Molecular biomarkers of glial activation and injury in epilepsy

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Increasing evidence from fluid biopsies suggests activation and injury of glial cells in epilepsy. The prevalence of clinical and subclinical seizures in neurodegenerative conditions such as Alzheimer's disease, frontotemporal dementia, and others merits review and comparison of the effects of seizures on glial markers in epilepsy and neurodegenerative diseases with concomitant seizures. Herein, we revisit preclinical and clinical reports of alterations in glial proteins in cerebrospinal fluid and blood associated with various types of epilepsy. We consider shared and distinct characteristics of changes in different age groups and sexes, in humans and animal models of epilepsy, and compare them with those reported in biofluids in neurodegenerative diseases. Our analysis indicates a significant overlap of glial response in these prevalent neurological conditions.

Keywords: neurodegeneration; GFAP; S100b; IL-1b; IL-6; TNF-a; HMGB1; fluid biopsy; status epilepticus; neuroinflammation

Introduction

Epilepsy is one of the most common neurological disorders, characterised by unprovoked and repeating seizures. It is caused primarily by the pathologically enhanced excitability of glutamatergic neurons in the neocortex, limbic system, and parts of the brain stem, leading to synchronised activity of large groups of neurons. Over recent years, epilepsy has emerged as a common comorbidity of the most prevalent neurodegenerative diseases (NDDs), including Alzheimer's disease (AD),[(p1),(p2)] frontotemporal lobar degeneration (FTLD), Parkinson's disease (PD), and dementia with Lewy bodies (DLB).[(p1),(p3)] The increasing incidence of clinical and subclinical seizures with the progression of NDDs suggest a mechanistic relationship, with growing data implying synergistic effects between epileptic seizures (ES) and neurodegenerative processes.[(p4)] In AD, for instance, the prevalence of seizures from the early stages predicts faster cognitive decline.[(p5),(p6)] Inversely, the early signs of cognitive decline in AD are associated with a higher incidence of seizures. Increasing clinical data also suggest that better pharmacological control of comorbid epilepsy in AD, especially in older age, mitigates cognitive deficits and reduces seizure-related mortality.[(p2),(p7)] Similar trends have also been reported in DLB.[(p1),(p8)]

The recognition of the impact of ES on the primary pathology and clinical manifestations of AD and related dementias, FTLD,

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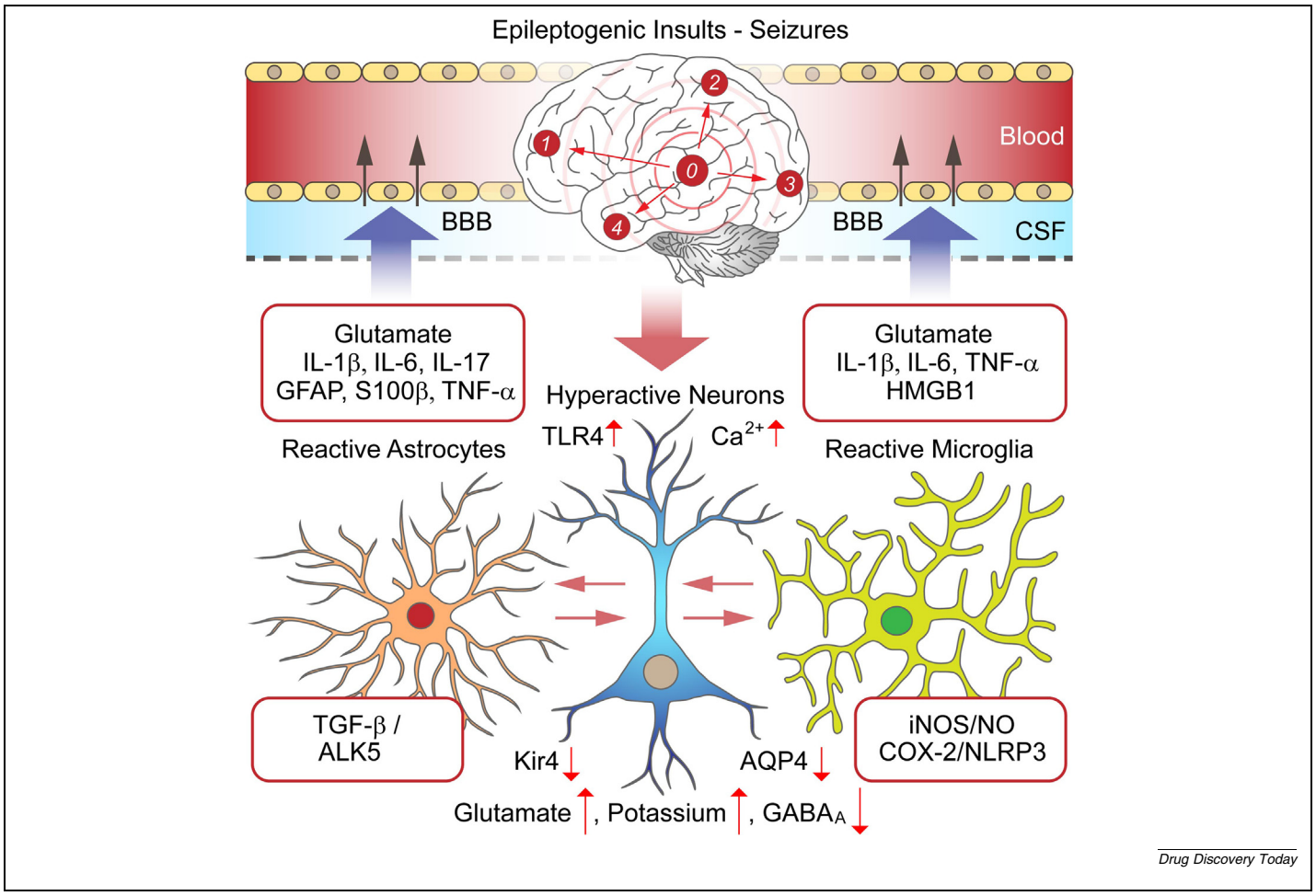


