

Astrogliosis instigated signature in neurodegenerative diseases: Supremacy of GFAP in forecasting structural pathology and disease progression in Alzheimer's disease

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Abstract

In Alzheimer Disease, accumulation of A β and tau aggregates in the vicinity of neuro-synaptic junction is bound to stir up a potpourri of compensatory glial modifications that tend to backpedal the neurotoxic damage and revitalize the physiological equilibrium. In this regard, astrogliosis is a protective mechanism that serves to counterbalance these neuronal mutilations during various stages of pathogenesis in AD. A spin-off of astrogliosis is exuberant production of markers such as GFAP. GFAP is a 50kDa cytoskeletal protein comprising of head domain, rod domain and tail domain. The sovereign function of GFAP is to promote neuronal growth, synaptic plasticity, and myelination. Its cardinal isoform is GFAP- α but it can exist in different isoforms based on alternative splicing of parent mRNA. Throughout physiological conditions, GFAP absconds from the brain by fleeing through para-vascular route and glymphatic pathways to enter the systemic circulation. Unfortunately, CSF GFAP does not live up to the expectations as reliable marker for broadcasting subtle cellular and structural changes in AD. On the contrary, serum GFAP championed itself as an acclaimed marker that can consistently omen accumulation of structural changes, stockpiling of A β 42/40 and tau aggregates in PET scan, cognitive impairment, and dementia in pre-clinical and clinical AD patients. This review contributes to bridge the current knowledge gap in physiology, excretory pathways, and clinical significance of plasma GFAP as a glorious biomarker in AD. Hopefully, we propound further research studies for cementing its position as a high-powered marker for auguring disease initiation, evolution, progression in AD.

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Introduction

Blood and CSF biomarkers that were previously used to monitor the disease progression in the preclinical and clinical AD include tau, phospho-tau (p-tau), and A β 42 (1-3). Since levels of tau and A β aggregates fluctuate as the disease progresses, understanding the relationship between the stage of disease and their levels is crucial is interpreting their relative significance to disease pathogenesis and clinical progression in AD. Accordingly, upregulation of p-tau and downregulation of A β in the blood/CSF was previously used as a reliable indicator for neurodegeneration in symptomatic AD patients (4). With synaptic loss occurring in parallel to neuronal

damage, markers such as neurogranin and neurofilament (NFL) in CSF and blood were used to predict axonal instability and synaptic transmission disturbances in preclinical and clinical AD(5, 6). Visinin-like protein 1 (VILIP-1) which is a neuronal calcium sensor is also demonstrated to be in higher levels in the blood/CSF of AD patients as compared to control patients(7). Furthermore, measuring presynaptic and postsynaptic proteins such as synaptosome-associated protein 25 (SNAP-25), and synaptotagmin in the blood/CSF yielded marginal success in estimating the neurodegeneration in the AD patients as compared to control patients (8, 9). Predicting neuroinflammation in AD can be successfully accomplished by assessing blood biomarkers such as MCP-1 and sTREM2

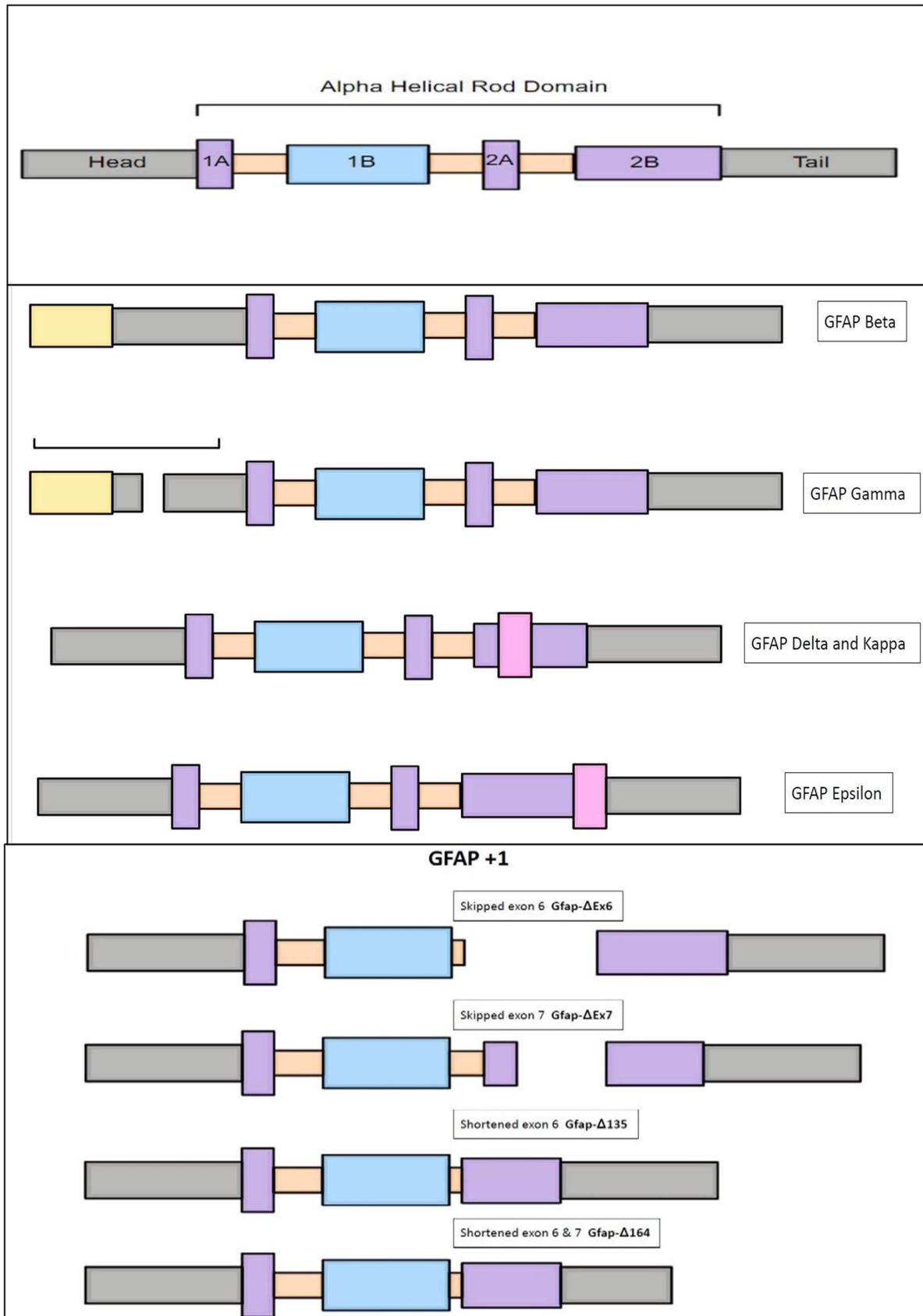


Figure 1. Structure of principal isoform of GFAP and alternate isoforms: The structure of principal isoform GFAP- α is composed of head, rod, and tail domain. GFAP-α, GFAP-β, GFAP-γ, GFAP-δ, GFAP-κ, and GFAP-ζ are the different isoforms that were widely expressed in the cortex, striatum, and cerebellum. These isoforms are derived by alternative splicing of GFAP- α. The pathological relevance of these uncommon GFAP isoforms in the context of AD disease pathogenesis is open to question and needs further investigation. But some of these isoforms were implicated in the pathogenesis of neurological diseases including Alexandria disease, astrocytoma, and glioblastoma.

(10). Flotillin, which is an exosome marker was previously characterized for using it as a blood biomarker for gauging disease changes in AD(7). Previous reports established the fact that accumulation of A β aggregates in the brain tissue results in attenuated exosome secretion resulting in decreased serum flotillin levels (11, 12).

Positive emission tomography (PET) is an imaging modality that can utilize radioactive tracers for delivering crucial information regarding the shifts in the biochemical and metabolic activity of the brain tissues. PET has been used previously to ascertain and study A β aggregates, senile plaques, tau astrocytes, microglia, and synapses in AD with modest success(13-16). In this regard, the most studied PET tracer that targets MAO-B (mono amino oxidase-B) expressed in astrocytes for is 11C-deutrium-L-deprenyl (11C-DED)(17, 18). Tracers that are currently in the development for studying the subtle alterations in the levels of GFAP, tau and A β in the postmortem AD and control brain sections include 3H-PIB, 3H-THK5117 and 3H-florbetaben respectively(19-21).

GFAP: a future dependable marker for assessing disease-related brain tissue change in AD.

