# Glial fibrillary acidic protein is a body fluid biomarker for glial pathology in human disease

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**Abstract**

This review on the role of glial fibrillary acidic protein (GFAP) as a biomarker for astroglial pathology in neurological diseases provides background to protein synthesis, assembly, function and degeneration. Qualitative and quantitative analytical techniques for the investigation of human tissue and biological fluid samples are discussed including partial lack of parallelism and multiplexing capabilities. Pathological implications are discussed in view of immunocytochemical, cell-culture and genetic findings. Particular emphasis is given to neurodegeneration related to autoimmune astrocytopathies and to genetic gain of function mutations. The current literature on body fluid levels of GFAP in human disease is summarised and illustrated by disease specific meta–analyses. In addition to the role of GFAP as a diagnostic biomarker for chronic neurodegenerative conditions, there are important data on the prognostic value for acute neurodegeneration. The published evidence permits to classify the dominant GFAP signatures in biological fluids. This classification may serve as a template for supporting diagnostic criteria of autoimmune astrocytopathies, monitoring disease progression in toxic gain of function mutations, clinical treatment trials (secondary outcome and toxicity biomarker) and provide prognostic information in neurocritical care if used within well defined time–frames.

*Keywords:* intermediate filaments, glial fibrillary acidic protein, GFAP, body

fluid biomarker, neurodegeneration, autoimmune astrocytopathies.

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**Contents**

## 1. Introduction

The discovery of glial fibrillary acidic protein (GFAP) by Lawrence F. Eng in 1969 published in this Journal, represented the first step to unravel the chemical properties of those fibres giving rise to the distinctive intra-cytoplasmic features of astrocytes[1,2]. The GFAP protein equips astrocytes with a nematic liquid crystal hydrogel, able of rapid fibre reorganisation. Like other cellular fibres, GFAP is classified by fibre diameter (8-12 nm) as *intermediate* between the smaller microfilaments (7 nm) and the larger microtubules ($\approx$ 25 nm)[3]. Expression of GFAP in the human brain occurs pre–dominantly in astrocytes and is about 10 times higher compared to rodent astrocytes[4]. It is the highly cell–type specificity and stability which qualifies this class III intermediate filament (IF) as a biomarker for human disease.

This review on GFAP as a protein biomarker (1) discusses protein synthesis and assembly; (2) introduces quantitative and qualitative analytical methods; (3) explains the clinico–pathological relationships underlying the biomarker hypothesis; and (4) reviews the evidence to use GFAP biomarker signatures as supportive diagnostic criteria, monitoring disease progression and improving prognostic accuracy.

## 2. GFAP structure and function

GFAP is a relatively non–soluble acidic cytoskeletal protein. It is the principal IF of the human astrocyte. First, viewed with the electron microscope, GFAP appear as bundled fibres of 8-12 nm diameter in the astrocyte. With the availability of specific antibodies, GFAP can be visualised using routine immunohistochemistry. The specificity of GFAP for astrocytes is such that GFAP has become one of the most useful proteins for identifying astrocytes in the brain[5]. There is heterogeneity in astrocytes. Expression of GFAP is higher in white matter compared to grey matter astrocytes[4]. In the retina GFAP is specific for Müller cells and astrocytes[6].

### *2.1. Genetics*

The human GFAP gene was cloned in 1989 and is mapped to chromosome 17q21.1-q25 (about 10 kb DNA)[7,8]. The gene consists of 8 introns and 9 exons, with 4 alternative exons and 2 alternative introns (3 kb, mRNA). Alternative splicing leads to 6 GFAP isoforms[9] (Figure 1). Of these $\alpha$–GFAP is most abundant in the human CNS[9]. The calculated protein length in aminoacids is 432 for $\alpha$–GFAP, $\geq$ 321 for $\beta$–GFAP, 431 for $\gamma/\epsilon$–GFAP, 438 for $\kappa$–GFAP, 374 for $\Delta$135–GFAP, $\leq$ 366 for $\Delta$164–GFAP and $\leq$ 347 for $\Delta$exon6–GFAP. The complex regulatory mechanisms governing alternative splicing of the GFAP gene have not yet been fully unravelled[10]. It is not yet clear if all of these get translated into protein, but there is good evidence for $\alpha$–GFAP, $\beta$–GFAP and $\Delta$–GFAP.

Over 80 mutations of GFAP have been described [1], notably for patients with Alexander disease of which 95% harbour gain of function mutations[11]. This is interesting because pathology seems to be almost entirely associated with gain of function. Loss of function or even complete GFAP knock out does not appear to be related to *immediate* substantial pathology. Notably and with one single exception, demonstrating hydrocephalus, the majority of GFAP null mice did not show any signs of *spontaneous* pathology at all[12]. In contrast, long–term potentiation was demonstrated. In addition, the GFAP knock out mice do however, show an altered response to induced pathology such as larger infarcts after ischaemic stroke[13,14]. For Alexander disease there is a need for longitudinal data on body fluid GFAP levels in these mutations in order to better understand the relationship of individual mutations with the variable pattern of disease progression.

### *2.2. Structure*

The structure of GFAP has not yet been determined. Like with other IF such as neurofilament proteins were the structure remains unknown[15], this

[1] For an updated database with references of the ever extending list of mutations see www.waisman.wisc.edu/alexander-disease/ or http://www.interfil.org/

may in part be related to the presence of intrinsically unstructured domains[16]. These domains permit a number of properties, related to Ca$2+$ binding polyanion tails[17]. Despite this limitation, molecular modelling of GFAP has been undertaken (Figure 2) and guided the development of specific ligands[18].

### *2.3. Synthesis and assembly*

Expression of GFAP mRNA is rapidly induced following acute brain injury and modified by neurological disease[19]. In addition, transcription of GFAP mRNA increases with age[20]. Translation of $\alpha$-GFAP mRNA results in polypeptides which are capable to self–assemble into homomeric filaments with aid of their amino–terminal head domains[21,22]. More often, however filaments appear to be heteromeric with for example vimentin and nestin, for example during early in development. The complex expression of GFAP during development is beyond de scope of this review.