FIGURE 1

Pathological cascade relating epileptic seizures in the brain with the rise in glial markers in biofluids. The onset of epileptic activity in seizure focus (0) and spread over extended networks (1–4) drives brain circuits into a hyperactive state, causing a change in the local environment by releasing transmitters, trophic factors, and signalling molecules from various cell types. Activated astrocytes and microglia cooperate and release inflammatory molecules and mediators, contributing to the hyperexcitability and generation of seizures. IL-1 β released by microglia and astrocytes enhances glutamate release, lowers glutamate reuptake, and decreases gamma-aminobutyric acid A receptor ($GABA_A$) currents. IL-6 secreted from activated astrocytes and microglia promotes glutamate release. In contrast, IL-17 secreted from astrocytes and microglia promotes the infiltration of peripheral immune cells into the brain and inhibits GABAergic transmission, causing excitation-inhibition imbalance. TNF-α secreted by microglia and astrocytes promotes T lymphocyte infiltration, triggers microglial glutamate release, and induces $GABA_A$ endocytosis. HMGB1 released by activated astrocytes, microglia, or neurons interacts with Toll-like receptor 4 (TLR4), promotes proinflammatory cytokine secretion, increases Ca^{2+} influx, upregulates N-methyl-D-aspartate receptor (NMDAR), and disrupts the BBB. Activation of transforming growth factor (TGF)- β/activin-like receptor kinase 5 (ALK5) signalling in astrocytes might downregulate the K^+-inward rectifier 4.1 (Kir4.1) channel and impair the aquaporin 4 channel (AQP4), whereas cyclooxygenase 2 (COX-2)/NLR family pyrin domain containing 3 (NLRP3) upregulation activates caspase-1 and promotes the release of cytokines. In combination, these changes lead to a rise in glial markers in the interstitial fluid, followed by leakage into the CSF and peripheral circulation.

and potentially other NDDs with likely mechanistic links merits careful consideration of comorbid epilepsy when evaluating patients with neurodegenerative conditions. In NDDs with comorbid epilepsy, seizures, in addition to detrimental effects on the course of the disease, also complicate the analysis of pathobiology based on fluid biomarkers. Indeed, some of the proteins used as markers of injury and loss of neurons and synaptic connections found in biofluids of NDDs also respond to a single episode of self-limiting seizures without neurodegeneration.[p9] Although the mechanistic relationship between the injury and loss of neurons in neurodegenerative conditions with comorbid epilepsy remains elusive, growing data support the role of astrocyte deficits and associated disruption of glutamate homeostasis, which can lead to neuronal hyperactivity and cytotoxicity.[p7],[p10],[p11] Increasing evidence also supports a role of the activation of microglia and endothelial cells as part of the neuroinflammatory response in NDDs and epilepsy[p12],[p13],[p14],[p15] (Figure 1). Contrasting with the increase in neuron-specific markers in biofluids, which are viewed primarily as indicators of the neurodegenerative process,[p16],[p17] changes in glial proteins are considered to be associated with the hyperactivity and injury of glial cells.[p12],[p18] It remains to be shown if and how the response of glia to seizures contributes to the clinical manifestations of AD and other NDDs with comorbid epilepsy.

In this review, we revisit reports of changes in glial markers in the cerebrospinal fluid (CSF) and blood of patients with epilepsy

and several NDDs, as well as in animal models. Our analysis suggests a convergence of the glial response in these prevalent neurological diseases and urges further studies of the glial reaction to seizures to elucidate the role of astrocytes and microglia in the pathobiology of epilepsy and neurodegenerative conditions with concurrent seizures.