Any changes in the GFAP expression are deemed to suggest a disruption in the functional dynamics of astrocyte cytoskeleton, morphology, and their trophic protective function on neurons(22). This indirectly indicates that the onset of astrocytic response secondary to accumulation of A β and tau aggregates in AD(22). Indeed, astrocytes are deemed to be considered as a first line of brain immune cellular defenses that are discharged upon appearance of A β aggregates in the neuronal tissues. Upon encountering the neuronal damage, young astrocytes tend to migrate near injured site and curtails further A β induced neuronal damage by their inherent secretory, phagocytic and trophic functions (21). As the inflammatory assault continues these young clusters of astrocytic populations will ultimately give up, but is slowly followed by a second wave of aged and resilient astrocytes with very high GFAP positivity thus increasing the chances of glial scar formation at the sites of neuronal injury(21). In parallel with these findings, a meta-analysis that examined the postmortem brain tissues concluded that there is a positive correlation between GFAP immunoreactivity and astrocytic response in AD(22). According to a three-center cross-sectional study conducted in Canada, plasma GFAP was a sensitive and reliable than CSF GFAP for assessing reactive astrogliosis and A β pathology in the early stages of AD (23). These findings are against the common logic where one would expect the CSF GFAP to better mirror unfolding neuronal damage in its vicinity rather than plasma GFAP which has crossed the blood-brain barrier into the systemic circulation. Although plasma GFAP test is FDA-authorized for characterizing intracranial damage in traumatic brain injury (TBI), their potential usage in neurodegenerative and neuroinflammatory diseases warrants further validation. Studies examining the levels of GFAP in the CSF and blood

conducted so far revealed encouraging albeit mixed and enigmatic results. This sets the precedence for reviewing the current literature on GFAP for its untapped potential as a CSF and blood biomarker in predicting the onset and progression of neurodegeneration in AD. Grasping, comprehending, and analyzing the current literature not only sets the stage for developing future basic science and clinical research studies for developing a reliable marker for diagnosing preclinical AD but also enables its potential usage as non-invasive marker for monitoring treatment in parallel with PET scan imaging modalities. A knowledge gap in understanding the relative importance of GFAP can be addressed a review that enlightens regarding its physiological structure, function, and current clinical studies involving GFAP in AD diagnosis and treatment monitoring

Given what has been said, this review was drafted to discuss systematically various topics ranging from GFAP structure, function, GFAP splice variants, physiological excretory pathways, post-translational modifications, importance of CSF and blood GFAP in relation to amyloid plaque deposition in AD. Another important aspect that needs further discussion is its head-to-head comparison with A β and phospho-tau levels to explore its sensitivity and specificity with and without these markers for predicting hippocampus atrophy in AD patients. Furthermore, it also be prudent to discuss whether GFAP monitoring needs to be supplanted with PET scans for earlier detection and risk stratification of AD. Taken together, the main purpose of this review is to update the current knowledge regarding GFAP so that it can kick-start future research studies to cement its position as one of the important and stable biomarker for early diagnosis as well as treatment monitoring in AD.

GFAP isoforms and their importance

There are several isoforms of GFAP reported to be expressed in the different brain regions. The underlying mechanism for generation of these different isoforms is through alternative splicing of GFAP mRNA(24). GFAP- α , GFAP- β , GFAP- γ , GFAP- δ , GFAP- κ , and GFAP- ζ are the different isoforms that were widely expressed in the cortex, striatum, and cerebellum(25, 26) (Fig 1). In AD mice, GFAP- α was most highly expressed isoform whereas other isoforms (GFAP- γ , GFAP- δ , GFAP- κ , and GFAP- ζ) were shown to have a variable expression with some reports establishing increased expression as compared to wild-type mice(25). Interestingly some prior studies show that, astrocytes which are positioned in proximity with amyloid plaques were stained positive for less common isoforms namely GFAP- α and GFAP- δ (25). The pathological relevance of these uncommon GFAP isoforms in the context of AD disease pathogenesis is open to question and needs further investigation.

Expression of some of these GFAP isoforms in the astrocytes leads to deleterious consequences due to their effect on filament assembly, function and cellular morphology. Expression of GFAP isoforms (GFAP- γ , GFAP- δ , GFAP- κ) in the astrocytes of AxD transgenic mice resulted in a spectrum of abnormalities ranging from attenuated filament solubility,

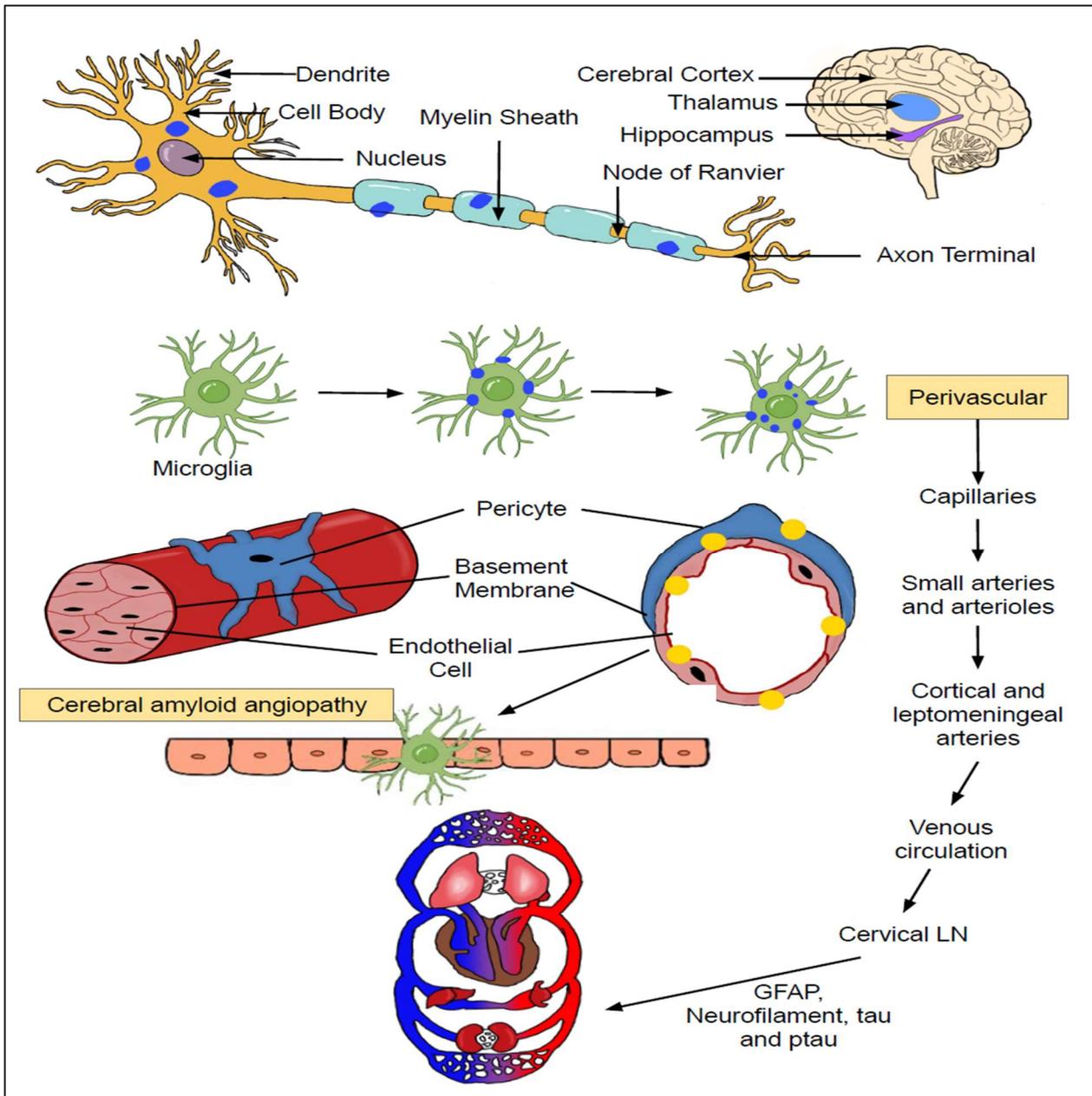


Figure 2. The pathological basis for emigration of GFAP and other toxic aggregates from brain circulation into blood: Amyloid deposits accumulating on the neuronal cells is primarily engulfed by scavenging microglial cells. These microglial cells then regurgitate these toxic aggregates upon the vascular endothelium of micro-vasculature thus triggering the onset of vasculitis and blood-brain barrier (BBB) dysfunction. The escape of GFAP from the brain circulation into the systemic circulation is dependent upon the presence of BBB dysfunction as well as its passage through the glymphatic and vascular pathways. The presence of an obstruction in these excretory clearance pathways can impede the efficient migration of GFAP in the systemic circulation, thus preventing its ability to be used as a biomarker for mirroring disease changes in AD.

increased formation of cytoplasmic aggregates, derailed endogenous filament network, disordered astrocyte morphology, altered glutamate processing to disrupted trophic effect on the neighboring neurons(27). These findings exemplify the pathogenic influence of these GFAP isoforms in the pathogenesis of neurodegenerative diseases including AxD and AD.

