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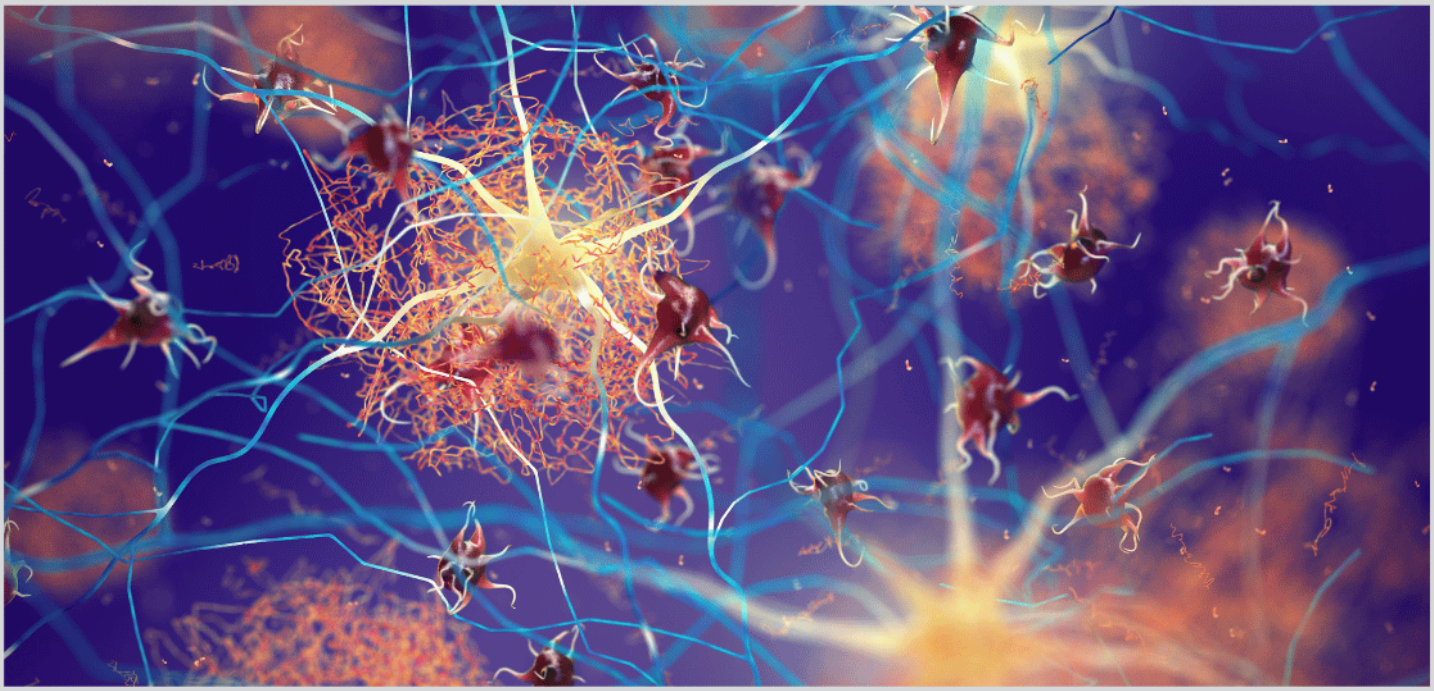
Pathophysiological convergence of epilepsy and Alzheimer's disease



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Epilepsia™

RESEARCH ARTICLE

Neurofilament light, glial fibrillary acidic protein, and tau in a regional epilepsy cohort: High plasma levels are rare but related to seizures

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Abstract

Objective: Higher levels of biochemical blood markers of brain injury have been described immediately after tonic–clonic seizures and in drug-resistant epilepsy, but the levels of such markers in epilepsy in general have not been well characterized. We analyzed neurofilament light (NfL), glial fibrillary acidic protein (GFAP), and tau in a regional hospital-based epilepsy cohort and investigated what proportion of patients have levels suggesting brain injury, and whether certain epilepsy features are associated with high levels.