Literature review and data presentation

All authors conducted a literature search using scientific databases such as PubMed and ScienceDirect. Google Scholar, Academia, and ResearchGate were utilised as additional information sources where necessary. Keywords used for the search were 'glial biomarkers of epilepsy', 'glial fibrillary acidic protein (GFAP)', 'S100β in biofluids of epilepsy', 'epilepsy with neurodegenerative diseases', 'effect of epileptic seizures on glial protein', 'interleukin-1β (IL-1β) and epilepsy', 'interleukin-6 (IL-6) and epilepsy', 'high mobility group box one (HMGB1) protein and epilepsy', 'tumour necrosis factor-alpha and epilepsy', 'tumour necrosis factor-α (TNFα) in epilepsy' and 'glial biomarkers of neurodegenerative diseases'. The list of articles was scanned to identify information relevant to the current analysis. A summary of references was drafted, followed by thematic grouping and manuscript writing. Figures were prepared using Adobe Illustrator Artwork 16.0 in the Adobe Creative Suit Version 6 program. Tables were generated using Microsoft Word. EndNote X8.2 was used for reference formatting per journal guidelines.

Glia-specific markers in biofluids from patients with epilepsy

Based on their origin, glia-specific proteins of biofluids are loosely classified into astrocytic, oligodendroglia, and microglial, with some originating from more than one cell type.[p18],[p19],[p20]

GFAP

An increase in GFAP in biofluids indicates activation or injury of astrocytes, leading to its release into the interstitial space and leakage from there to the CSF and blood (Figure 1). In rat and mouse models of pilocarpine-, kainic acid-, and 4-aminopyridine (4-AP)-induced status epilepticus (SE), GFAP expression in the hippocampus and entorhinal cortex is increased, suggesting an astrocytic response.[p21],[p22],[p23] In a nonconvulsive SE rat model induced by electrical stimulation, there was also an increase in astrocyte and microglial activation at 1 and 4 weeks after seizure, associated with neuronal loss.[p24] In a mouse model of pilocarpine-induced SE, there was also strong upregulation of GFAP in the hippocampus at 1 and 3 weeks post-SE.[p25] Interestingly, some reports showed a reduction in the GFAP level in the brain in a mouse and rat pilocarpine-induced SE model, possibly attributed to the degeneration of astrocytes.[p26],[p27] Mechanistic analysis in a rat model of epilepsy showed that a minimum of nine seizures or a 250-s episode is necessary to induce reactive astrocytes, attested to by their hypertrophy and increased expression of GFAP.[p28]

Clinical evidence suggests elevated GFAP in the serum and CSF of subjects affected by different types of seizures, with changes therein correlating with the severity and duration of epilepsy (Table 1). Analysis of GFAP in serum and CSF showed a significant increase after prolonged ES as compared with psychogenic nonepileptic seizures (PNES) and healthy controls.[p29] Nass and coworkers compared the levels of GFAP in subjects with autoimmune epilepsy (AIE), genetic generalised epilepsy (GGE), and PNES. They showed similar levels of GFAP in the serum and CSF in all types of epilepsy, with no comparison with controls presented.[p30] The concentration of GFAP in the serum of the epilepsy groups in that study, however, exceeded those reported in controls.[p29] Mochol *et al.* also reported higher serum GFAP levels in epilepsy patients with generalised tonic–clonic seizures (GTCS) and focal epilepsy (FE) after adjustment for potential confounders (sex, age, and body mass index).[p31] The level of GFAP in that analysis was not associated with epilepsy duration, seizure type or severity, or recurrent seizures in the preceding 6 months.[p31] Schulz and coworkers analysed autoantibodies in the CSF and serum of those with new-onset epileptic seizures (NES) or chronic epilepsy of unknown aetiology. The study reported increased GFAP autoantibodies associated with AIE compared with controls.[p32] Elhady and colleagues observed significantly higher levels of GFAP in the serum of children after focal-onset epilepsy (FOE) and generalised-onset epilepsy (GOE), with the GFAP response reflecting the severity of seizures in the previous 6 months and predicting active seizures.[p33] Of note, longitudinal studies found that GFAP levels in serum in paediatric cases of GTCS, focal motor seizures (FMS), and epileptic spasms can remain elevated over several months after seizures.[p33] Overall, from the discussed reports, it emerges that epilepsy can lead to the activation of astrocytes with GFAP upregulation and release (Table 1). The disrupted blood–brain barrier (BBB) in epilepsy can lead to the leakage of GFAP from interstitial fluid and CSF into the bloodstream, causing an increase in its levels therein (Figure 1).