Generally, expression of GFAP peaks in early development which is followed by a slow decline[23,24]. A secondary rise of GFAP expression is related to development of pathology, such as Alzheimer disease or non–specific gliosis[23,25]. Not all studies consistently demonstrated this bi–phasic expression of GFAP, partly due to methodological issues and different experimental models[26–28].

Cytoskeletal GFAP is tightly packed into polymers. GFAP assembly is organised hierarchically, starting with monomers (single polypeptides of about 49.8 kDa), dimers and staggered tetramers to form a complex, cross–linked cytoskeletal network[21,29,30]. At each of these steps, GFAP assembly can be influenced by mutations, post–translational modifications, competing GFAP isoforms and other proteins such as S100, annexin, vimentin and $\alpha$-crystallin[30–34]. A much simplified sketch of this dynamic and reversible hierarchical process is summarised in Figure 3. For a more in detail review of GFAP synthesis and assembly the reader is referred to the pioneering Danish and Dutch groups an references therein[9,35,36].

### 2.4. Non astrocytic expression and function of GFAP

As with most proteins dominant expression within one body compartment does not preclude systemic expression. There is mainly immunocytochemical evidence for GFAP in the lens, non–myelinating Schwann cells, testicular Leydig cells, stellate cells of the liver and pancreas, enteric glia, podocytes, mesangial cells and chondrocytes[37,38]. The quantitative contribution of such non–CNS derived protein was found to be negligible for neurological biomarker studies[39].

Interestingly, the same anti–GFAP antibodies used in ELISA[40–43] also stained fibroblasts and keratinocytes of the rodent and human epidermis[44]. Nevertheless, the pattern of non astrocytic GFAP expression at the air–tissue interface may provide functional insight[44]. The authors speculate on a possible protective role as part of an immune-barrier[38,44,45]. These results will need confirmation using a GFAP-KO model as control.

### 2.5. Post–translational modifications

The dominant 3 post-translational modifications are citrullination, glycosylation and phosphorylation. Phosphorylation is targeted to 6 serine and threonine residues, the first 5 of which are located in the carboxy–terminal tail: Thr-7, Ser-8, Ser-13, Ser-17, Ser-34, Ser-389[46–52] (Figure 3). Snider and Omary suggest that disease–causing alterations in GFAP may lead to aberrant protein phosphorylation[53].

### 2.6. Degradation

It has long been known that degradation of the GFAP polymer causes the release of more soluble fragments of GFAP ranging from 39-50 kDa, with predominance of a $\approx$ 41 kDa fragment[54,55]. Responsible is calpain–mediated digestion, a broadly expressed calcium–dependent protease[56,57]. There is also evidence for caspase-3 and caspase-9 GFAP cleavage products[58–61]. In addition, the cystein protease *caspase 6* cleaves GFAP at $Asp^{225}$ resulting in two proteolytic fragments of 24 kDa and 26 kDa[62].

### *2.7. Function*

The GFAP head domain is relevant for IF assembly[29]. The C–terminal tail domain influences IF spacing and the IF network[29,62,63]. These processes are influenced by post-translational modifications such as phosphorylation of the N–terminal head region at Thr-7, Ser-8, Ser-13, Ser-17 and the C–terminal tail domain at Ser-34 (Figure 3).

A remarkable feature of the GFAP network is the speed with which it is modified in response to stimuli and injury[19]. This is required to support the variable morphological changes seen in acute and reactive astrocytosis as well as astrogliosis.

## 3. GFAP tests

### *3.1. Qualitative techniques*

Qualitative and semi–quantitative techniques are frequently required for proof–of–principle studies, but do not qualify for reproducible, quantitative analysis of GFAP from biological fluids.

#### *3.1.1. Western & immunoblotting*

Most authors use western and immunoblotting techniques as a highly specific, semi-quantitative approach[1,2,21,22,29–34,46–52,54,55,62,63]. Epitope–specific antibodies permit to distinguish post translational modifications and proteolytic break–down products of GFAP. A limitation of the method is that gel entrance is not possible or challenging for insoluble GFAP fragments, GFAP aggregates, cross–linked GFAP or larger GFAP polymers.

#### *3.1.2. Immunohistochemistry*

Immunocytochemical detection and semi–quantification of GFAP allows for indirect assessment of astrocytosis and astrogliosis from the tissue, but reproducibility of the published methods remains poor[64]. Like for all immunological methods the quality of the anti–GFAP antibodies chosen for immunohistochemistry is important.

### *3.2. Quantitative techniques*

A summary of the quantitative technologies suitable for detection of GFAP in biological fluids is given in Table 1. A limitation of current immunoassay is that there is a lack of testing for the potential effect of post–translational modifications, aggregate formation, cleavage products and presence of auto–antibodies from body fluid levels.

### *3.3. Single analyte technologies*

#### *3.3.1. ELISA*

There are a number of sensitive, robust, quantitative and high throughput are ELISA techniques[40–43,65–67]. Commercially available ELISA have been made available by BioVendo, Millipore, Biotech AG, AbcamCellBiolabs and GenScript. The sensitivity of ELISA is superior to immunoblotting and soluble oligo-/polymers may also be detected. Insoluble GFAP fragments/aggregates remain problematic. In addition, presence of anti–GFAP autoantibodies may be an overlooked analytical limitation as epitopes relevant for the immunoassay may be masked. In analogy to other intermediate filament biomarkers it is therefore recommended to carefully check for presence of parallelism[68,69].

An important limitation of the ELISA methods is that multiplexing is not possible. This remains a challenge if one attempts to measure protein and auto–antibodies in parallel, focus on GFAP isoforms or investigate post–translational modifications. It was shown that phosphorylation improves the detection of GFAP from the CSF in ELISA[70].

#### *3.3.2. RIA*

Radio–immunoassays (RIA) pre-date the ELISA technology by about a decade. Because of logistic reasons and health–and–safety regulations the use of RIAs is limited to specialised laboratories. An advantage of the technology over ELISA is the low detection limit. But in view of more recent technologies with even lower detection limits, also capable of multiplexing, RIA technology is unlikely to play a role for the quantification of GFAP from biological fluids.

### *3.4. Multiple analyte technologies*

#### *3.4.1. Mass–spectrometry*

Mass–spectrometry has been used to detect small amounts of GFAP and better characterise the post–translational modifications of GFAP[49,71–76]. An important finding from these studies was that compared to other proteins GFAP is an extremely stable and robust candidate biomarker for mass–spectrometry[76].