Methods: Biomarker levels were measured in 204 patients with an epilepsy diagnosis participating in a prospective regional biobank study, with age and sex distribution correlating closely to that of all patients seen for epilepsy in the health care region. Absolute biomarker levels were assessed between two patient groups: patients reporting seizures within the 2 months preceding inclusion and patients who did not have seizures for more than 1 year. We also assessed the proportion of patients with above-normal levels of NfL.

Results: NfL and GFAP, but not tau, increased with age. Twenty-seven patients had abnormally high levels of NfL. Factors associated with such levels were recent seizures ($p = .010$) and epileptogenic lesion on radiology ($p = .001$). Levels of NfL ($p = .006$) and

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GFAP ($p=.032$) were significantly higher in young patients (<65 years) with seizures ≤2 months before inclusion compared to those who reported no seizures for >1 year. NfL and GFAP correlated weakly with the number of days since last seizure (NfL: $r_s=-.228$, $p=.007$; GFAP: $r_s=-.167$, $p=.048$) in young patients. NfL also correlated weakly with seizure frequency in the last 2 months ($r_s=.162$, $p=.047$).

Significance: Most patients with epilepsy do not have biochemical evidence of brain injury. The association with seizures merits further study; future studies should aim for longitudinal sampling and examine whether individual variations in NfL or GFAP levels could reflect seizure activity.

KEYWORDS

blood biomarkers, glial fibrillary acidic protein, neurofilament light, plasma biomarkers, tau

1 | INTRODUCTION

Whether seizures injure the brain is one of the fundamental questions in epilepsy. Clinically, many persons with epilepsy do not show evidence of progressive brain damage; cognitive functions remain intact and seizures well controlled. Some persons with epilepsy do, however, experience memory decline, cognitive problems, and progressive seizure worsening. This clinical heterogeneity is reflected in emerging biomarker research. Tau depositions typically found in traumatic brain injury (TBI) or dementia are also observed in a worrying proportion of resected temporal lobe specimens, and associated with poor cognitive function.[1–3] Uncontrolled temporal lobe epilepsies (TLEs) have been associated with progressive brain atrophy until epilepsy surgery.[4,5] Recent multi-center studies have investigated this, utilizing event-based modeling and topographic analysis to demonstrate patterns of atrophy in the brain in this patient population.[6,7] The conventional wisdom that seizures by themselves (if not prolonged like status epilepticus) do not cause brain damage may be correct in some cases but oversimplified in others. In addition to seizures, epilepsy often entails use of anti-seizure medication (ASM), among which at least valproic acid (VPA) can have structural side effects like brain atrophy or slowly developing encephalopathy. VPA use in epilepsy has been associated with reduced total brain and white matter volume, as well as occipital lobe cortical changes.[8,9]

Advances in dementia and TBI research have led to the discovery of blood biomarkers reflecting brain injury and neurodegeneration. Among these, neurofilament light (NfL) seems the most universal and a sensitive marker of brain damage in, for example, dementias, TBI, multiple sclerosis, and stroke.[10–13] Glial fibrillary acidic protein (GFAP) and tau reflect other aspects of astrocyte activation and brain damage, respectively.[14–16] In epilepsy, NfL levels can rise after prolonged febrile seizures,[17] status epilepticus,[18] or tonic–clonic seizures.[19] Higher levels of NfL were recently reported in patients with drug-resistant epilepsy,[20] but not in patients with autoimmune encephalitis and epilepsy.[21] NfL levels in general epilepsy populations is less well researched.

The possibility of studying brain injury on a biochemical level has many potential applications in epilepsy: ASM evaluation, early identification of detrimental epilepsy trajectories (warranting intensified therapy or surgical evaluation), need for cognitive testing, and early detection of ASM side effects are some possibilities. Monitoring and reassurance that there is no biochemical evidence of brain injury may also be an important part of epilepsy care in the future. Before that, however, more information on blood biomarkers of brain injury in epilepsy is needed.

We investigated plasma NfL, tau, and GFAP in patients with epilepsy and asked what proportion of patients have levels suggesting brain injury, and if certain epilepsy features are associated with high levels. We used a regional biobank of hospital-based patients with a broad representation, including well-controlled as well as difficult-to-treat epilepsy.