S100β

Like GFAP, S100β is enriched in astrocytes, where it serves as the principal calcium-binding protein, regulating intracellular Ca^{2+} dynamics and signalling.[p34] S100β controls various functions, including enzyme activity, cell cycle and differentiation, proliferation, migration, and apoptosis.[p35],[p36],[p37] Several reports have demonstrated S100β increase in the CSF and blood of subjects with epilepsy (Table 1). In patients with mesial temporal lobe epilepsy (MTLE), the plasma level of S100β measured more than 5 days after the last epileptic seizure was significantly higher compared with healthy controls. Notably, the S100β level in female patients exceeded that in males. No sex difference in S100β was found in healthy controls.[p38] Analysis of the level of S100β in serum within 6 h of typical seizures in patients with ES and PNES showed markedly higher levels of protein in ES *vs* PNES, whereas its level in PNES was higher compared with healthy controls.[p39] S100β was also examined in serum samples collected within 30 min after seizures in children with temporal lobe epilepsy (TLE), with its level exceeding that in healthy children.[p40] In children with MTLE, the S100β level was significantly higher compared with seizure-free age-matched controls.[p41] Post-seizure follow-up studies showed that the levels of S100β were elevated in the blood in most patients with SE after an average of 7 to 11 days.[p42] Eighty-four percent of patients with serum S100β above a specific cutoff point

TABLE 1

Glia markers in fluid biopsies of epilepsy

Biomarker, cell of origin	Epilepsy type	Fluid source	Age (years)	Sex, n	Biofluid	Collection phase	Response to seizures	Refs
GFAP, astrocytes	ES, PNES	Human	∼30.2, ∼34	F = 19, M = 24; F = 15, M = 5	Serum	Postictal	Increase	(p29)
	AIE, GGE, PNES,	Human	∼64, ∼29, ∼43	N = 26	Serum and CSF	Postictal	Increase	(p30)
	GTCS, FE	Human	Not specified	N = 119	Serum	Postictal	Increase	(p31)
	AIE, idE	Human	∼45	F = 15, M = 24	CSF	Ictal	Increase	(p32)
	GOE, FOE	Human	∼7	F = 14, M = 16	Serum	Postictal	Increase	(p33)
S100β, astrocytes	MTLE	Human	∼27	F = 14, M = 14	Plasma	Postictal	Increase	(p38)
	ES, PNES	Human	∼30, ∼34	F = 19, M = 24; F = 15, M = 5	Serum	Ictal (within 6 h)	Increase	(p39)
	TLE	Human	∼11	F = 7, M = 12	Serum	Postictal	Increase	(p40)
	MTLE	Human	4–30	F = 15, M = 15	Serum	Postictal	Increase	(p41)
	SE	Human	∼49	N = 82	Serum and CSF	Postictal	Increase	(p42)
	FIE	Human	∼10	F = 19, M = 13	serum	Postictal	Increase	(p43)
	GSE, FSE	Human	∼7	F = 44, M = 54	Serum	Postictal	Increase	(p44)
	SE	Human	∼70	F = 54, M = 33	Serum	Ictal	Increase	(p45)
IL-1β, astrocytes, microglia	PTE with TBI	Human	18–70	F and M = 35	Serum and CSF	Ictal, postictal	Bidirectional	(p51)
	DrE	Human	∼8.9	F = 47, M = 68	Serum	Postictal	Increase	(p53)
	FIRES with HLH	Human	4–16	F = 2, M = 3	Serum and CSF	Postictal	No change	(p54)
	stE, idE	Dog	Not specified	F and M = 73	CSF	Postictal	Increase	(p57)
IL-6, astrocytes, microglia	RSE	Human	<8	M = 11	Serum and CSF	Ictal, postictal	Increase	(p61)
	rS	Human	1.6–26	F = 5, M = 8	Plasma and CSF	Postictal; 24 h	Increase	(p62)
	FIRES	Human	18	M = 1	Serum and CSF	Ictal, postictal	Increase	(p63)
	idE, AE	Dog	0.75–15	F = 5, M = 12	CSF	Postictal; 24 h	Increase	(p64)
TNF-α, astrocytes, microglia	idE, AE	Dog	0.75–15	F = 5, M = 12	Serum and CSF	Postictal	Increase	(p64)
	gS, pS, SE	Human	∼22–25	N = 36	Serum	Postictal	Increase	(p68)
	AERRPS	Human	∼7.5	M = 1	Plasma	Ictal, postictal	Increase	(p69)
	IPE	Human	∼15	F = 18, M = 32	CSF	Postictal	Increase	(p70)
HMGB1, astrocytes, microglia	FS, AS	Human	∼1–3	F = 66, M = 70	Serum	Ictal, postictal	Increase	(p74)
	FS, AS	Human	∼6.0	N = 20; N = 20	Serum	Postictal	Increase	(p75)
	sE, mE	Human	∼10	F = 43, M = 41	Serum	Postictal	Increase	(p76)
	DrE	Human	35–46	N = 27; N = 56	CSF	Postictal	Increase	(p77)
	DrE	Human	∼34	F = 37, M = 28	Serum	Postictal	Increase	(p78)
	idE	Dog	<0.3 and >0.3	N = 40	Serum	Postictal	Increase	(p79)

