diseases was

poorly analysed: we could only find two cohorts of participants with sporadic CJD[95] and FTD[93]. 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 promising approach for improving the precision of differential diagnosis. The association of higher blood GFAP concentration 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 *GRN* mutation carriers 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. These raise 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.

[H1] 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[112-116], 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[117,118]. In participants with GBM, blood GFAP concentration correlated with preoperative tumour volume[112,113,115,119,120], volume of necrosis[112,120], and GFAP expression levels in tumour tissue[112,120]. 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 healthy controls[121].

Data regarding the prognostic value of blood GFAP in individuals with brain tumours seem to be inconsistent. Evidence indicates that blood GFAP levels rise shortly after operative treatment compared with preoperative samples[122], before ultimately decreasing[123]. One study found that blood GFAP levels did not correlate with the amount of malignant tissue that remained postoperatively[124]. In several studies, blood GFAP levels did not help predict postoperative tumour recurrence or overall survival[113,117,123]. However, two studies reported an association between high blood GFAP levels and poor progression-free survival[114,115]. The major limitations of the studies discussed here are that they used immunoassays with low sensitivity and low readout resolution compared with the highly sensitive bead-based assays like 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.

[H1] 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[125], 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[17]. Site-specific phosphorylation can be disease-relevant and has been reported to be associated with disease severity, for example in Alexander disease[126] and following hypoxic injury[127]. The effect of post-translational modifications on the analytical performance and clinical utilization of different GFAP assays, which use different proprietary antibody pairs, has been poorly characterised.

Similarly, compared with healthy participants, blood levels of GFAP breakdown products seem to be elevated following TBI and follow a similar diagnostic and prognostic pattern as the blood levels of the full GFAP protein[128-130]. 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 recognise (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[126,131-133]. 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[134]. Although some subclasses of astrocytes (for example, neurotoxic astrocytes) are toxic to neurons and oligodendrocytes, other subclasses promote CNS repair[134,135]. Data suggest that harmful pan-activated astrocytes at the rim of MS lesions are GFAP positive, whereas direct neurotoxic astrocyte subpopulations are not[79,82,136]. So far, the expression of GFAP over the spectrum of astrocyte subclasses remains poorly characterised, 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[137], the half-life of GFAP in blood is around 24–72 hours[35,138]. 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[139]. In this case, blood GFAP levels are likely to reflect ongoing astrocytic activation in addition to the possible astrocytic damage, as has been shown in NMOSD[92]. In chronic neuroinflammatory (for example, progressive MS)

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.

[H1] Conclusions

Unprecedentedly, the FDA recently authorised a panel test for blood-derived brain protein biomarkers, including GFAP, for clinical use in a neurological disease. GFAP is a well-established marker of astrocyte injury and activation in CNS diseases and represents 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 injury 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 prehospital and acute hospital settings if integrated into standard care. In inflammatory neurological diseases, blood GFAP has promising applications in progressive MS, as the marker could reflect and predict long-term disability worsening and, therefore, contribute to the treatment decision algorithm. In the elderly population, GFAP seems to predict the rate of cognitive decline and conversion to overt dementia, which makes it an attractive marker to recognise individuals at risk and enable rapid initiation of future preventive, and eventually therapeutic measures. Finally, recent insights suggest that blood GFAP levels have 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.

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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

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Peer review information

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Key points:

- GFAP levels reflect the clinical severity and extent of intracranial pathology after traumatic brain injury (TBI).
- In 2018, the FDA authorised 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, 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.

Review criteria

For this Review, we screened the published literature in PubMed using the following terms in the title/abstract: 'GFAP' or 'Glial fibrillary acidic protein', 'blood' or 'plasma' or 'serum', and the disease of interest. Hence, we added the following terms: 'MS', 'Neuromyelitis optica', 'NMO', 'MOG Antibody Disease', 'MOG associated disease', "Traumatic brain injury", 'TBI', 'Spinal trauma', 'Spinal injury', 'Stroke', 'Cerebral ischemia', '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', 'Meningitis'. Animal studies, previous reviews, and studies reporting only GFAP values in CSF were considered beyond the scope of this article.