A new approach relies on antibody labelling–free technology based on reversed–phase HPLC followed by mass–spectrometry, called selected reaction monitoring (SRM)[77]. This method permits multiplexing and the diagnostic value of SRM has been shown for GFAP[78].

#### *3.4.2. Fluorescence based multiplexing*

Fully automated analyses of femtolitre sample volumes is possible with single molecule arrays (Simoa)[79,80]. This method relies on enzyme–induced fluorescence and also permits for multiplexing.

Another multiplexing method using fluorescence is the xMAP technology (Luminex). Again multiplexing is possible[81].

At time of writing there are to the best of my knowledge no validated multiplexing tests available for quantification of GFAP. The technology is potentially suited to simultaneously test for post–translational modifications of GFAP, GFAP isoforms and presence of anti–GFAP autoantibodies.

#### *3.4.3. Electrochemiluminescence based multiplexing*

A large analytical range and excellent detection limit for protein biomarkers can be achieved using solid phase electrochemiluminescence (ECL)[82,83]. An established platform in the field comes form Meso Scale Discovery (MSD). This technique is particularly well suited for interesting analytical developments making use of nano–crystals, nano–tube arrays, synthetic receptors using polymers and microfluid analysis for point–of–care diagnostics[84]. Another advantage of the MSD platform over other multiplexing technologies is that it only takes about 70 seconds to read a 96–well plate.

For ECL, as for fluorescence based multiplexing there are to the best of my knowledge no validated multiplexing tests available for quantification of GFAP. Again, the technology is potentially suited to simultaneously test for post–translational modifications of GFAP, GFAP isoforms and presence of anti–GFAP autoantibodies.

Table 1: *Summary of quantitative technologies suitable for analysis of GFAP from biological fluids.*

| Method | Multiplexing | Detection | Volume | Reference |
|---|---|---|---|---|
| ELISA | no | Colour–reaction | microlitres | [43] |
| MSD | yes | ECL | microlitres | [82] |
| Sensors | yes | ECL | femtolitres | [84] |
| xMAP | yes | Fluorescence | microlitres | [81] |
| Simoa | yes | Fluorescence | femtolitre | [80] |
| SRM | yes | label-free | microlitres | [77] |

### *3.5. GFAP Auto–antibodies*

The presence of anti-GFAP auto–antibodies has been observed[75,85–91]. The reproducible quantification of auto–antibody titres is challenging. Presence of heterophile antibodies need to be excluded. Heterophile antibodies are of weak avidity and multi-specific activity which may interfere with immunological assays and cause false positive test results[92]. There is a clear need of SRM generated data on anti–GFAP antibodies[78]. Particularly, because development of anti-GFAP IgG auto–antibodies was suggested to be significant within only four days following traumatic brain injury[91]. There is a need to adopt the same rigorous methodological approach as for other auto–antibodies[93,94].

Post–translational modifications of GFAP epitopes may be relevant. The diagnostic relevance of these auto–antibodies remains elusive. It will be important to investigate if binding of auto–antibodies to GFAP influenced the physiological half–life of GFAP and potential clearance through the reticuloendothelial system (RES). There have been no studies to date to investigate for presence of GFAP in the RES. Specifically, there have been no post–mortem studies

of the spleen from patients with pathologies were GFAP auto–antibodies have been observed[75,85–91]. Careful examination of the immunoblot of patient sera against human tissue homogenate (Figure 1D in reference[91]) suggests presence of a single band in the molecular range of GFAP, not commented on by the authors.

### *3.6. GFAP hook effect*

Albrechtsen *et al.* were first to make the observation of a GFAP hook effect for serial dilutions of CSF and blood samples from patients[95]. Parallelism could be established in most cases after 4 dilution steps, but some samples require up to 10 dilution steps. A good example for the latter was shown in their Figure 2 for a serum sample from a patient with dementia. The authors speculated the cause to be inhibitory factors[95]. In view of recent data on a neurofilament hook effect caused by protein aggregate formation in neurodegenerative disease[68,69], it will be important to test whether GFAP aggregate formation also occurs in neurodegeneration. Both homomeric aggregates and heteromeric aggregates related to anti–GFAP autoantibodies may co–exist.

## 4. GFAP body fluid levels

Biological fluid levels of GFAP will be reviewed as relevant for acute and chronic neurodegenerative diseases. Negative data will be included as well to enable a critical analysis of sensitivity and specificity of GFAP as a biomarker in the presented disease specific meta–analyses. In the literature data were reported as median, mean or frequencies (normal/abnormal). There were good reasons for this presentation because data were non–Gaussian, but it complicated a easily accessible visual overview comparison between studies for the purpose of this review. Therefore authors of all papers were contacted by email and available data were presented as mean and standard deviation in disease specific meta–analyses. The standard deviation was calculated from the standard error of a mean by multiplying with the square root of the sample size.

Taken together, the data permit to classify the GFAP biological fluid levels in main patterns which will be summarised at the end of the section.

### *4.1. Neuromyelitis Optica*

Immunocytochemical there is combined loss of staining for AQP4 and GFAP[96]. AQP4 is expressed at the astrocytic foot processes and of particular supramolecular aggregate density within the optic nerve and spinal cord[97]. Both, complement mediated auto–immune antibody and cellular mediated glial toxicity are hold responsible for astrocytic apoptosis[98–102].

The group from Sendai was first to report the serendipitous finding of a several magnitude increase of CSF GFAP levels in acute NMO[103]. This finding has since been reproduced by different groups[104–107]. An elevation of CSF GFAP levels can be observed with a large number of pathologies as reviewed here. Also CSF GFAP levels were high in NMO they did not permit to generally separate patients with NMO from a highly heterogeneous ($p<0.0001$) group of control patients ($p=0.07$, Figure 4 A). Importantly, CSF GFAP did however permit to separate NMO from a homogeneous ($p=0.29$) group of patients with MS ($p=0.003$, Figure 4 B). This meta–analysis provides class I evidence that CSF GFAP levels are of diagnostic value for separating NMO from MS.