Abbreviations: AE, acquired epilepsy; AERRPS, acute encephalitis with refractory, repetitive partial seizures; AIE, auto-immune epilepsy; AS, afebrile seizures; CSF, cerebrospinal fluid; DrE, drug-resistant epilepsy; ES, epileptic seizures; F, female; FE, focal epilepsy; FIE, focal intractable epilepsy; FIRES, febrile infection-related epilepsy syndrome; FOE, focal-onset epilepsy; FS, febrile seizures; FSE, focal seizures epilepsy; GGE, genetic generalised epilepsy; GOE, generalised-onset epilepsy; gS, generalised seizure; GSE, generalised seizures epilepsy; GTCS, generalised tonic–clonic seizures; HLH, hemophagocytic lymphohistiocytosis; iDE, idiopathic epilepsy; M, male; mE, mild epilepsy; MTLE, mesial temporal lobe epilepsy; PNES, psychogenic nonepileptic seizures; pS, partial seizure; PTE, post-traumatic epilepsy; rS, refractory seizures; RSE, refractory status epilepticus; sE, severe epilepsy; SE, status epilepticus; stE, structural epilepsy; TBI, traumatic brain injury; TLE, temporal lobe epilepsy.

presented with SE, whereas in most patients without SE, the S100β levels were lower than the cutoff point. Serum S100β levels were not significantly different according to SE aetiology, semiology, or refractoriness. Notably, higher serum S100β predicts confusion or decreased alertness in patients. The authors recommend that serum S100β levels be added to the clinical evaluation and electroencephalogram to identify the difficult-to-diagnose form of SE.[(p42)]

TABLE 2

Glia markers in fluid biopsies of patients with NDD and preclinical models

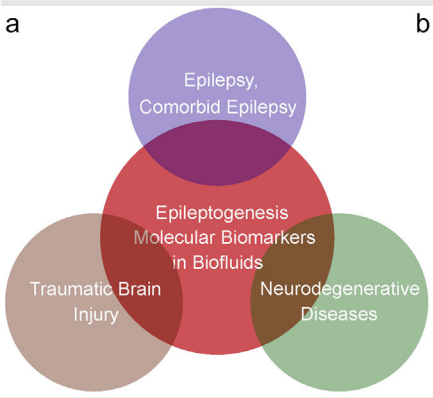
Biomarker, cell of origin	NDD	Fluid source	Age (years)	Sex, n	Biofluid	Response to seizures	Refs
GFAP, astrocytes	AD	Human	~66	F = 21, M = 24	Plasma and CSF	Increase	(p80)
	AD, Dem	Human	~70	N = 27	CSF	Increase	(p82)
	AD	Human	~73	F = 9, M = 7	CSF	Increase	(p83)
	AD	Human	~38	F = 16, M = 11	Serum	Increase	(p84)
	AD, Dem	Human	~62–76	N = 230	Serum	Increase	(p85)
	AD + MCI	Human	~67–78	N = 28	Serum and CSF	Increase	(p86)
	AD + MCI	Human	~61	F = 235, M = 149	Plasma and CSF	Increase	(p87)
	AD, Dem	Human	~51–84	F = 11, M = 17	CSF	Increase	(p89)
	PD + MCI	Human	~59	F = 22, M = 41	Plasma	Increase	(p96)
	PD	Human	~68	N = 29	Plasma	Increase	(p99)
S100β, astrocytes	AD	Human	~77	F = 36, M = 18	Serum	Increase	(p81)
	AD	Human	~57	F = 15, M = 16	CSF	Increase	(p88)
	PD	Human	~65	F = 28, M = 30	CSF	Increase	(p93)
	PD	Human	~63	F = 13, M = 27	Serum	Increase	(p95)
	AD	Human	~69	F = 18, M = 50	CSF	Increase	(p102)
IL-1β, astrocytes, microglia	AD	Human	53–80	M = 9, F = 2	CSF	Increase	(p46)
	PD	Human	42–76	M = 12, F = 10	CSF	Increase	(p46)
	PD	Mouse	4 months	M = 18	Serum	Increase	(p47)
	AD	Human	Not specified	M + F = 197	Plasma	Increase	(p105)
IL-6, astrocytes, microglia	VaD	Human	~73	M + F = 67	Serum	Increase	(p90)
	VaD	Human	~74	M + F = 11	CSF	Increase	(p97)
	VaD	Human	~ 65	M + F = 30	Serum	Increase	(p100)
	ALS	Human	~ 60	M + F = 68	Plasma	Increase	(p107)
TNFα, astrocytes, microglia	PD	Human	~64	F = 35, M = 51	Blood	Increase	(p92)
	ALS	Human	~57	M = 28, F = 23	Serum	Increase	(p94)
	VaD	Human	~ 65	M + F = 30	Serum	Increase	(p100)
	AD	Human	~68	M + F = 55	Serum	Increase	(p106)
	ALS	Human	~ 60	M + F = 68	Plasma	Increase	(p107)
HMGB1, astrocytes, microglia	AD	Human	Not specified	M + F = 8	CSF	Increase	(p91)
	ALS	Human	Not specified	M + F = 5	CSF	Increase	(p108)
	MCI + AD	Human	63–84	M + F = 24	Serum	Increase	(p116)

Abbreviations: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; CSF, cerebrospinal fluid; Dem, dementia; F, female; M, male; MCI, mild cognitive impairment; MS, multiple sclerosis; PD, Parkinson's disease; VaD, vascular dementia.