GFAP-α:

Most of the literature published so far regarding the physiological and pathological functions of GFAP in the brain

tissues is related to this predominant as well as principal isoform(28). This isoform has all the 9 canonical exons of a prototype GFAP gene(25, 28). Its protein length is approximately 432 amino acids (aa)(22, 28). In response to the neuronal injury, astrocytes respond with reactive gliosis which is characterized by morphological changes as well as increased expression of this main isoform(25). In sub-cellular mRNA localization studies, it was evident that most of the GFAP-α was localized to the astrocyte protrusions as compared to Gfapδ which can be attributed to subtle differences in 3'-exon sequences in their respective

mRNA(24). Although this predominant isoform is responsible for supporting most of the astrocyte functions, any alterations in its physiological levels can trigger deleterious consequences. Astrocytes isolated from transgenic mice overexpressing GFAP- α displayed a wide spectrum of abnormalities ranging from cytoplasmic inclusions, derailed filament network, attenuated proliferation, cell death to dysfunctional proteasomal system(29). High malignant grade (IV) astrocytoma is known to express low levels of GFAP- α and thus has higher GFAP δ / α ratio as compared low grade astrocytoma (II)(30). This change in GFAP isoform ratio might be detrimental as it can make astrocytes more motile, adhesive and promote their ability to invade the neighboring tissues.

This functional change in astrocytes might have some relevance to the behavior of astrocytomas. In this regard, gliomas having higher GFAP δ / α ratio were more invasive due to their increased cell adhesion and motility properties of astrocytes(31).

Forced overexpression of GFAP- α in the glioma cells lines resulted in decreased cell motility whereas its downregulation restored their motility and invasiveness(32). These findings underscore the importance of the GFAP δ / α ratio in controlling the expression of genes associated with cell proliferation, cell-ECM interactions, and protein phosphorylation in astrocytoma(30, 31). Using ex vivo brain slice model, it was shown that astrocytes with higher GFAP δ / α ratio displayed persistent invasion whereas those with lower GFAP δ / α ratio had disorganized migration(33). Previous studies demonstrated that GFAP δ / α ratio regulates physiological properties of astrocytes including cellular morphology, mitotic cycle extracellular matrix production, adhesion, 2D migration, phosphorylation, and proliferation(34). In a study a Moeton, M. et al, silencing of GFAP- α and increasing the GFAP δ / α ratio resulted in decreased motility via downregulation of plectin, increased ECM laminin and alteration of integrins(35). Therefore, it can be hypothesized that alteration of GFAP mRNA splicing and shifting the GFAP δ / α ratio in astrocytoma can be beneficial as it has the potential to impede the progression to highly malignant grade tumor, attenuate invasiveness, afford better clinical outcomes and prolong patient survival rates(30).

GFAP- δ :

GFAP- δ is distinguished from GFAP- α by the presence of an intron 7-8 sequences(25). Structurally, the exons 8 and 9 of GFAP- α isoform are replaced by exon 7a resulting in the generation of a new C-terminal tail domain(36). Due to incorporation of this C-terminal tail domain, it loses its ability to self-assemble into the intermediate filaments(37). As compared to GFAP- α , association and dissociation of GFAP- δ from the IF networks is also reported to be sluggish(38). This delayed filament dynamics can be explained due to the presence of coil-2B binding site in the C-terminal tail domain in contrast to GFAP- α (38). Nevertheless, GFAP- δ can still self-assemble in the physiological conditions when its threshold concentration is not breached (10%-15% of total

GFAP concentration)(37). Incorporation of GFAP- δ into assembly compromised GFAP constructs (AxD GFAP mutant) results in deleterious effects such as increased protein aggregation, derailment of IF networks and increased association with α B-crystallin (protein chaperone) and p-JNK(37, 39). This disruption of IF networks results in alteration of astrocyte morphology with increased appearance of round cells with extended focal adhesions due to the shift in integrin and ECM interactions(38).

Overexpression of GFAP- δ tends to alter the cytoskeleton and filament assembly properties in a manner where there is a tendency towards intermediate filament collapse(37, 40). These findings highlight the fact that changes due to the incorporation of GFAP- δ might be due to the modification of filament-filament as well as filament-cytoplasmic protein interactions(37).

Astrocytes localized to the subpial zone of the cerebral cortex, spinal cord, olfactory bulb, human rostral migratory pathway, sub-granular zone of the hippocampus and along the ependymal layer of the cerebral ventricles express this GFAP- δ isoform(37, 40, 41). GFAP- δ positive astrocytes are found most abundantly in the sub-ventricular zone (SVZ) or along lateral ventricles, where they function as multipotent neuronal stem cells(40, 42, 43). Although both GFAP- δ and GFAP- α were documented to be upregulated in the AD lesions, GFAP- δ upregulation only transpires via alternative splicing from GFAP- α isoform in the likely setting of enhanced GFAP gene expression(40, 44). Other than AD, its expression is documented in pathologies such as astrocytoma, vanishing white matter syndrome, and stroke(39, 45, 46).

According to Heo, H.D. et al, the intensity of GFAP- δ staining is directly proportional to the grade of spinal cord astrocytoma and thus it has the potential to become a reliable marker for monitoring the histological grade, tumor behavior and invasiveness(45).

Higher GFAP δ / α ratio leads to poor prognosis in gliomas as it promotes increased astrocyte-ECM interactions via DUSP4 (Dual specificity phosphatase 4)(31). DUSP4 which regulates MAPK pathway has a role in a variety of biological processes including migration, invasion, proliferation, ECM degradation and chemotherapy-induced cytotoxicity(36). Alternatively, higher GFAP δ / α ratio leads to increased invasiveness in the malignancies through laminin mediated upregulation of phospholipase-D, phosphatidic acid, and matrix metalloproteinase-2 (MMP-2)(35, 36, 47, 48). MMP2 which is a type IV collagenase is utilized by glioma cells to invade across ECM and thus its overexpression increased the likelihood of metastasis and poor survival in the gliomas(36, 47, 49).

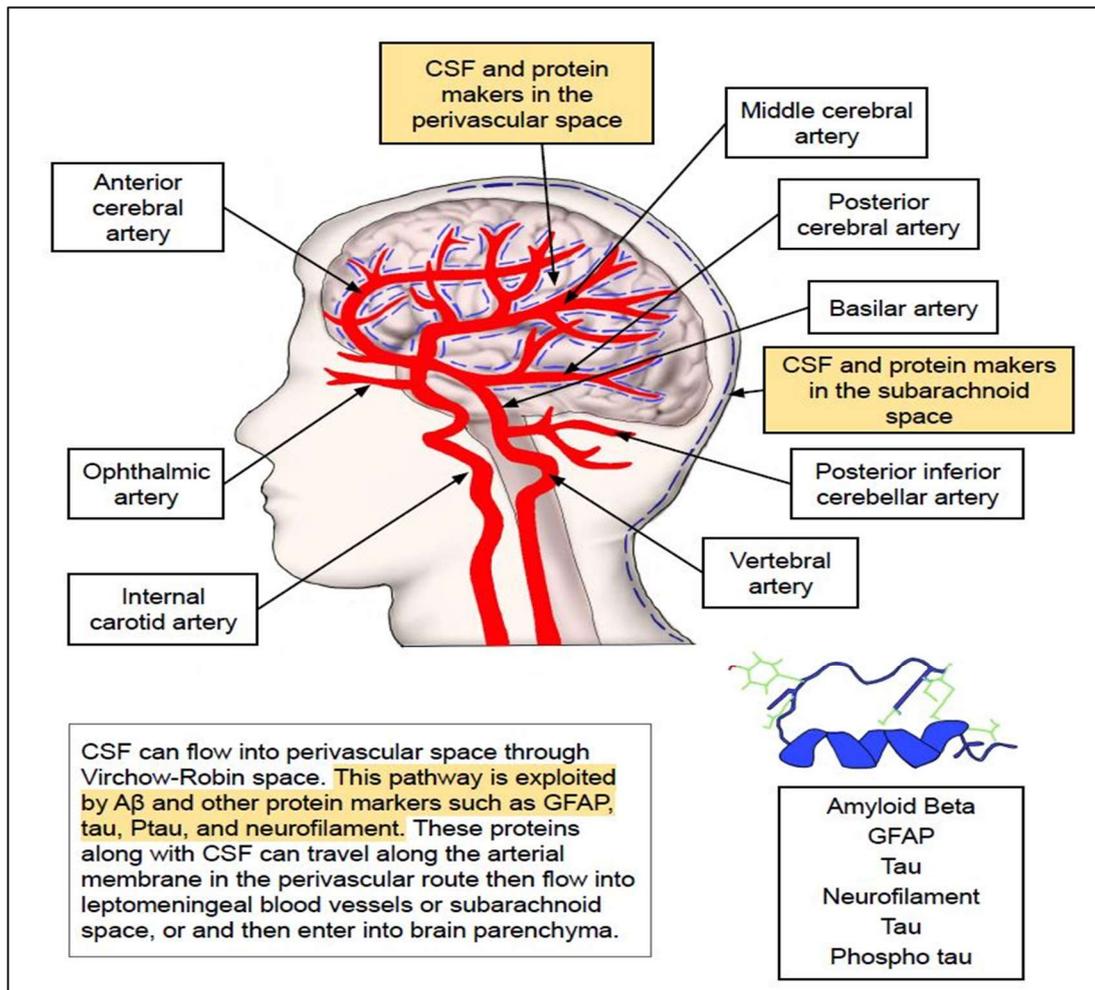


Figure 3. Arterial phase of GFAP excretory pathway. The peri-vascular excretory pathways of GFAP are powered by peri-arterial pulsations, sleep and respiration. This pathway is briefly classified into arterial phase, capillary phase, and venous phase. In the arterial phase, accumulated solutes and GFAP floating in the extracellular space will eventually travel closely along the endothelial basement membrane and vascular smooth muscle of arterial vasculature. The aquaporin channel (AQP4) which is present on the astrocytic end feet and very closely juxtaposed to the vascular endothelial basement membrane. This AQP4 channel will be responsible for filtering and secretion of these solutes including GFAP from the peri-arterial space into the brain interstitial fluid by peri-arterial efflux.