Of note, CSF GFAP levels were only high during an acute exacerbation in NMO[103–105]. There was a highly significant inverse correlation between the time lag from relapse to sampling ($R=-0.62$, $p=0.009$)[105].

For this reason, depending on timing of sampling the diagnostic sensitivity ranges from 85%–100% and the diagnostic specificity from 77%-100%[104–106,108]. The critical time–frame for sample collection is likely within 10 days following an event as discussed further down for cerebrovascular pathology. Because high CSF GFAP levels were so closely related to the acute phase, larger, multi–center study on longitudinal CSF levels will be required in order to inform whether or not revised diagnostic criteria for the NMO spectrum disorder might consider incorporating CSF GFAP levels as a supportive diagnostic test[109]. Consequently, it has been suggested to classify NMO as an autoim-

mune astrocytopathy[110].

In contrast to the CSF, studies on blood GFAP levels in NMO have been negative[111–113]. The diagnostic specificity for blood GFAP levels was about 33% only.

None of these studies investigated GFAP isoforms, post–translational modifications or proteolytic breakdown products in body fluids of patients with NMO.

### *4.2. Multiple sclerosis*

Immunohistochemistry demonstrates white-spread astrocytic activation and astrogliosis in MS[46,114–116]. In fact, GFAP was discovered in the MS brain[1].

The majority of studies on GFAP levels in multiple sclerosis (MS) were from cross–sectional CSF samples. CSF GFAP levels were slightly higher in MS compared to controls[106,114,117–124]. The data for clinical subgroups are summarised in a meta–analysis (Figure 5).

A correlation between CSF GFAP levels and clinical scales for disability was found by the majority[114,120–122,125,126], but not all studies[127]. Likewise CSF GFAP levels tended to be higher in MS patients with a progressive disease course in some[125,128], but not all studies[114] and one group found lower CSF GFAP levels during the progressive disease course compared to relapsing remitting disease[120]. The point may be made that CSF GFAP levels might have been increased following an acute relapse in the latter study[120], but no such relationship was found in another study specifically investigating this question[122].

In MS longitudinal CSF GFAP levels were found to be stable over time and even after receiving disease modifying therapies[127,128]. The jury on these data is still out, because the same group, using the same methods, also described an 2-3 fold increase of CSF GFAP with accumulating disability[126] and a mean annual increase of CSF GFAP of 18.9 ng/mL in secondary progressive MS (SPMS) compared to only 6.5 ng/mL in control patients[125].

Serum GFAP levels were not found to be of much diagnostic value in MS[111,112].

One semi–quantitative analysis suggested higher serum compared to CSF GFAP levels[118]. It is not known if possible presence of anti-GFAP auto-antibodies[75,85–90] in this chronic disease might have influenced these data.

One study demonstrated altered post-translational modifications of GFAP in the brain tissue from patients with secondary progressive MS[46]. An increase of citrullinated GFAP was found in MS brain tissue affected by lesions, but interestingly also in areas which apparent normal appearing white matter. No data are yet available on other post–translational modifications, aggregation or breakdown products of GFAP in the body fluid of patients with MS.

### *4.3. Alexander disease*

Alexander disease is caused by mutations in the GFAP gene[11]. This results in altered GFAP assembly, GFAP aggregate formation, astrogliosis and pathological intracellular GFAP deposits know as Rosenthal fibres[11]. There is solid experimental data demonstrating the link between cellular over expression of GFAP mRNA and protein, intracellular GFAP aggregate deposition and increased CSF GFAP levels[24,71]. CSF GFAP levels were elevated in children with Alexander disease[129]. Targeted treatment strategies to interrupt this process and improve protesomal clearance of mutant GFAP are already in the experimental pipeline[130–132].

CSF and blood GFAP levels will need to be tested as a potential secondary outcome measure for emerging human treatment trials for this devastating condition. In analogy to what is known for another IF, the neurofilament proteins, any such studies are advised to take the possibilities for presence of anti–GFAP auto–antibodies or an aggregation related "GFAP hook effect" into account[68,69].

### *4.4. Cerebrovascular pathology*

Data on GFAP levels in ischaemic stroke[78,133–136], subarachnoid haemorrhage (SAH)[78,137–140], cerebral vasculitis[141] and intracranial haemorrhage (ICH)[78,133–136]. Data from the ICH studies suggest that blood GFAP levels may be of diagnostic value if taken within 1–6 hours after onset of ICH[133–135,142,143].

Likewise, longitudinal data from SAH indicates a wash–out time of 7–10 days for CSF GFAP levels[137]. Therefore studies making use of GFAP should consider this time frame in their sample collection protocol.

None of these studies investigated GFAP isoforms, post–translational modifications or proteolytic breakdown products in body fluids of patients with cerebrovascular pathology.

### *4.5. Traumatic brain injury*

Elevated serum GFAP levels following severe head trauma were first reported by Missler *et al.* using a highly sensitive ELISA[144]. The prognostic value of early serum GFAP levels were found to be better compared to S100B[145–147]. A pre–analytical problem with S100B is that expression occurs in a wide range of tissues which are easily damaged during trauma[148]. This does not appear to be a problem with GFAP.

Following traumatic brain injury (TBI), serum GFAP levels were predictive for poor outcome[91,149–153]. In addition, serum GFAP levels were found to be higher with focal compared to more diffuse pathology as assessed with the Marshall classification[154,155]. In support of this concept are essentially normal GFAP levels in human blast traumatic injury (bTBI) know to cause diffuse damage[156]. Repeated, experimental bTBI does however compromise cell membrane integrity followed by an increase of GFAP levels[157]. Finally, a rise of GFAP levels may indicate secondary brain damage due to an increase of intracranial pressure (ICP)[146,154].

Consequently, serum GFAP was included as the main body fluid biomarker in a large multi-centre trial, TRACK-TBI (NCT01565551)[158].

As mentioned before, there is a lack of studies on GFAP clevage products and post–translational modifications in body fluids following TBI.