Calik and coworkers conducted a case–control study of the S100β level in children diagnosed with focal intractable epilepsy (FIE). The higher S100β level in FE *vs* age-matched controls led the authors to conclude that this protein can be a reliable peripheral biomarker for neuronal damage in patients with intractable epilepsy.[(p43)] Alterations of S100β in serum and CSF were also compared in children with generalised seizure epilepsy (GSE) and focal seizure epilepsy (FSE), with the S100β level in serum being significantly higher over 6 h after the epileptic attack in the former.[(p44)] Monitoring S100β in serum showed that its level gradually declined from 6 h to 24 h after the epileptic attack. Based on statistical analysis and predictive values, the authors conclude that serum S100β could provide a sensitivity index for evaluating nerve damage in children with epilepsy, which can be used as a serum biomarker for diagnosis and assessment of the severity of the disease.[(p44)] A retrospective analysis of the serum levels of S100β during the 72-h postictal period in patients with SE demonstrated higher levels in the serum compared with healthy controls.[(p45)] Elevated S100β was associated with stupor/-coma before treatment, independently from aetiology, age, and sex. No differences in S100β serum levels were found between patient samples acquired within 24 h and those acquired 24 h after SE onset.[(p45)]

IL-1β

One of the critical pathobiological features shared by NDDs and epilepsy is the neuroinflammatory response.[(p46),(p47),(p48)] In the inflamed brain, IL-1β is produced by activated microglia and astrocytes,[(p49),(p50),(p51),(p52)] with its level in fluids responding to various seizure types (Table 1). Diamond and coworkers showed that there is an increase in IL-1β in the CSF of individuals who have posttraumatic epilepsy (PTE) associated with traumatic brain injury (TBI) and inflammation.[(p51)] A rapid and significant seizure-related rise in IL-1β has been reported in the serum of children with afebrile drug-resistant epilepsy (DrE), suggesting an inflammatory response with microglial activation after seizure attacks.[(p53)] Remarkably, in 4–16-year-old subjects with febrile infection-related epilepsy syndrome (FIRES) and hemophagocytic lymph histiocytosis (HLH), CSF and serum collected during seizures and 6 months thereafter showed no change in IL-1β



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Biomarker types	Biomarker candidates	Biofluids
miRNA	miR-106b-5, miR-146a, miR-301a-3p, miR-194-5p	Blood, CSF
Proteomic	HMGB1, S100β, GFAP, Aβ-42, Aβ fragments, tau, NF-L, α-SYN	Blood, CSF
Metabolomic	MMP-2, MMP-3	Blood, CSF
Hormones	Creatine kinase, NSE, Prolectin, nesfatin-1, ghrelin	Blood, CSF
Cytokines	IL-1β, IL6, IL-17, TNF-α	Blood, CSF

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FIGURE 2

Molecular biomarkers of epileptogenesis in liquid biopsies. **(a)** Venn diagram demonstrating the overlap of molecular markers of epileptogenesis in epilepsy, neurological and psychiatric diseases with comorbid epilepsy, NDD, and traumatic brain injury. **(b)** Representation of emerging molecular biomarker types and candidates detected in the biofluids of people with epilepsy. Among the biomarker candidates, those released by reactive and injured glia are coloured red. Part of the data included in the figure were adapted with permission.[p116] (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

levels.[p54] Neuroinflammation has also been evident in animal seizure models.[p55],[p56] Analysis of the serum of dogs with either PTE associated with structural abnormalities (stE) in the brain or idiopathic epilepsy (idE) without structural changes showed an increase in IL-1β.[p57] Of note, in dogs with epilepsy and healthy dogs, IL-1β was not measurable in CSF.[p57] Indeed, attempts to measure IL-1β in the CSF of dogs with nonspecified types of epilepsy and TBI showed no detectable IL-1β.[p57] Overall, IL-1β increases in the blood of humans and dogs affected by epilepsy support shared neuroinflammatory pathways and neurobiological mechanisms with NDDs (see below). Given that IL-1β plays a crucial role in neuroinflammation, changes therein could shed light on the underlying pathobiology and facilitate diagnosis and therapeutic interventions. Further research is warranted to validate the utility of IL-1β as a glial activation biomarker in epilepsy and NDDs.