GFAP+1

This isoform comprises of four variants namely GFAP Δ Ex6 (exon-6 of GFAP- α deleted), GFAP Δ Ex7 (exon-7 of GFAP- α deleted), GFAP Δ 164 (abbreviated exon-6&7 of GFAP- α deleted along with absent Coil 2B), and GFAP Δ 135 (abbreviated exon-6 of GFAP- α deleted along with absent Coil 2B)(22, 25, 26, 28). GFAP+1 positive astrocytes were identified with purified GFAP+1 positive antibody in the sub-ventricular region, hippocampus, striatum, spinal cord of the elderly non-demented controls, AD and Parkinsonism (PD) patients(44, 50). Recent reports were successful in identifying these out of frame splice variants in the pyramidal neurons of hippocampus in the AD and down syndrome patients(51). The researchers in study speculate that the appearance of these splice variants can be reasoned by occurrence of increased GFAP gene stimulation along with impairment of proteasome degradation(51). A previous study correlated the presence of GFAP+1 positive astrocytes in the hippocampus to the

accumulation of A β 1–42 oligomers and fibrils in the AD patients(44). Alternative mechanisms hypothesized for the appearance of these isoforms include somatic mutations, genetic modification, DNA mutations and stable epigenetic alterations with increasing age (44, 52). GFAP+1 was also highly expressed in the focal brain lesions of chronic epilepsy associated with astrogliosis (53). The immunostaining pattern of GFAP+1 positive astrocytes appear to extend 1-5 long cellular processes (1 mm) that can be traced to nearby blood vessels or neutrophils in contrast to GFAP δ positive astrocytes which appear as stained cellular bodies with predominant peri-nuclear pattern (28, 40, 50, 53, 54). Another distinguishing feature of GFAP+1 positive astrocytes is the absence of evenly positioned varicosities on the long cellular processes emanating from these astrocytes (55). Transfection of GFAP+1 isoform into the SW-13cl.2 cells which lack the endogenous expression of intermediate filament proteins resulted in the collapse of the intermediate filament network along with altered filament-filament interactions thereby

underscoring its pathological role in hampering normal filament-filament coordination (44). Furthermore, the predominance of this isoform over canonical GFAP α in the astrocytes accelerates the wreckage of IF network leading to disfigured astrocyte morphology(44).The impact of GFAP+1 isoform on the filament assembly, cellular morphology and physiological function needs further clarification and research in the near future.

GFAP γ

This GFAP isoform is formed by deleting exon 1 and replacing it with intron 1-2(25, 56). In mice, it is expressed in the astrocytes, bone marrow and spleen whereas in humans it is only detected in the brain(56). Due to lack of amino-terminal head domain, it might not be able to function as intermediate filament (56). The significance of its expression and its primary physiological function in the astrocytes needs further validation.

GFAP κ

This isoform can be differentiated from GFAP- α by the presence of exon 7b formed by a linking of sequences including exon 7, intron 7a and exon 7a(57). It is produced by combination of events namely alternative splicing and polyadenylation(57). Its expression is documented in the adult human frontal cortex, human glioblastoma tumors and human astrocytoma cell lines (U343)(57, 58). GFAP κ is less capable to form homomeric filaments and this compromised filament assembly can be attributed due to the absence of RDF motifs in the C-terminal tail and positive charge of the tail domains(59, 60). In fact, co-expression of GFAP κ and GFAP α resulted in the formation of an unstable IF network and protein aggregates(57). Previous reports indicate that GFAP κ /GFAP ϵ ratio is a marker for glial cell differentiation and any increase in this ratio signifies the aggressiveness of glioblastoma tumors(57). This can be reasoned by the hypothesis that GFAP κ fine-tunes the IF filament assembly and revamps the shape in a manner that makes the tumor cells more motile, adhesive and invasive. These changes might increase the chances of developing aggressive tumors, and resultant worse prognosis and poor clinical outcomes(57). Regardless, the influence of this isoform on the IF assembly, network and associated cellular functions in the physiological and pathological states needs further attention. Furthermore, it would also be worthwhile to investigate the impact of this isoform on the disease progression as well as neurodegeneration in AD.

GFAP β

GFAP β is identified by the presence of secondary upstream transcription site at the classical 5' UTR region normally present in the canonical GFAP α isoform(25, 61). Northern blot analysis confirmed the expression of this isoform in the primary astroglial cultures(61, 62). It is also isolated from rat schwannoma cell lines, rat cerebral cortex, hamster brain and human brain(62). Neurotoxic brain injury in the rat hippocampus was documented to stimulate the expression of both isoforms GFAP α & GFAP β within the astrocytes(61).

These findings underscore the existence of analogous post-transcriptional mechanisms in place for modulating the expression of these isoforms in response to neural injury(61). Treatment of rat astrocyte cultures with 25U of Interferon (IFN) γ resulted in increase in the GFAP β levels along with small decrease in the total GFAP levels(62). Under normal conditions, this isoform accounts for at least 5-10% of total GFAP(62). In rat brain slice cultures Treatment of rat brain cultures with 25U of IFN- γ did not have any effect on the expression of GFAP β levels while decreasing the total GFAP levels by 10-fold, thereby increasing its relative concentration to 50%(62). According to Benveniste et al, actions of IFN- γ can be explained by stimulation of protein kinase C (PKC) in the astrocytes of rat brain slice cultures(63). This relative upregulation of this isoform with IFN- γ might represent a compensatory response for activating different set of astrocyte populations to defend against the inflammatory stimulus(62). Further studies should be focused on understanding the transcriptional regulation of this GFAP β isoform in physiological and pathological conditions.

GFAP ζ

This isoform is characterized by the presence of 284 bp intron 8-9 interspersed within the GFAP gene(25). Its physiological and pathological role in the astrocytes and brain tissues is currently unknown. The importance of this isoform and its relative effect on the filament assembly, dynamics and functional aspects needs further elucidation.

Mechanisms proposed for dribbling of GFAP from cerebrospinal fluid into the systemic circulation:

Astrocytes are the primary glial cells that respond to TBI or neuronal injury secondary to accumulation of toxic A β and tau plaques (64-66). Reactive astrogliosis is a term highlighting the morphological changes in the astrocytes for mounting a robust immune response to contain and thwart any neurological damage secondary to inflammatory or toxic stimulus (67). Reactive astrogliosis can encompass a mosaic of changes ranging from alteration of gene expression, change in the morphology, and cytoskeleton hypertrophy, to upregulation of GFAP(67). It is important to understand that the degree of GFAP upregulation is proportional to the degree of CNS tissue damage in disorders such as neurodegenerative diseases, inflammation, infection, stroke and autoimmune diseases(67).

Unfortunately, astrocyte damage and cell death can be a by-product of moderate to severe astrogliosis, and this will result in spilling of GFAP from astrocytes into the brain interstitial fluid (ISF) and cerebrospinal fluid (CSF)(67, 68). These upregulated levels of GFAP in the brain interstitial fluids can be a reliable signature that can signify the severity of reactive astrogliosis but it might not show a direct relationship with the severity of neurodegeneration in dementia patients(69). Once GFAP enters the CSF, it circulates and courses along the vascular channels and eventually decamps from the brain into the systemic circulation secondary to structural abnormalities or due to efficient excretory systems in place.

GFAP circulates in the ISF, and CSF makes it way towards the systemic circulation either due to BBB dysfunction or via seeping through brain's glymphatic system or meningeal lymphatic vessels(70-74). In the following sub-sections, each of these excretory pathways utilized for GFAP will be discussed in detail.