1101 **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-month post-injury, GOSE and RTW 6-month post-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)[129]	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 post-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)[143]	ELISA	209 with MMTBI, 188 without MMTBI	Prospective cohort study: serum collected <4 h post-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 hours post-injury injury; repeated sampling at 4 h, 8 h, 12 h and then each 12 h up to 180 h post-injury	GFAP was detectable <1 h post-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)[144]	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 post-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 day 1, 2, 3, and 7 post-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	34 with TBI, 69 controls	Prospective multicentre cohort study: blood samples < 24 h, and 30 and 90 days post-TBI, head CT and GCS at admission; GOSE 6 months post-injury	GFAP levels were highest at day 0 and distinguished complicated mild TBI from controls with an AUC of 0.936. Elevated GFAP levels up to 90 days post-injury compared with controls
Bazarian et al. (2018)[36]	CLIA	2,011 (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 post-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 hours post-	Inclusion of GFAP improved ($p \leq 0.05$) prognostic capacity of GOSE scores compared with a model containing only baseline patient

			injury; GOSE at 1–4 and 6 months post-injury	variables and characteristics. The best prognostic capability (AUC 0.85) was achieved by also incorporating blood S100 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 post-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 0–8 h. GFAP was a predictor of pathological head CT results (0–8 h: 0.89 sensitivity, 0.62 specificity;12–32 h: 0.94 sensitivity, 0.67 specificity). 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 post-injury, brain MRI 7–18 days post-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
Anderson et al. (2020)[52]	ELISA	243 with TBI (PH GCS score 3–12, SPB > 90)	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 hours (SD = 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 to good functional outcome. Similar serum (AUC = 0.814) and plasma (AUC = 0.778), 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 post-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 TBI with cognitive impairment than TBI without cognitive impairment (p = 0.06).
Shahim et al. (2020)[53]	Simoa	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 controls (p <0.001). GFAP levels decreased during the first 6 months of 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; DRS, Disability Rating Scale; GCS, Glasgow Coma Scale; GOSE, Glasgow Outcome Scale Extended; GFAP, glial fibrillary acidic protein; ICH, intracranial haemorrhage, IL-6, interleukin 6; IL-10, interleukin 10; MAP2, microtubule-associated protein 2; MMTBI, mild or moderate traumatic brain injury; NfL, neurofilament light chain; NRGN, neurogranin; pTAU, phosphorylated tau; RTW, return to

work; S100B, calcium-binding protein S100 beta; SBDP150, Spectrin Breakdown Product of molecular weight 150; TNFα, tumour necrosis factor α; UCH-L1, ubiquitin C-terminal hydrolase L1. [a] Average time from most recent TBI = 37 years

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 healthy control participants	GFAP level in PMS compared with RRMS	Treatment effect (% participants treated)	Correlation with other markers (correlation coefficient or beta value)		
						Clinical disease severity (EDSS)	MRI T2 lesion load	Blood NfL levels
Abdelhak et al., (2018)[25]	Simoa (singleplex)	RRMS+ (18) RRMS- (24) SPMS (13) PPMS (25)	ns[a] Higher[a] (p<0.05)	Higher in PMS than RRMS (p < 0.05)	ns (8.8%)	ns ns r = 0.5 (p < 0.001)	Higher GFAP levels in participants with > 9 lesions than in participants with 2–9 lesions (p < 0.05)	r= 0.4 (p < 0.01)
Högel et al., (2018)[78]	Simoa (singleplex)	RRMS (46) SPMS (33)	ns Higher (p<0.01)	Higher in SPMS than 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)
Abdelhak et al., (2019)[8]	Simoa (singleplex)	PPMS (71)	NA	NA	ns (40.9%)	β = 0.3 (p < 0.01)	NA	NA
Watanabe et al., (2019)[77]	Simoa (singleplex)	RRMS (38) PMS (11)	Higher in RRMS+ (p<0.01) Higher (p<0.01)	ns	ns (55.1%)	β = 1.1 (p < 0.05)	ns	NA
Lee et al., (2020)[85]	Simoa (singleplex)	MS (112)	NA	NA	NA	r = 0.3 (p = 0.001)	NA	r = 0.6 (p < 0.001)
Thebault et al., (2020)[86]	Simoa (multiplex)	RRMS (12) SPMS (10)	Higher (p<0.001)	NA	Higher after treatment with IAHSCT compared to baseline p < 0.01)	NA	NA	NA
Ayrignac et al., (2020)[87]	Simoa (singleplex)	PPMS (18) RRMS (111)	NA	Higher in PPMS than 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)