IL-6

Like IL-1β, IL-6 levels have been reported to increase in biofluids in those with epilepsy, AD, and other NDDs.[p58],[p59] This versatile cytokine is released from activated microglia and astrocytes by various pathogenic stimuli, contributing to neuroinflammatory responses and changes in the microenvironment.[p60] Several studies have demonstrated an increase in IL-6 levels in the CSF and blood of subjects with epilepsy (Table 1). A report on refractory SE (RSE) in children under the age of 8 demonstrated an increase in the levels of IL-6 in both the CSF and serum during and after ES.[p61] Intrathecal dexamethasone infusion resolved the RSE, with levels of inflammatory markers gradually normalising over subsequent days.[p61] Billiau and coworkers tested the fluids of subjects in multiple age groups suffering from refractory seizures (rS) and discovered that 24 h after the seizure episodes, the level of IL-6 subsided but remained significantly elevated in both the CSF and plasma.[p62] Another study similarly found that in patients suffering from FIRES, there was an increase in the concentration of IL-6 in the CSF and serum during the ictal and postictal periods.[p63] Assessment of the IL-6 level and activity in fluids of dogs with idE or acquired epilepsy (AE) showed a significant rise in the CSF and serum, followed by a gradual decline over 24-h, 48-h, and >48-h periods following the last epileptic episode.[p64] The higher levels of IL-6 in fluid biopsies support the hypothesis that inflammatory processes involving cytokines play a crucial role in the pathogenesis of epilepsy.

TNF-α

TNF-α is another major player in neuroinflammation, and it is also implicated in the hyperactivity of neurons and the generation of ES. As a proinflammatory cytokine, TNF-α is produced mainly by activated microglia and astrocytes[p65],[p66] and is known to regulate neuronal excitability, the activity of immune cells, and the modulation of synaptic plasticity.[p67] A growing number of reports have demonstrated a rise in TNF-α in the CSF and blood of humans and animals with epilepsy (Table 1). Like IL-1β and IL-6 in humans, in dogs suffering from stE and idE, there was a notable increase in TNF-α in the CSF and serum 24 h and 48 h after the last seizure episode.[p64] Higher TNF-α levels in biofluids of dogs substantiate the hypothesis that inflammatory processes involving cytokines play a crucial role in the pathogenesis of seizures.[p64] Sinha and coworkers showed that in young adult patients suffering from a generalised seizure (gS), partial seizure (pS), SE, or localisation-related epilepsy (LRE), there was an elevated level of TNF-α in the serum for 1–24 h after the last seizure episode.[p68] This finding is in line with the notion of a rise in cytokines in the serum of postictal patients with several epilepsy syndromes. In addition, in a study of a boy with acute encephalitis and refractory and repetitive partial seizures (AERRPS), an increase in the levels of TNF-α in the plasma during and after seizure attacks was reported.[p69] These

findings suggest that AERRPS, a major immune-mediated component, involves an autoimmune response and exaggerated cytokine production and release.[p69] Patients in their teens with intractable partial epilepsy (IPE) also showed an increase in TNF-α concentrations in the CSF shortly after seizure.[p70] Notably, upon therapy with the antiseizure matrix metalloproteinase (MMP)-9 inhibitor pranlukast, TNF-α was reduced. Pranlukast is likely to have pleiotropic effects, countering the leakage of cytokines from brain tissue fluids into biofluids and inhibiting their release from glial cells.[p70] As a potential biomarker of epilepsy, TNF-α also responds to neuroinflammation in NDDs, which might signify shared mechanisms involving the activation of microglia and astrocytes.

HMGB1

The nuclear protein HMGB1, released from microglia and astrocytes, plays a crucial role in the immune response and neuroinflammation in the central nervous system (CNS).[p71],[p72],[p73] Like other biomarker candidates, many studies showed an increase in HMGB1 levels in response to episodes of seizures (Table 1). A report in infants with either febrile seizures (FS) or afebrile seizures (AS) showed an increase in HMGB1 in the serum during and 15 min after ES.[p74] The authors suggest HMGB1 might lower the action potential firing threshold and promote neuronal excitability. A notable surge in the serum HMGB1 level was also observed in children with either FS and AS, which was detected ~10 min after GTCS or myoclonic seizure (mS) episodes and remained high during the first 24 h after seizures.[p75] A comparative study of HMGB1 in various types of seizures revealed that its level in severe epilepsy (sE) cases was higher than in milder epilepsy (mE) cases and controls, implying that it could be a potential indicator of the intensity of ES.[p76] A rise in HMGB1 was reported in the serum and CSF of adults with drug-refractory epilepsy (DrE) and newly diagnosed epilepsy (NDE) 24 h after epileptic bouts, with HMGB1 changes correlating with the severity of seizures and their resistance to antiseizure medications.[p77]

Comparison of HMGB1 in adults with refractory epilepsy (RE), well-controlled chronic epilepsy (WCE), and NDE showed a more robust seizure-related increase in the marker in the serum of subjects with DrE.[p78] Higher levels of serum HMGB1 in RE as compared with drug-responsive and healthy subjects suggest that HMGB1 can distinguish the former from the latter.[p78] Similar to inflammatory mediators, HMGB1 was also elevated in the serum of dogs with idE within the first hour after epileptic bouts compared with healthy dogs.[p79] Notably, the serum HMGB1 concentrations in dogs with nonepileptic brain diseases did not differ from those of healthy dogs, indicating specific role of seizures in increasing the level of this protein in serum.[p79] In dogs with chronic epilepsy (>3 months), the HMGB1 concentration was higher than in dogs affected by the condition for ≤3 months.[p79]