### *4.6. Traumatic spinal cord injury*

Following traumatic spinal cord injury (SCI), serum GFAP levels were significantly elevated within 24 hours compared to controls[159]. This observation is consistent with post

Serum GFAP levels remained significantly elevated for 72 hours. Importantly, serum GFAP levels were also significantly related a clinical measure, the American Spinal Injury Association (ASIA) Impairment Scale. Already 24 hours after injury, patients with a severity score of A or B had significantly higher serum GFAP levels compared to those with a milder injury severity score[159]. These longitudinal serum GFAP data extend on earlier cross–sectional CSF GFAP data in SCI and lumbar disc herniation[160–162]. Again, higher CSF GFAP levels were related to more severe SCI[161,162]. This is a relevant finding because at present there is no method for rapid assessment of injury severity.

### *4.7. Alzheimer's disease*

There is evidence for gliosis demonstrated by increased immunocytochemical staining for GFAP and mass–spectrometry in Alzheimer's disease (AD)[48,50,54,163]. In patients with manifest AD there is evidence for increased citrullination and oxidation of GFAP[47,73,164]. Likewise, anti-GFAP auto–antibodies were found to be elevated in the serum of patients with AD[85].

Importantly there was correlation of CSF GFAP levels with higher age[65,66,165–168]. This needs to be taken into account for the interpretation of CSF GFAP data in dementia studies in an ageing population. An important limitation of these studies is that we do not know about the relationship between parenchymal and body fluid GFAP levels.

At present there is no clear role for CSF GFAP levels in the diagnostic work-up of patients with suspected AD, despite encouraging data from a number of studies and groups over the past two decades[124,168–172].

Again, there are no data on modifications of GFAP from body fluids in patients with dementia. Because post–translational modifications such as phosphorylation occur in neurodegenerative dementias, it will be interesting to learn if such information on GFAP phosphorylation will be of diagnostic or prognostic value. For another biomarker in dementia, tau, such efforts have been made successfully[173].

### *4.8. Hydrocephalus*

Substantially elevated CSF but not serum GFAP levels were reported in severe hydrocephalus[174]. Interpretation of this data will need to consider if the CSF was of ventricular or lumbar origin, as there is evidence of a rostro-caudal gradient with higher CSF GFAP levels in ventricular CSF[175]. Likewise, presence of blood in the CSF was related to higher CSF GFAP levels in hydrocephalus[176]. This may partly explain the considerable elevation of ventricular CSF GFAP levels following SAH which required extra ventricular drainage for management of secondary hydrocephalus[137].

### *4.9. Miscellaneous conditions*

Elevated amniotic fluid levels of GFAP were found in experimental models of menignomyelocele (MMC) and human neuronal tube defects[177,178]. The data on the value of CSF GFAP levels for the differential diagnosis of Parkinsonian syndromes remains controversial in view of negative data[179,180]. Patients with schizophrenia had normal GFAP levels[181]. Elevated CSF GFAP levels were found in patients suffering from narcolepsy[182].

## 5. Classification of GFAP body fluid biomarker patterns

Taken together, three dominant patterns of the GFAP biomarker signature in body fluids emerge, type I to type III (Table 2). In addition to these three main patterns there is a range of diseases with moderately elevated GFAP levels which, given the large overlap of data, may be regarded as rather non–specific.

Table 2: *Dominant patterns of GFAP body fluid levels and related diseases. NMO = neuromyelitis optica, ICH = intra-cerebral haemorrhage, MS = multiple sclerosis, SAH = subarachnoid haemorrhage, AD = Alzheimer disease, MMC = menignomyelocele, TBI = traumatic brain injury, SCI = spinal cord injury, DP = disc prolapse.*

| Type I | Type II | Type III | Non–specific |
|---|---|---|---|
| NMO | Alexander disease | ICH | MS |
| Vasculitis | Hydrocephalus | SAH | AD |
| Autoimmune-astrocytopathies | MMC | TBI | PD |
| | | SCI | Schizophrenia |
| | | | Narcolepsy |
| | | | DP |
| | | | Ischaemic stroke |

The first GFAP signature (type I) is of diagnostic value in the newly recognised group of autoimmune astrocytopathies to which NMO belongs (Figure 6A). The spectrum of autoimmune astrocytopathies is likely to expand as new auto–antibodies are discovered and close the gap of a so called "sero–negative spectrum disorder"[183].

The second GFAP signature (type II) is of value for monitoring disease progression in the many toxic gain of function mutations in the GFAP gene (Figure 6B). Any future clinical trail designed to halting disease progression may consider body fluid GFAP levels as a secondary outcome measure.

The third GFAP signature (type III) exemplifies the prognostic value of GFAP body fluid levels in acute neurocritical care conditions during the acute

phase (Figure 6C, light green curve) and due to complications in the disease course (Figure 6C, dark green curve). Potentially, the last curve may also serve as a template for testing if there are astroglial toxic side–effects of treatment interventions we may not be aware of. To do so, one will need to test if the intervention (grey shaded box) is followed by an increase of GFAP levels (light green curve in Figure 6C). The here idealised style of the curves must not deflect from the importance that each laboratory will need to to establish their own reference values and preferably also participate in external quality control schemes to optimise performance and safeguard against pitfalls in determining body fluid GFAP levels. Taken together the three dominant GFAP body fluid signatures have the potential to be of future diagnostic and prognostic value for diseases of the present review (Table 2)

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**Conflict of Interest**

I wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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## 6. Figures