Key points

- Most patients with epilepsy do not have biochemical evidence of brain injury.
- Twenty-seven patients of 204 had above-normal levels of neurofilament light (NfL).
- Factors associated with higher NfL levels were recent seizures and epileptogenic lesion on radiology.
- Absolute levels of NfL and glial fibrillary acidic protein (GFAP) were also increased significantly in patients (<65 years) with recent seizures and epileptogenic lesion.

2 | METHODS

2.1 | Cohort

Participants in this study were selected from the Prospective Regional Epilepsy Database and Biobank for Individualized Clinical Treatment (PREDICT), a biobank study of epilepsy in Region Västra Götaland (VGR), Sweden (clinicaltrials.org NCT04559919). The aims of PREDICT are to identify biomarkers of value in epilepsy care, study longitudinal medical and psychosocial outcomes, and assess the quality of epilepsy care. Recruitment to PREDICT is not consecutive, but opportunistic; patients with epilepsy or single seizures were recruited at five (all but one) different outpatient clinics in VGR from December 2020 and onwards. Criteria for participation in the PREDICT study is age over 18 years and an unprovoked seizure within the last year, or an epilepsy diagnosis according to the current International League Against Epilepsy (ILAE) definition. Individuals with an expected survival of less than 2 years or the inability to give informed consent are excluded. Recruitment started on November 23, 2020; at the time of analysis for this study (December 2021), 242 participants had been recruited to PREDICT.

Inclusion criteria for this study were an epilepsy diagnosis ($n = 204$), whereas patients with missing PREDICT information ($n = 4$) and no epilepsy diagnosis (single or multiple seizures, $n = 34$) were excluded. Comparison to the National Patient Register, which contains all outpatient appointments in specialized care in Sweden, was done by a search in open statistics available at www.socialstyrelsen.se for patients seen for a diagnosis of G40 in 2019 in VGR.

2.2 | Blood collection and plasma preparation

After recruitment to PREDICT, blood was drawn into ethylenediaminetetraacetic acid (EDTA) tubes and centrifuged for 10 min at 2000 *g* at room temperature. The time between a patient's last clinic visit to blood collection (median = 1 day) was 1 week or less for most patients (131), with 119 patients having their blood collection on the day of their last visit. Plasma supernatant was collected and aliquots were stored at −80°C at the regional health care–integrated biobank Biobank Väst (registration number 890) until analysis.

2.3 | Clinical data collection

Clinical data from the medical records, including the recruitment appointment where physicians are encouraged to document according to a PREDICT template, were collected into a pseudonymized clinical report form (CRF) by a neurologist (F.A. or J.Z.). Collected variables used in the present analysis include age, sex, result of latest brain imaging, result of latest electroencephalography (EEG), cause of epilepsy if deemed symptomatic, date of last seizure, number of seizures in the last 2 months, and ASMs.

2.4 | Quantification of biomarkers

Plasma NfL, total-tau (t-tau), and GFAP were quantified using the commercially available single molecule array (Simoa) N4PB kit (Quanterix). The coefficients of variation were about 5% for all markers. This kit also measures ubiquitin C-terminal hydrolase L1, but this assay failed our quality control criteria with coefficients of variation above 30% for most samples. The kits were run according to manufacturer instructions in the Clinical Neurochemistry Laboratory at Sahlgrenska University Hospital, Mölndal, Sweden.

2.5 | Statistical analysis

All statistical analyses were performed using SPSS (IBM SPSS Statistics for Macintosh, Version 29.0.0). Because the markers were not normally distributed, we used Spearman

rank for correlation analyses (Spearman Rho = r_s) and Mann–Whitney *U* for comparisons of absolute levels ($p \leq .05$). For NfL, we also described the proportion of patients with levels above an age-adjusted cutoff based on healthy individuals.[22] For GFAP and tau, there is currently no such discriminative threshold between healthy individuals and patients with nervous system disease. Clinical factors were compared with a column proportion test. Because of the explorative nature of the study, we did not correct for multiple comparisons.

We conducted a multiple linear regression analysis to examine the relationship between biomarker levels and independent variables seizure status, radiological finding, and epilepsy duration (years). The models were adjusted for the age and sex. In a second set of models, we also adjusted for the number of ASMs. All dependent variables were log-transformed to achieve a normal distribution of the residuals in linear regression models. Regression coefficients (*B*) were presented as a relative change in percent (B_{rel}) to facilitate interpretation of the results.