Blood-brain barrier disruption:

Traumatic brain injury can disrupt the BBB and allow the seepage of albumin, fibrinogen, and thrombin from the systemic circulation into the brain(70). These factors will ultimately stimulate astrocytes and microglia adjoining the vascular endothelium of BBB leading to secretion of reactive oxygen species (ROS), Nitric oxide (NO), tumor necrosis factor-alpha (TNF- α), IL-1 β , IL-6, IL-12, glutamate, chemokines, and MMPs(70). These factors will cause apoptosis of the vascular endothelial cells and derail the integrity of BBB(70). This will allow more inflammatory cells seepage into the brain to further exacerbate the BBB damage already initiated as well as instigate post-traumatic neuroinflammation(70). In AD, a different mechanism of BBB disruption has been proposed. Accumulation of toxic A β aggregates seems to trigger activation of vascular endothelium. Energized vascular endothelium then upregulates adhesion molecules like ICAM-1 (Intracellular adhesion molecule-1) & VCAM-1 (Vascular cell adhesion molecule-1) and chemo-attractants which then sparks the immigration of neutrophils from the systemic circulation into the brain(75, 76). These drifted neutrophils will be activated by surrounding A β & senile plaques thereby acquiring a virulent phenotype(77). These stimulated neutrophils will secrete a spectrum of toxic factors including ROS, cytokines, chemokines, and neutrophils extracellular traps (NETs)(76, 78). These pro-inflammatory agents are alleged to trigger BBB dysfunction, chronic inflammation, reciprocal microglial activation, neuronal degeneration, and synaptic dysfunction(76). Furthermore, oligomeric A β accumulated within the neurons and synapses will be ingested and shuttled by neighboring microglial cells to the endothelium of microvasculature thereby instigating the onset of cerebral amyloid angiopathy (CAA)(79)(Fig 2). Initiation of CAA on the cerebral microvasculature by deposition of amyloid plaques will cause the breakdown of BBB and the subsequent structural abnormalities would spark the leakage of GFAP from brain interstitial fluids into the systemic circulation.

During CAA, A β accumulation starts initially on the basement membrane and progresses gradually inwards to replace the entire arterial wall (Tunica interna, Tunica media and Tunica externa)(80)(Fig 2). Studies demonstrate that this occurrence of CAA varies among different brain regions with increasing age and AD(81). In mouse models of AD, hippocampus, thalamus, and cerebral cortex exhibit higher levels of CAA whereas striatum is least effected in terms of vessel wall changes(81). This CAA can itself contribute to BBB dysfunction via decreased expression & mis-localization of tight junction proteins (Claudin-5 & Zonula occluden-2), and

increased expression of MMPs(82-85). Furthermore, it has been documented that amyloid beta is directly translocated across the BBB utilizing receptors LDL receptor-related protein-1 (LRP-1) and alpha (2)-macroglobulin(86). ATP binding cassette (ABC) transporters such as ABCB1 (also known as P-glycoprotein 1 or MDR1) is capable to transporting A β across the BBB into the systemic circulation(87). Internalization and elimination of A β across the brain capillary endothelial cells at BBB is enforced by insulin and human natriuretic peptide (hANP) via engagement of insulin degrading enzyme (IDE)(88). It is quite possible these receptors in BBB will be damaged and dysfunctional in AD patients due to continuous onslaught by these toxic inflammatory mediators secondary to profuse accumulation of A β aggregates within the brain tissues(86, 89-91).

Perivascular route and Glymphatic clearance pathways:

Markers such as GFAP, tau, synuclein, neurofilament and amyloid beta can passively slide through along the basement membrane of the smooth muscle of microvasculature in a peri-vascular route and drain into the cervical lymph nodes (LNs)(81, 82, 89, 92, 93). This peri-vascular route is energized by the low frequency peri-arterial pulsations and spontaneous vasomotion which was recently demonstrated with in-vivo two-photon microscopy by van Veluw SJ et al(94, 95). Additionally, respiration and CSF pressure gradients were known to play atleast a partial role in rapid inter-exchange of fluids between CSF and ISF(96, 97). Other physiological factors that are hypothesized to play a significant role in the operation of perivascular and glymphatic clearance pathways include APOE* ϵ 4, arterial age, molecular size /weight of solutes, AQP4 expression and sleep(98).

It has been shown that sleep directly accounted for 40% clearance of A β aggregates via the glymphatic clearance pathway during physiological conditions(98). This is because there is 60% increase in the interstitial space during sleep resulting in the exuberant exchange between CSF-ISF compartments promoting the A β clearance via lymphatic pathways(98, 99). Continuous cardiac output is essential for peri-vascular drainage of soluble solutes and fluid without any cells via capillary and arterial basement membrane which function as surrogate lymphatics of the brain(100).

Broadly, this glymphatic pathway can be divided into five phases namely peri-arterial influx, peri-arterial efflux, CSF-ISF exchange in the brain parenchyma, peri-venous influx, and peri-venous efflux(101-103)(Fig 3-5).

In this pathway, accumulated extracellular solutes in the brain that need to be cleared from CSF circulation initially enter an arterial peri-vascular space where they travel closely abutting along the endothelial basement membrane and vascular smooth muscle (peri-arterial influx) (Fig 3) (82, 96-98, 101). Preston et al, argued that there is a slightly modified perivascular drainage pathway whether solutes from the extracellular surface of the brain initially move into the pericapillary spaces and later into the peri-arterial space of cortical and leptomeningeal arteries(89). From peri-arterial space,

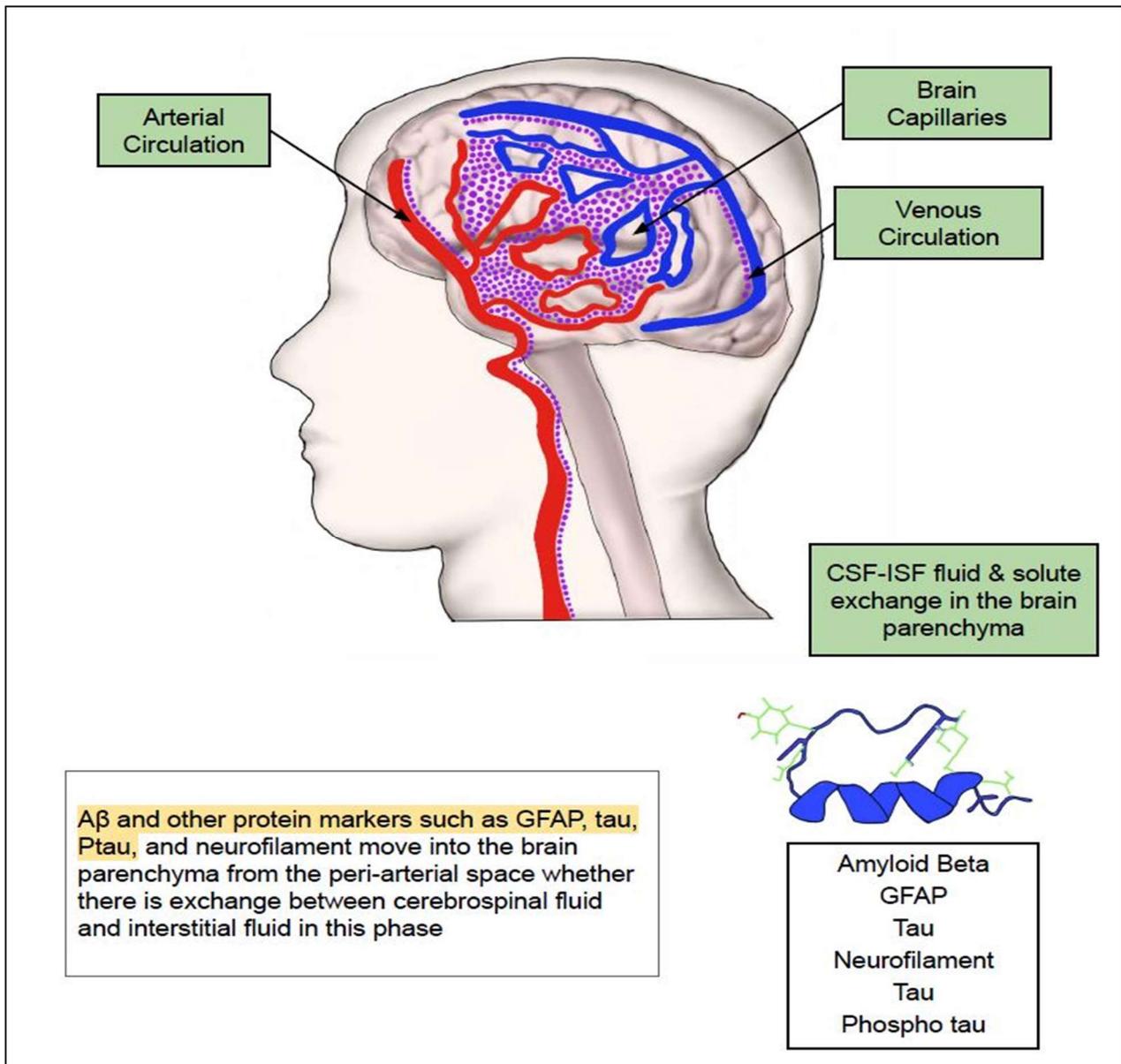


Figure 4. Capillary phase of GFAP excretory pathway. In the capillary phase, the solutes secreted will traverse through the capillary network of the arterial and venous vessels. In this phase, there will be inter-mixture of solutes secreted from the cerebrospinal fluid (CSF) and brain interstitial fluid and they travel across the brain parenchyma. This phase precedes the onset of venous phase from where these solutes will be finally making their way into the systemic circulation by squeezing through the lymphatic vessels and lymph nodes.