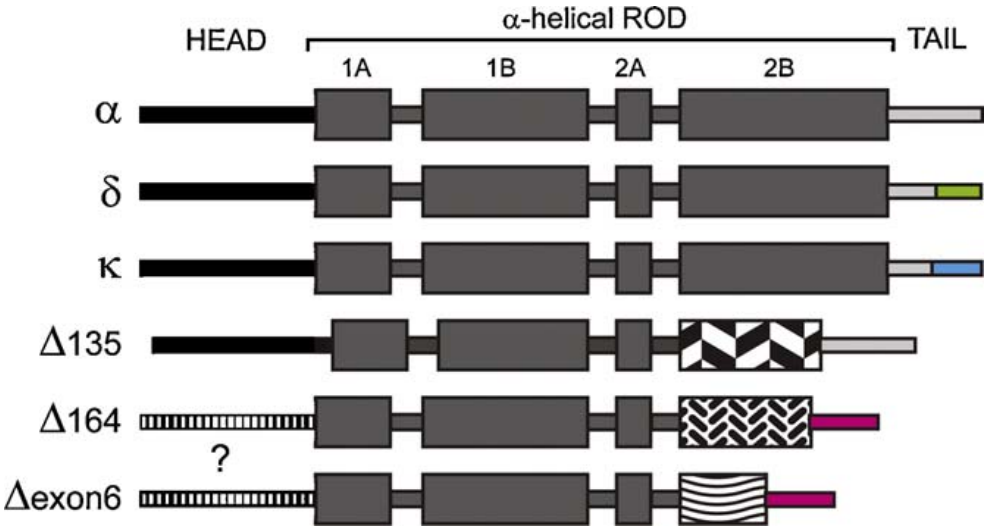



Figure 1: ***The GFAP isoforms.*** *A schematic drawing of the protein structures of the GFAP isoforms shows the overlap and difference between the GFAP isoforms. All isoforms are composed of a head (amino–terminal), a rod (with coils 1A, 1B, 2A and 2B) and a tail (carboxy–terminal) domain. Different patterns and different colours indicate differences between the individual isoforms. Although it is known that the tail of GFAPD164 and GFAPDexon6 are identical (pink), the exact composition of the head domain is not known, due to the unknown initiation site, hence the question mark. (reproduced with permission from reference[9]).*

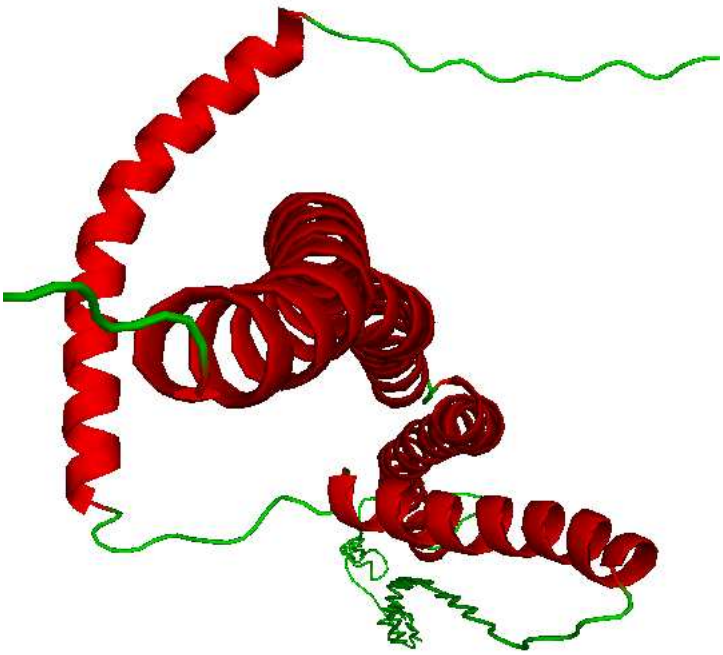


Figure 2: *Modelled structure of GFAP. Reprinted with permission from*[18].

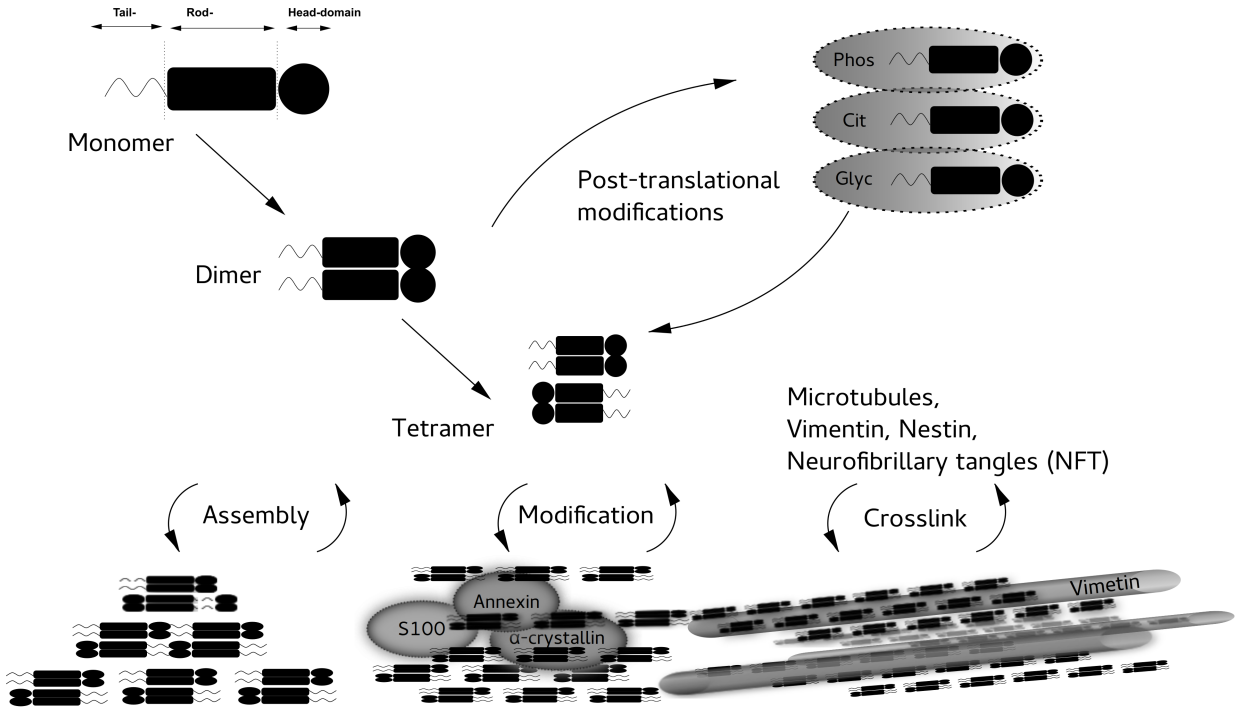
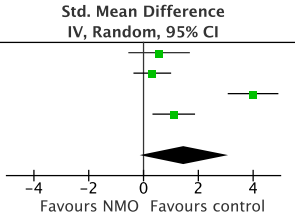



Figure 3: *Intracellular organisation of GFAP. The dynamic and reversible steps between the GFAP monomer and the assembled, functional and cross–linked cytoskeletal network are shown in this simplified sketch. Phos = phosphorylation, Cit = citrullination, Glyc = glycosylation.*