3 | RESULTS

Compared to the National Patient Register, the cohort was representative regarding age and sex for patients seen in outpatient care in the VGR region (Figure 1). Of 204 participants, 131 (64%) had focal epilepsy, 37 (18%) had generalized epilepsy, and 36 (18%) had epilepsy of unknown cause (Table 1). Eighty-five (42%) had been seizure-free for more than 1 year and 89 (44%) had experienced seizures in the last 2 months (Table 1).

3.1 | Brain injury marker levels

The median concentrations were 7.89 (1.71–128) pg/mL of NfL, 89.1 (20.4–2190) pg/mL of GFAP, and 6.75 (.47–23.4) pg/mL of tau. None of the markers were normally distributed. NfL increased with age ($r_s = .73$, $p < .001$) and with GFAP ($r_s = .67$, $p < .001$) (Figure 2). GFAP increased with NfL (as above) and with age ($r_s = .60$, $p < .001$), whereas tau did not increase significantly with any of the other markers or with age.

3.2 | Clinical factors associated with high NfL levels

A total of 177 patients (87%, 95% confidence interval [CI] 82–91) had normal levels of NfL, and 27 patients (13%, 95% CI 9–18) had levels above the selected cutoff. Clinical factors more common in patients with elevated levels for NfL were seizures in the last 2 months ($p = .010$) and epileptogenic lesion on radiology ($p = .001$) (Table 2). In total, 18 of 89 (20%, 95% CI 13–29) patients with seizures in the last 2 months, 4 of 30 (13%, 95% CI 5–29) patients with seizures between 2 and 12 months, and 5 of 85 (6%, 95% CI 2–12) patients with no seizures in the last year were above the cutoff for NfL (Figure 2A). Fifteen of 27 patients with high NfL levels had epileptogenic lesion on radiology (26%, 95% CI 16–38), whereas 5 patients had normal imaging (7%, 95% CI 3–15) (Figure 2B).

3.3 | Seizure status

In patients <65 years of age, the levels of NfL and GFAP were significantly higher with seizures in the last 2 months compared to those who were seizure-free for more than 1 year (NfL mean 12.7 pg/mL vs 6.1 pg/mL, median 7.4 pg/mL vs 5.6 pg/mL, $p = .006$; GFAP mean 124 pg/mL vs 78.9 pg/mL, median 82.8 pg/mL vs 72.2 pg/mL, $p = .032$; Figure 3). No significant differences were found for tau between patients with recent seizures and those seizure-free (mean 7.1 vs 7.3 pg/mL, median 6.5 vs 6.7 pg/mL). In patients ≥65 years, no significant differences were reported between patients with recent seizures and patients who were seizure-free (NfL mean 28.0 pg/mL vs 20.7 pg/mL, median 23.0 pg/mL vs 18.4 pg/mL; GFAP mean 198.3 pg/mL vs 208.3 pg/mL, median 140.0 pg/mL vs 163.0 pg/mL; tau mean 6.7 pg/mL vs 7.0 pg/mL, median 6.4 pg/mL vs 7.3 pg/mL).

In patients <65 years, NfL correlated weakly with the days since last seizure ($r_s = -.238$, $p = .005$), as did GFAP ($r_s = -.213$, $p = .011$), whereas tau showed no correlations. In addition, NfL correlated weakly with the number of seizures in the last 2 months ($r_s = .162$, $p = .047$). There were no correlations with the number of seizures for GFAP and tau (<65 years), or among older patients (≥65 years) for any marker with the number of seizures in the last 2 months or time since last seizure.

3.4 | Clinical characteristics and non-stroke patients

Clinical characteristics of participants with values of NfL above the cutoffs (Table 2) showed that 8 of 27 patients with high levels had post-stroke epilepsy. When restricting the analysis to young (<65 years) non-stroke participants, NfL levels were still significantly higher in patients with recent seizures than in those who were seizure-free (mean 8.7 pg/mL vs 6.1 pg/mL, median 7.0 pg/mL vs 5.2 pg/mL, $p = .022$), but the difference in GFAP was no longer significant. In non-stroke patients, NfL was correlated with the days since last seizure ($r_s = -.206$, $p = .019$) in younger patients (<65 years), and GFAP with the number of seizures in the last 2 months ($r_s = .176$, $p = .037$).