these solutes are then secreted by AQP4 (Aquaporin-4) channel present on the astrocytic end feet which is closely juxtaposed to the arterial endothelial basement membrane into the ISF (peri-arterial efflux) (82, 96-98, 101). Downregulation of astrocyte water transport by blocking the expression of AQP4 resulted in 70% reduction in CSF influx into the brain interstitial space and 40% attenuated interstitial solute drainage via the glymphatic system(101, 104, 105). Next, these secreted solutes move across the brain parenchyma where is an intermixture of solutes from CSF & ISF by convection (Fig 4). In the subsequent phase, they enter the perivascular space of venous circulation closely bordering the endothelial basement membrane (peri-venous influx) (82, 96-98) (Fig 5).

By intrathecal administration of contrast agents, researchers were able to visualize the peri-arterial CSF influx and CSF-ISF fluid changes in the extracellular space of the rat brain with the help of dynamic contrast-enhanced MRI(106). By sliding across venous circulation these solutes are eventually transported to the cervical LNs by (peri-venous efflux). Once they enter the lymphatic circulation, these are emptied into the right lymphatic duct and thoracic duct. From the lymphatics, these solutes are drained into the sub-clavian vein and finally into the systemic arterial circulation.

Implications for impaired peri-vascular drainage:

Any impedance of this peri-vascular drainage is detrimental as it facilitates the accumulation of these toxic aggregates within the brain parenchyma thus raising the chances of accelerated

neuronal death as well as neuroinflammation in neurodegenerative diseases (Fig 2). Mice overexpressed with apolipoprotein E (APOE) ϵ 4 allele developed profuse accumulation of A β (40) in the hippocampus secondary to blockage of peri-arterial drainage pathways via collagen deposition in vascular endothelial basement membrane as compared to wild-type mice(107). According to Nimmo et al, age-related decline in the peri-arterial drainage pathways can be the underlying mechanism for the manifestation of various synucleinopathies and tauopathies(108). Research studies indicate that the most important factor governing the efficacy of peri-vascular drainage in aged mice is the presence of CAA(95, 109). The degree of peri-vascular drainage varies across different brain regions, and this can be partly explained by disparities in the intensity of CAA present in their respective regional vasculature(110). Failure of peri-vascular drainage due to CAA can ultimately result in the rupture of the A β laden arteries (Intra-cerebral hemorrhage), deposition of A β in the brain tissues, disarray of brain homeostasis, which in combination incites neurodegeneration in AD(95).

Significance of CSF GFAP in amyloid plaque deposition in pre-clinical and clinical AD.

Assessing and characterizing CSF biomarkers including GFAP for neurodegenerative diseases is important because their changes are commensurate with and might provide indirect evidence of ongoing pathological processes in the brain including protein misfolding, protein aggregation, astrogliosis, microglial overactivity, synaptic dysfunction and neuronal death(111). Immunological determination of CSF GFAP levels by Fukuyama et al in 2001 revealed that their levels are higher in the moderate to severe AD patient cohorts (13.2 ± 9.10 ng/ml) as compared to mild to moderate AD groups (6.85 ± 5.76 ng/ml)(112). Jesse S, et al that appraised the CSF levels found that GFAP levels in the AD patients were significantly higher than Creutzfeldt-Jakob disease (CJD), and non-demented control patients (CON), underscoring its fluctuation with neuronal tissue damage in neurodegenerative diseases(113). Accordingly, CSF levels of GFAP, YKL-40 (chitinase-3-like protein 1), CHIT1 (chitotriosidase-1) were elevated in the prion disease, AD, and frontotemporal dementia (FTD), as compared to controls(114). Nevertheless, none of them except YKL-40 performed better in terms of sensitivity and specificity for offering a reliable diagnostic value for differentiating high risk groups from controls(114). In multiple sclerosis (MS), CSF GFAP levels are higher than controls, with its values more unequivocal and dependable for progressive MS as compared to relapsing-remitting MS for forecasting disease evolution, prognosis, and patient survival(115). In MS patients, elevated GFAP is a marker of pathological changes including severe astrogliosis, and irreversible neurological damage, a combination that might be perceivable as a harbinger for future neurological disability in these patients(111, 116, 117). In a study performed by Jany, P.L. et al analyzing the GFAP levels with ELISA immunoassay in 24 patients in AxD, CSF GFAP levels were significantly and consistently elevated as compared to controls in contrast to

blood GFAP levels which were only upregulated in a few subsets of patients(118). AxD patients have underlying astrocytic injury, seizures, hydrocephalus, and cavitation which might inherently amplify the spillage of GFAP from astrocytes into the extracellular space and CSF within the brain(118).

Not to mention, AQP4 water channel of the astrocytic end feet might be disintegrated in these AxD patients thus forestalling the exuberant clearance of this extracellular GFAP floating around in the brain ISF through the peri-vascular and glymphatic pathways into the systemic circulation(118, 119). In a recent observational cross-sectional study where they prudently planned to gauge the blood and CSF biomarkers from 3 centers from July 2014 to January 2020, CSF GFAP was overmatched by blood GFAP in accurately portraying the A β pathology in different AD continuum groups(23). The authors speculate that, CSF GFAP provides a more acceptable mirror image of the astrocytic counterattack in response to microglial activation instead of encumbrance in the AD continuum groups(23). Moreover, augmented spilling of the GFAP from CSF into the systemic circulation secondary to blood brain barrier dysfunction and peri-vascular drainage is the most probable explanation for under-performance of CSF GFAP in relaying A β pathology in AD patients (23). In parallel with these findings, in a study completed by Montoliu-Gaya L et al, CSF GFAP levels demonstrated insignificant fold change and were found to be a second fiddle for predicting pre-symptomatic as well as symptomatic AD with respect to cortical thinning and amyloid A β uptake (120). Additionally in a clinical trial recently performed by Pereira, J.B. et al, CSF GFAP underperformed as a biomarker as it did not correlate with hippocampal A β accumulation, cognitive impairment, and PET findings in different AD groups (121). Definitively, it exhibited a lesser precipitous upsurge with A β burden on PET scans and was also increased in non-AD neurodegenerative disorders(121). Regrettably, even after combining with other markers such as sTREM2 and YKL-40 the performance of CSF GFAP as a marker for inferring A β burden on PET scan was very displeasing and unfavorable (121). In contrast in a clinical research study performed by Ferrari S. J.P. et al, CSF GFAP were accurate enough to accurately predict A β conglomeration in the PET scan as compared to CSF YKL-40 which presaged PET tau agglomeration in the AD patients(122). These divergent findings draw attention to the fact that CSF GFAP elevation arising from astrogliosis can be inferred as non-credible and non-specific. Furthermore its elevation can surface with any neuroinflammatory or neurodegenerative clinical condition without any clear-cut attribution to A β stock-piling specifically associated with AD patients.