(A)

| Study or Subgroup | NMO Mean | NMO SD | NMO Total | Control Mean | Control SD | Control Total | Weight | Std. Mean Difference IV, Random, 95% CI | Year |
|---|---|---|---|---|---|---|---|---|---|
| Misu 2009 | 7,666 | 15,266.5 | 10 | 0.6 | 0.3 | 5 | 23.9% | 0.57 [-0.53, 1.67] | 2009 |
| Takano 2010 | 2,476.6 | 8,815 | 33 | 0.7 | 0.5 | 12 | 25.8% | 0.32 [-0.34, 0.98] | 2010 |
| Petzold 2011 | 5,028.7 | 5,905.6 | 7 | 4.9 | 23.2 | 129 | 24.9% | 4.00 [3.10, 4.90] | 2011 |
| Uzawa 2013 | 369.3 | 523.7 | 13 | 0.9 | 0.59 | 20 | 25.4% | 1.10 [0.35, 1.86] | 2013 |
| **Total (95% CI)** | | | **63** | | | **166** | **100.0%** | **1.49 [-0.11, 3.10]** | |

Heterogeneity: Tau² = 2.49; Chi² = 44.77, df = 3 (P < 0.00001); I² = 93%
Test for overall effect: Z = 1.82 (P = 0.07)




(B)

| Study or Subgroup | NMO Mean | NMO SD | NMO Total | MS Mean | MS SD | MS Total | Weight | Std. Mean Difference IV, Random, 95% CI | Year |
|---|---|---|---|---|---|---|---|---|---|
| Misu 2009 | 7,666 | 15,266.5 | 10 | 0.7 | 1.5 | 5 | 13.8% | 0.57 [-0.53, 1.67] | 2009 |
| Takano 2010 | 2,476.6 | 8,815 | 33 | 0.8 | 0.4 | 27 | 44.4% | 0.37 [-0.14, 0.89] | 2010 |
| Petzold 2011 | 5,028.7 | 5,905.6 | 7 | 0.69 | 0.84 | 15 | 15.6% | 1.50 [0.47, 2.52] | 2011 |
| Hyun 2014 | 2,078 | 3,805.1 | 17 | 0.5 | 0.6 | 13 | 26.2% | 0.70 [-0.04, 1.45] | 2014 |
| **Total (95% CI)** | | | **67** | | | **60** | **100.0%** | **0.66 [0.23, 1.10]** | |

Heterogeneity: Tau² = 0.04; Chi² = 3.77, df = 3 (P = 0.29); I² = 20%
Test for overall effect: Z = 2.99 (P = 0.003)

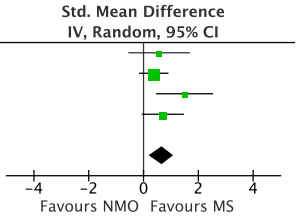



Figure 4: *Meta–analysis of CSF GFAP levels in patients with an acute exacerbation of NMO compared to (A) other neurological disorders (controls) and (B) MS.*

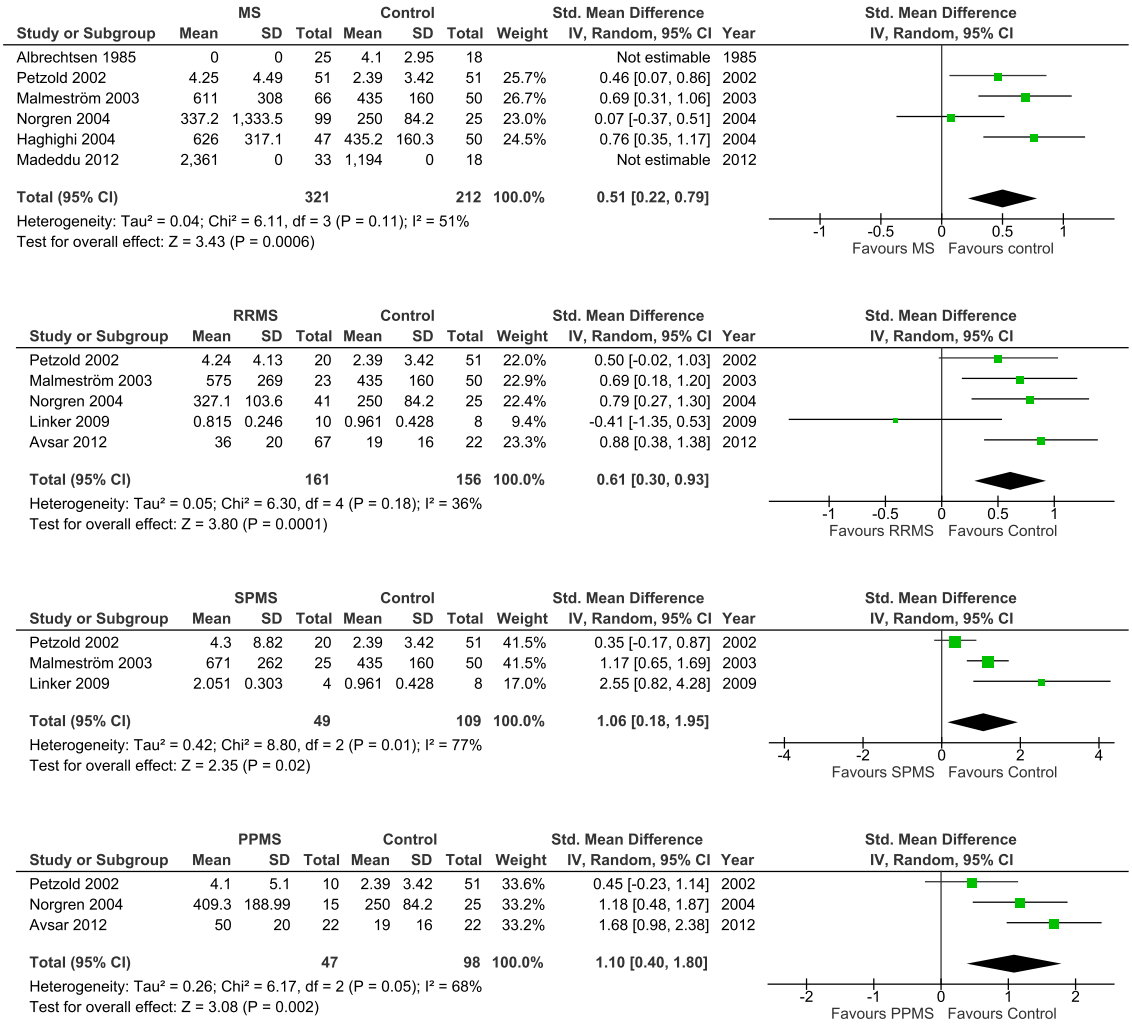



Figure 5: *Meta–analyses of CSF GFAP levels in* ***(A)*** *MS patients (pooled) compared to controls. Clinical subgroup analyses are also presented for* ***(B)*** *RRMS,* ***(C)*** *SPMS,* ***(C)*** *PPMS compared to controls.*