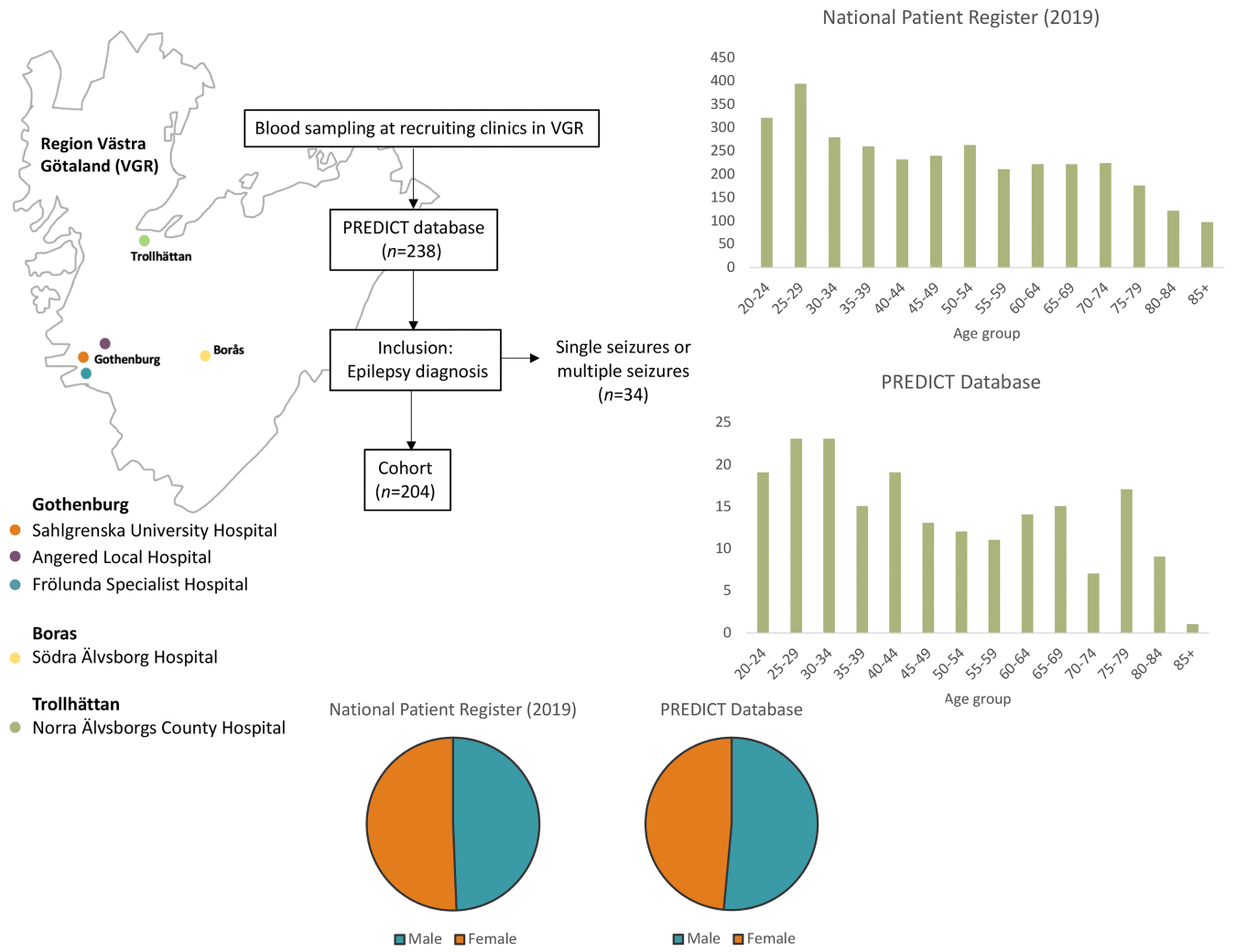

FIGURE 1 Recruiting health care clinics in Region Västra Götaland (VGR) and National Patient Registry data comparison to the Prospective Regional Epilepsy Database and Biobank for Individualized Clinical Treatment (PREDICT) database.