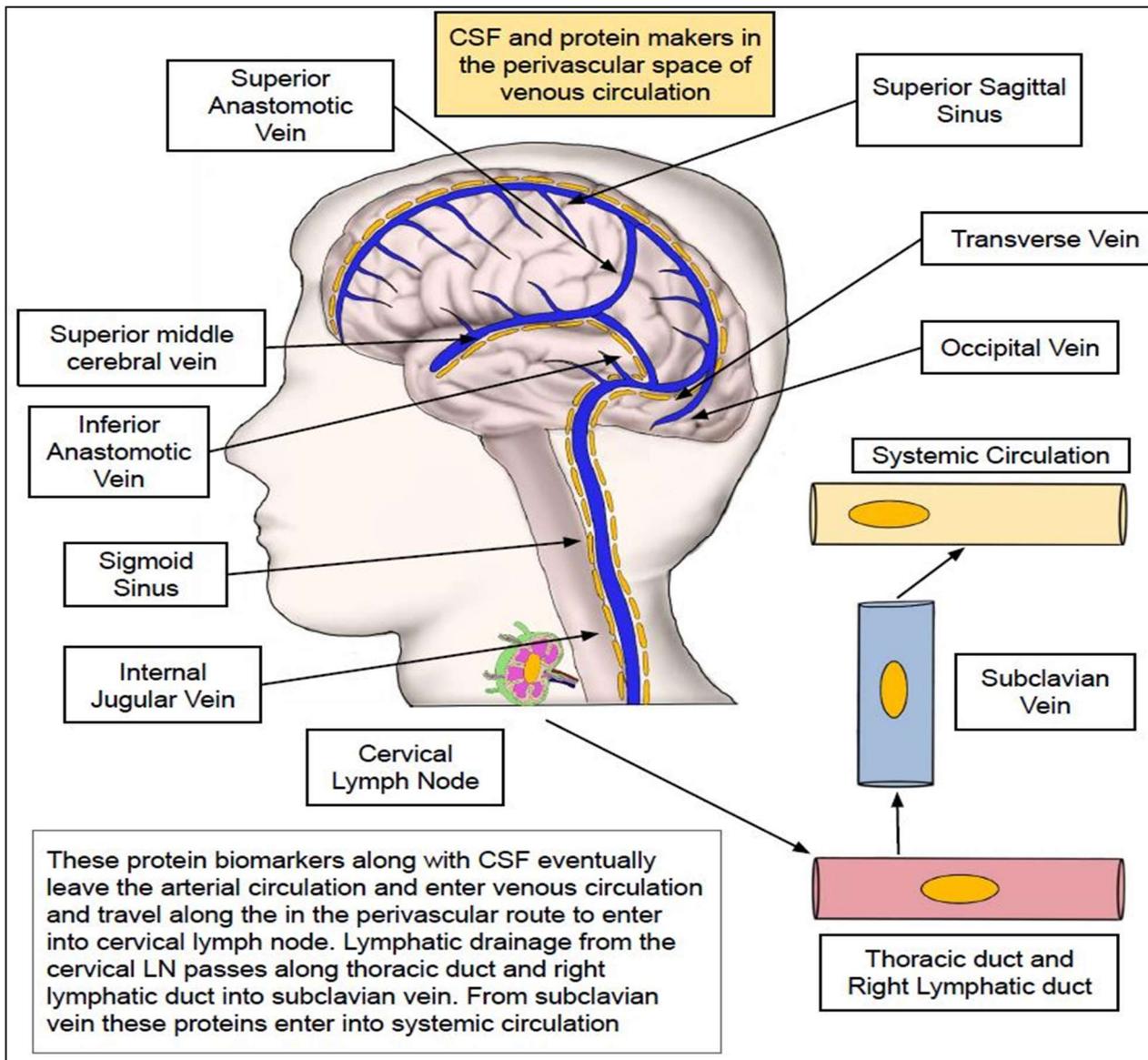


Figure 5. Venous phase of GFAP excretory pathway. In this phase, solutes enter the peri-vascular phase of the venous circulation where they course very closely to the endothelial basement membrane of the venous vessels. Eventually, from the venous vessels they will enter the lymphatic circulation and are ultimately emptied into the right lymphatic duct and thoracic duct. From here, the solutes are emptied into the sub-clavian vein and thereby finally emigrating into the systemic circulation.

Why CSF GFAP underperforms as a biomarker in predicting structural abnormalities and Aβ accumulation in pre-clinical and clinical AD groups as compared to blood GFAP

From the above discussion, it is obvious that there some conflicting opinions regarding the suitability of CSF GFAP as upright and unequivocal marker for guessing the underlying Aβ pathology in the preclinical and clinical AD groups. So, few researchers contended some speculative hypothesis and opined plausible guesswork for decreased stability as well as enhanced degradation of GFAP in the CSF thus making it a less viable option for monitoring disease progression-related brain changes in AD.

First, freeze thaw cycles and storage temperature might have a varying effect on the stability of GFAP in the blood and CSF

samples. In a study where paired blood and CSF samples from AD patients went through a series of freeze-thaw-cycles (FTC) followed by GFAP assessment by molecule array technology, CSF GFAP levels declined by about 188.12 pg/ml per each FTC while blood GFAP levels were untarnished due to their higher endurance capacity(123). Blood GFAP levels endured repeated four freeze-thaw cycles and were more resilient from degradation as compared to Aβ40 and p-tau181 levels which underwent degradation with more than three freeze-thaw cycles(124). It was previously demonstrated that GFAP is cleaved by calpain and caspases into BDPs including rod domain as well as N&C terminal fragments(22, 125). ELISA anti-GFAP antibodies were previously shown to detect the GFAP and its fragments in the CSF and blood samples within the molecular weight range 38-50 kDa(126). It is conceivable that pattern of GFAP cleavage by calpain and caspases is quite inconsistent as well as unequal in CSF and blood samples. Due to this erratic GFAP cleavage, generated divergent GFAP

fragments might have contrasting molecular weights (20 kDa) in these respective body fluids (127). This might make the GFAP fragments (20kDa) evading discovery by routinely used in ELISA detection kits for being far-flung from the detection range of anti-GFAP antibodies routinely used by them(126). This might partly account for differences in detecting GFAP levels in the CSF and blood. Moreover, it has been estimated that the amount of time taken by CSF GFAP to traverse the cerebral vasculature to reach the lumbar sac is approximately 4h(128). It is quite possible that GFAP might have been partially degraded in the CSF by the time it reaches its final destination (lumbar sac).

Research studies should be focused on the direction of accurately accounting for the different GFAP fragments generated in the CSF / blood and appraising their specific molecular weights. This will enable the researchers to design and tailor specific GFAP fragment specific antibodies which can accurately track down these spliced breakdown products. This will ultimately uncover reliable biomarkers capable of ferrying out disease progression in AD continuum groups.

Second, GFAP released from the damaged astrocytes will initially take refuge in the CSF and brain interstitial fluids. This GFAP retreat in the CSF might be abbreviated by its high turnover rate secondary to its massive degradation rate and enhanced seepage pathways into the systemic circulation. Lower levels of CSF GFAP levels as compared to blood can be partially explained by the operation of adept deporting mechanisms through peri-vascular route and glymphatic pathways(129). Furthermore, BBB dysfunction which frequently accompanies AD patients also facilitates augmented spillage of GFAP from CSF into the systemic circulation(130-132). Thus, once GFAP is released extracellularly from astrocyte damage, it might quickly escapes from CSF into systemic circulation through the above-mentioned channels.

Previous reports state that phosphorylation of GFAP kindles depolymerization of filamentous subunits and affords buffering from enhanced proteolysis in the body fluids including blood and CSF(133). It is probable that the ratio of phosphorylation-dephosphorylation of GFAP in the CSF is doctored so that dephosphorylation supersedes thus yielding towards augmented proteolysis as well as enhanced degradation of GFAP in the CSF. On the contrary, dephosphorylation might take a back seat allowing the phosphorylation to overshadow in the blood thereby bolstering the filament integrity and structural plasticity of the GFAP filaments. These changes in the filament assembly due to post-translational modification of GFAP might partly explain the discrepancy in the GFAP levels in the CSF and blood.

Although these are some of the speculations for the mechanisms that can conceivably happen behind the curtain for exaggerated degradation, as well as amplified spurting of GFAP from CSF, the actual reasons are still enigmatic and

cryptic. The need of the hour is launching basic science and clinical research studies to comprehend these unsolved mysteries. This will ultimately lay the appropriate foundation for shedding the light on GFAP stability, turnover, phosphorylation status and excretory pathways. Discerning this crucial information will enhance the practical utility of CSF GFAP as a reliable biomarker for predicting onset and clinical progression of disease pathology in AD.

Upper hand of serum GFAP as a biomarker in predicting structural abnormalities and A β accumulation in pre-clinical and clinical AD groups

A wide variety of clinical research studies conducted so far underscore the importance of blood GFAP as a crucial biomarker that can appraise and capture the structural brain abnormalities, disease evolution and disease progression in AD. Currently guidelines suggest that it can be used as screening as well as disease monitoring tool for making informed treatment decisions in AD when used carefully in tandem with clinical assessment and imaging modalities. In this section, we will summarize the clinical research studies that highlight its potency as biomarker in gauging clinical progression in AD.

Blood GFAP was shown to predict the excessive accumulation of A β 42/40 and A β 42/T-tau in the brain tissues in patients with AD pathology(134). In addition to that it was also demonstrated to be efficient in predicting the evolution of this high-risk cohort into overt AD with mild cognitive impairment (MCI)(134).

According to a study by Ranjan, K.B. et al, patients with baseline blood GFAP values > 232 pg/ml of GFAP exhibited a 130% greater reduction in hippocampal volume as compared to those with baseline values less than 160 pg/ml(135). In the analysis of blood samples from 300 patients from Amsterdam Dementia Cohort which were studied for 3 years, both blood GFAP and amyloid β 42/40 were superior signals for presaging abrupt decline in memory, attention, and executive functioning as compared to blood neurofilament (NfL)(136). These authors bring forward the proposition that GFAP exemplifies and acts as a proxy for over-energized astrocytes displaying a pro-inflammatory phenotype which are responsible for thwarting neuronal loss secondary to accumulation of A β aggregates within the brain tissues(136). Thus, the authors of this study propose putting into practice assaying serum GFAP as an early biomarker for identifying high-risk cases in the incipient stages of brain tissue damage as well as for monitoring disease pathology, progression & prognosis of AD(136).