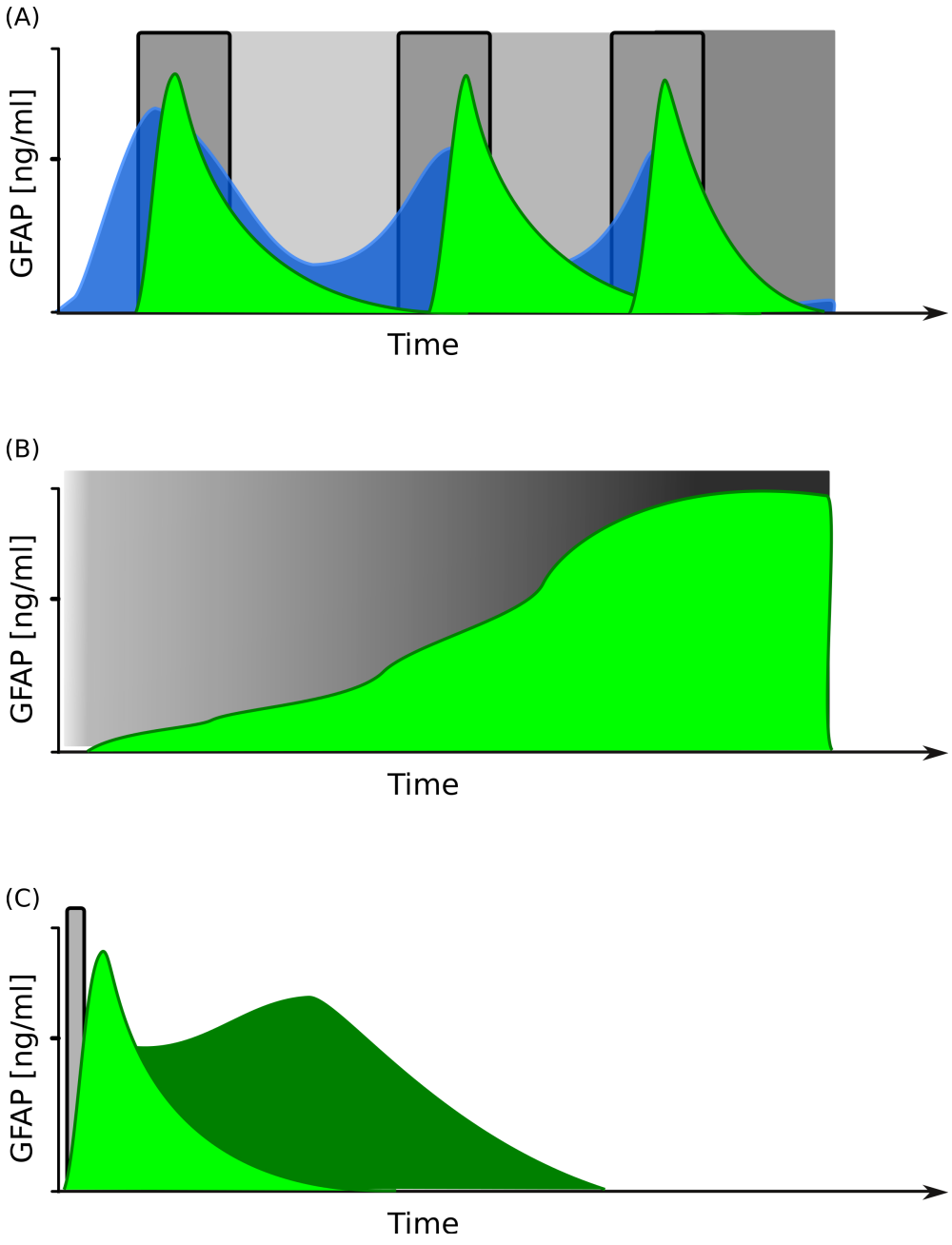



Figure 6: ***The three dominant GFAP body fluid biomarker signatures.*** *Isolated clinical events in a patient are indicated by grey shaded boxes. Severity and speed of irreversible disease progression is indicated by a grey shaded area with darker shades of grey signalling more severe disease. The relative quantity of GFAP body fluid levels in ng/mL is indicated on the y-axis and time on the x–axis (both axes not drawn to scale). The green shaded curves indicate the idealised pattern of GFAP levels over time as derived from the literature review.* ***(A)*** *The emerging entities of autoimmune astrocytopathies such as an acute episode of neuromyelitis optica (NMO) are characterised by relapsing clinical episodes during which disability accumulates. Each episode is associated with a significant titre of auto–antibodies directed at astrocytes (blue curve) such as anti-AQP4. Destruction of astrocytes leads to high GFAP levels in the acute phase. In between relapses GFAP levels are normal.* ***(B)*** *Toxic gain of function due to mutations in the GFAP gene such as Alexander disease cause severe and rapid disease progression. A cumulative increase of GFAP levels is hypothesised which may be masked by a "hook effect".* ***(C)*** *In acute neurocritical care conditions such as an intracranial haemorrhage (ICH), a brief clinical episode such as a stroke is followed by a hyper acute release of GFAP levels. Typically these normalise within the physiological wash–out time of 7–10 days. In some cases complications of the disease course such as hydrocephalus, vasospasm or infections may cause a secondary rise of GFAP levels (dark green shaded curve).*