EDSS, expanded disability status scale; GFAP, glial fibrillary acidic protein; RRMS, relapsing-remitting MS; RRMS+, RRMS during clinical relapse; RRMS-, RRMS patients without evidence of clinical relapses; PMS, progressive MS; SPMS, secondary progressive MS; PPMS, primary progressive MS; CIS, clinically isolated syndrome (optic neuritis in MS); Simoa, single molecular array; MRI, magnetic resonance imaging; NfL, neurofilament light chain; NA, not assessed or not reported; ns, not significant; IAHSCT, Immunoablation and autologous hematopoietic stem cell transplantation; r, correlation coefficient (Pearson/Spearman); β: regression estimates.

[a] Compared with participants with non-inflammatory neurological diseases

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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 controls and participants with psychogenic non-epileptic seizures	145-147
Delirium	In cases of ICU delirium following COVID-19 infection, GFAP increased and correlated to delirium severity. GFAP might increase in association with postoperative delirium, but the evidence is conflicting	58,148-152
Sepsis-related encephalopathy	Elevated serum GFAP levels compared with participants with sepsis without encephalopathy	153
Cardiac arrest	GFAP levels and proteolytic fragments were elevated after cardiac arrest	154-157
SARS-CoV-2 infection	Serum levels of GFAP, but not of other markers like NfL, were increased in participants with moderate and severe COVID-19 infection, who were admitted to ICU	148,158
West-Nile Virus infection	CSF and serum levels of GFAP were significantly higher in individuals with West-Nile virus infection compared with controls. These findings correlated with the severity of post-mortem histopathology	159
Atrial fibrillation	Elevated circulating levels of GFAP in individuals with atrial fibrillation compared with control participants	160

GFAP, Glial fibrillary acidic protein; NfL, Neurofilament light chain; COVID-19, coronavirus disease 2019.

Figure 1 | Astrocytes have multiple physiological roles in the CNS.

a | Astrocytic end feet containing glial fibrillary acidic protein (brown filaments) are an essential component of the blood–brain barrier and the glymphatic system[140]. **b |** Astrocytes are critical in maintaining axonal metabolic homeostasis[141]. **c |** Astrocytes contribute to tripartite synapses[142].

Box 1 | Unmet needs on the way towards a clinical utilisation for 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 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 for 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 and age-specific and gender-specific reference range in healthy individuals
- Identification of clinically useful cut-off values
- Agreement on a "gold standard" measurement technique
- Co-ordination of multicentre studies to address the clinical applicability of GFAP in difference diseases
- Longitudinal studies assessing GFAP dynamics in subacute and chronic conditions

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 makers 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[161,162]. The expression and secretion of astrocyte biomarkers varies according to the age of the individual , cellular localization of the marker, and astrocyte subtypes[163]. Moreover, other cell types can contribute to the concentration of these biomarkers circulating in CSF and blood. For example, GFAP is secreted from renal tubular cells and enteric cells[17], S100B is secreted from skeletal muscles[164], and CHI3L1 can be secreted from microglia and macrophages[165]. In 2021, advanced single-cell sequencing techniques unravelled the complexity of astrocyte subpopulation heterogenicity[166]; however, the definition of a biomarker-based signature for these different subpopulations is still far from reach.

Glossary

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

In this Review, the authors provide an overview of the evidence regarding the use of blood levels of glial fibrilliary acidic protein as a biomarker in a range of neurological diseases, including traumatic brain injury, stroke, multiple sclerosis and Alzheimer disease.