Blood GFAP [Area under curve (AUC): 0.69-0.96] had outsmarted CSF GFAP [AUC: 0.59-0.76] in separating A β positive individuals from A β negative individuals in AD continuum groups(137). In an observational cross-sectional study in Montreal, Canada (TRIAD: The Translational Biomarkers in Aging and Dementia) the mean [Standard Deviation] values of blood GFAP in cognitive unimpaired

(CU) - A β -negative group, CU A β -positive group, mild cognitive impairment [MCI]-A β -positive group and AD group were 185.1 [93.5] pg/mL, 285.0 [142.6] pg/mL, 332.5 [153.6] pg/mL and 388.1 [152.8] pg/mL respectively(137). Blood GFAP was not only accurate in forewarning the transition from MCI status to full-blown AD dementia but also was sensitive to segregate AD from the fronto-temporal dementia (FTD)(138). In this regard, blood GFAP levels in AD dementia and FTD were (median 375 pg/mL, IQR 276-505 pg/mL) and (190 pg/mL, IQR 134-298 pg/mL, $p < 0.001$) respectively(138). The diagnostic potential of blood GFAP (sensitivity 98% & specificity 60%) in portending AD were much higher than blood phospho-tau (sensitivity 80% & specificity 87%) and serum NfL (neurofilament) (sensitivity 92% & specificity 49%)(138).

In autosomal dominant AD, it was uncovered that blood GFAP elevation happens at least 10 years before symptom onset in the asymptomatic phase of the disease (139). Blood GFAP but not CSF GFAP will anticipate the A β accumulation, neurodegeneration, and cognitive decline with more precision and will show an upsurge with a corresponding increase in clinical severity and disease progression in AD patients(121, 139). In study completed by Oeckl, P. et al, blood GFAP was successful in differentiating AD from frontotemporal dementia (FTD) with a higher sensitivity (89%) and specificity (79%)(140). A recent study revealed that, blood GFAP measurements can relay information regarding accumulated A β PET burden even in patients with normal CSF A β 42/40 levels(121). Alternatively, blood GFAP levels was also elevated in parallel to the CSF A β 42/40 levels in few patients and projected

A β PET accumulation in the neocortical regions thereby providing a reliable benchmark for differentiating cognitive unimpaired from cognitively impaired AD patients(121).

What is the relationship between A β , phosphor-tau and GFAP in the preclinical and clinical stages of AD? Does serum GFAP accurately predicts brain structural alterations.

Reports suggest that assessing blood GFAP levels is a better plan of action as it surpasses the quantification of blood A β -42/1-40 and phosphor-tau181 in portending longitudinal brain A β load as well as cognitive deficits in the preclinical and clinical AD patient populations(141, 142). Not only that, calibrating the blood GFAP is even more rewarding because it appraises clinicians regarding the AD related brain structural changes springing up in nascent stages of disease process at a relatively earlier timeframe as compared to utilizing plasma phospho-tau levels(143).

Specifically, it has been regarded as a paramount biomarker for analyzing the role of astrogliosis as well as evaluating the efficacy of therapeutic modalities to arrest the disease progression in AD(121). In a clinical research study by Chatterjee, P. et al it was disclosed that higher blood GFAP levels and lower A β 1-42/A β 1-40 ratios were demonstrated to

be a harbinger for higher PET A β load as well as poorer cognitive performance in the AD patients(144). AD patients exhibiting higher blood GFAP levels and significant A β burden were also known to have GFAP-IgG seropositivity thereby underling the presence of silent and dormant autoimmune diseases in these high-risk populations(144). Surprisingly even after adjusting PET A β load, blood GFAP positively correlated with progressive cognitive decline in the AD patients. (121, 145).

These findings reinforce the fact that astrocytosis transpiring irrespective of A β accumulation will be detrimental in instigating the neuropathological changes in the cerebral cortex and limbic system.

It has been long thought that astrocytes are inherently programmed to defend and safeguard against A β and tau accumulation within the neuronal tissues(146). Some studies even proved that blood GFAP levels accurately correlated with cumulative A β and tau PET burden in the AD patients(147). In contrast, recent studies suggest that blood GFAP accurately predicts the A β associated pathology more accurately than tau related neuro-fibrillary tangles (NFT) aggregation(121). In fact, the relationship between blood GFAP and PET-tau burden was nullified after controlling amyloid burden on PET(121). In parallel to these findings, in the study performed by Bellaver, B. et al abnormal reactivity and signaling of astrocytes abutting A β was the primordial event that subsequently triggered the spawning of tau aggregates within the preclinical AD groups(148). They surmise that A β amassment leads to proliferation of astrocytes which eventually become infuriated leading to discharge of inflammatory mediators such as ROS, cytokines, chemokines, and caspases. (148). These astrocyte released toxic secretions might directly or indirectly enkindle the inception of tau aggregates within the brain tissues in AD(148).

These findings highlight the perception that the accumulation of tau related NFT is a repercussion of astrocytic counter defense to the A β aggregation with the neuronal tissues in AD(137, 148). It is conceivable that that GFAP-positive astrocyte populations responding to A β and tau aggregates in the AD brain tend to have different genetic makeup for devising discrepant immunological defenses against each of these aggregates. Accordingly, a recent study showed that genetic fingerprint of GFAP-positive astrocyte population associated with A β aggregates was predominated with proteostasis and exocytosis genes whereas those juxtaposed to tau aggregates had prevalence of genes associated with DNA/RNA processing, insulin signaling and cytoskeleton dynamics(147). In the same study it was proved that blood GFAP levels are more likely to mirror image the accumulation of with A β aggregates rather than tau accumulation in mouse models of AD(147).

Blood GFAP otCSF GFAP in presaging amyloid A β PET positivity, cognitive decline and A β abetted brain structural alterations(121).

Similarly, blood GFAP outpaced CSF GFAP in foreboding A β & tau PET status as well as their respective accumulation status in the brain tissues of preclinical and clinical AD groups(137). Secondly, this study showed that both GFAP and YKL-40 were instrumental in the manifestation of hippocampal atrophy and cognitive impairment in the AD continuum groups(122). 200 patients from Mayo Clinic Study of Aging undergoing PET and MRI imaging were simultaneously investigated for blood GFAP levels(149). They were able to reinforce that GFAP levels were directly proportional to the cortical thickness, white matter hyperintensities (WMH) and lobar cerebral microbleeds (CMB)(149). Blood GFAP levels exhibited a significant positive correlation with white matter atrophy in the temporal & parietal regions and also for memory impairment(150, 151). Excessive A β accumulation was shown to provoke grey matter atrophy in the hippocampus, posterior cingulate gyrus, precuneus regions, and temporal lobes(152). These grey matter changes can be monitored by assaying blood GFAP with or without the assistance of PET imaging due to its accuracy in estimating A β assemblage.

Pivotal take home messages

- a) Astroglial fingerprint, GFAP is a 50kD cytoskeletal protein whose gene can be traced to chromosome 17.
- b) From a structural standpoint, it comprises of head domain, rod domain and tail domain.
- c) GFAP synthesized by astrocytes initially squirts into brain interstitial fluid. During physiological conditions it slowly elopes out of brain into the systemic circulation by working its way through paracellular passages and glymphatic clearance pathways.
- d) Even though CSF is the proximate refuge for GFAP after its spawning from astrocytes, it is less reliable for foretelling the relevant pathological processes taking place in AD due to various reasons including decreased stability, shorter half-life, masked epitopes, altered processing and disjointed degradation.
- e) Numerous abnormalities present in AD(cerebral amyloid angiopathy, changes in composition of ECM proteins, APOE4 allele expression, immune complex deposition and astrocyte-AQP4 expression alterations) can thrust GFAP out of CSF even more vigorously thus allowing its decampment and departure out of brain into the systemic circulation. This will make it more accessible to be detected in blood.
- f) Umpteen studies performed so far has cemented numero uno position of - blood GFAP in accurately portending early cellular changes, structural abnormalities, and disease progression in the pre-clinical and clinical cases of AD.

g) Surprisingly, bloodGFAP even outperformed blood phospho-tau, A β 42 and NfL with better sensitivity and specificity in foretelling early disease changes suggestive of AD.

h) Although blood GFAP positively correlates with PET A β and tau load in AD patients, its relationship with tau aggregates was nullified after controlling amyloid burden on PET scan. As a matter of fact, tau aggregates were a collateral damage secondary to primordial A β accumulation in the neuronal synaptic environment in AD.

Deceleration

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Conflict of interest

The authors declare no conflict of interest regarding the publication of this paper.

Availability of data and material

The datasets analyzed during the current study are available upon request with no restriction.

Consent for publication

This manuscript has been approved for publication by all authors.

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