Glial markers in biofluids from patients with neurodegenerative diseases

Ample data suggest changes in glial markers in the CSF and blood in AD[p46],[p80],[p81],[p82],[p83],[p84],[p85],[p86],[p87],[p88],[p89] and other NDDs[p46],[p47],[p90],[p91],[p92],[p93],[p94],[p95],[p96],[p97],[p98],[p99],[p100] (Table 2). An increased level of GFAP is viewed as an indicator of the activation and injury of astrocytes, with overwhelming evidence supporting their role in neuroinflammation.[p101] Elevated plasma and serum levels of GFAP are found in normal older adults with mild cognitive impairment (MCI), patients with AD, and patients with AD dementia, as estimated by the brain amyloid load, and correlate with cognitive decline.[p80],[p82],[p83],[p84],[p85],[p86],[p87],[p89] Higher levels of GFAP have also been reported in the serum and CSF in other NDDs.[p96],[p99] Like GFAP, S100β is expressed in astrocytes and has been explored as a candidate biomarker of injury and degeneration in these cells, with a rise therein reported in the biofluids of patients with AD and PD[p81],[p88],[p93],[p95],[p102] (Table 2).

Over the last decade, the contribution of glial proinflammatory factors to the pathogenesis of NDDs has also been increasingly recognised. The most common cytokines of the nervous system, IL-1β and IL-6, are primarily released by microglia but can also be secreted by reactive astrocytes, neurons, and endothelial cells.[p103],[p104] The increase in IL-1β and IL-6 in biofluids is taken to indicate neuroinflammation, with their rise reported in AD, PD, and other NDDs[p46],[p47],[p90],[p92],[p94],[p97],[p100],[p105] (Table 2). Like cytokines, several reports in NDDs suggest enhanced release of TNF-α by the activated microglia of inflamed neural tissue, which affects multiple signalling pathways and mechanisms within neurons and glia and contributes to apoptosis and neurodegeneration.[p92],[p94],[p100],[p105],[p106],[p107] Finally, biofluids of several patients with NDD have also shown increased HMGB1[p91],[p98],[p108] (Table 2). Overall, although alterations of glial markers in biofluids in NDDs are viewed mainly in relation to the neuroinflammation associated with the neurodegenerative process, glial markers also respond to different types of epilepsy without overt signs of neurodegenerative changes.

Concluding remarks and outlook

Unprovoked and repeated seizures represent the primary hallmark of epilepsy. Clinical and subclinical seizures are also prevalent in AD, DLB, FTLD, and, to a lesser extent, other NDDs. Our recent analysis of changes in neuronal markers in biofluids in epilepsy demonstrated significant overlap with those reported in several NDDs.[p9] In this review, we revisited the reports of glial marker changes in the CSF and blood in different types of epilepsy, with the results having implications for the pathobiology and diagnosis of NDDs without and with comorbid epilepsy. The correlation of the rapid rise in several glial-specific proteins in biofluids with the type of epilepsy implies glial activation and injury, likely interfering with molecular biomarkers used for diagnosis of NDDs and other conditions with concurrent epilepsy (Figure 2). As the current analysis shows, many types of ES activate glial cells, releasing an array of proteins in CSF and the bloodstream.

Although elucidation of the specific mechanisms driving the rise in glial markers in the CSF and blood in response to seizures warrants further research, they are likely to involve release from reactive and injured glial cells via exocytosis, exosome-mediated discharge, and leakage caused by cell damage. Because of the effects on the local microenvironment,

the release of glial cell cytokines, chemokines, and other mediators can potentially impair neuronal mechanisms and synaptic functions. Consequently, restoring local homeostasis and countering inflammation caused by reactive astrocytes and microglia in epileptogenic tissue might provide an avenue for therapeutic intervention. Major clinical and preclinical efforts are underway to identify the mechanisms and detrimental effects of glial inflammatory factors and mitigate the associated damage.[(p109),(p110),(p111),(p112)] The knowledge gained from these studies should aid in diagnosis and guide therapeutic interventions to counter neuroinflammation and restore the microenvironment of affected brain tissue. Detailed analysis of the glial response should also facilitate the identification of signalling pathways mediating the harmful effects in neurons and other affected cell types.[(p113),(p114),(p115)] Finally, careful studies of glial marker dynamics in various types of epilepsy and NDDs should assist with designing and validating precision therapies to counter the injury and degeneration of neurons associated with glial dysfunctions. In synergy with mechanistic studies using preclinical models, these developments are anticipated to improve the management of immunological and homeostatic aspects of various forms of epilepsy, AD, DLB, and other neurological conditions with comorbid epilepsy.

Author contributions

Conception and design, **RK**, **DK**, **DN**, and **SVO**; drafting, revising and critical comments, **RK**, **DK**, **SS**, **DN**, **ICG**, **SB**, and **SVO**; illustrations and tables, **RK**, **DK**, and **SVO**; review and approval of the final version, **RK**, **DK**, **SS**, **DN**, **ICG**, **SB**, and **SVO**.

Data availability

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Declarations of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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