3.5 | Epilepsy type and imaging

In patients younger than 65 years of age, NfL levels were significantly higher in patients with focal compared to generalized epilepsy (mean 12.6 pg/mL vs 7.1 pg/mL, median 7.4 pg/mL vs 5.1 pg/mL $p = .012$), as was GFAP (mean 126.4 pg/mL vs 72.6 pg/mL, median 84.7 pg/mL vs 66.7 pg/mL, $p = .013$). In older patients there were only four patients with generalized epilepsy, which precluded further analysis. In addition, we compared patients with recent seizures (≤2 months) that had ever experienced tonic–clonic seizures (focal, unknown, or generalized onset) to patients with focal seizures only. In younger patients, there were no significant differences for any marker (NfL $p = .609$, GFAP $p = .945$, tau $p = .389$). In older patients, the levels of NfL were higher in patients who had epilepsy with focal seizures only ($p = .010$).

Regarding radiological findings, levels of NfL and GFAP were higher in younger patients with an epileptogenic lesion (NfL: mean 19.8 pg/mL vs 7.0 pg/mL, median 8.1 pg/mL vs 5.8 pg/mL, $p < .001$) (GFAP: mean 129 pg/mL vs 103 pg/mL, median 95.6 pg/mL vs 62.4 pg/mL, $p < .001$). In older patients, levels of GFAP, but not NfL, were significantly higher in patients with normal radiological findings (mean 223 pg/mL vs 149 pg/mL, median 173 pg/mL vs 143 pg/mL, $p = .048$). The results remained significant also when patients with stroke were excluded. The levels of tau were not significantly different in patients with different epilepsy type or radiological result.

3.6 | Multiple linear regression

Multiple linear regression was used to investigate the relationship between NfL, tau, and GFAP and the variables age, sex, epilepsy duration, seizure status, and radiological findings (Tables S1–S3). The model was a good fit for NfL ($R^2 = .49$, $p < .001$) and GFAP ($R^2 = .32$, $p < .001$), but a

TABLE 1 Demographics and clinical characteristics of study cohort.

Characteristics	*n*	%
Gender		
Male	106	52.0
Female	98	48.0
Age (years)		
Range (median)	18–92 (44)	
Epilepsy duration		
Range (median)	<1 to 73 years (8 years)	
25th Percentile (Q1)	2 years	
75th Percentile (Q3)	20 years	
Seizure onset and semiology		
Focal onset	131	64.2
Focal to bilateral tonic–clonic	80	39.2
Aware	41	20.1
Impaired awareness	52	25.5
Unknown	17	8.3
Generalized onset	37	18.1
Tonic–clonic	17	8.3
Myoclonic	7	3.4
Unknown	20	9.8
Unknown onset	36	17.6
Tonic–clonic	15	7.4
Unknown	21	10.3
Seizure status		
Seizures ≤2 months	89	43.6
Seizure frequency (within ≤2 months before inclusion)		
Median (range)	2 (1–98)	
25th Percentile (Q1)	1	
75th Percentile (Q3)	8	
Seizures >2 months to ≤1 year	30	14.7
No seizures (>1 year)	85	41.7
Days since last seizure (last seizure to last clinic visit)		
25th Percentile (Q1)	21	
75th Percentile (Q3)	1531	
Median	195	
Days between last seizure to blood sampling		
25th Percentile (Q1)	23	
75th Percentile (Q3)	1599	
Median	248	
Radiological finding		
Normal	71	34.8
Epileptogenic lesion	58	28.4
Abnormal, unrelated	34	16.7
No imaging	41	20.1
EEG Result		
Normal	49	24.0
Epileptiform activity	67	32.8
Slowing	38	18.6
No EEG	50	24.5
Etiology		
Infection	2	1.0
Trauma	7	3.4
Stroke	20	9.8
Tumor	4	2.0
Other	27	13.2
Unknown	144	70.6
Number of anti-seizure medications at inclusion		
1	115	56.4
2	47	23.0
3	26	12.7
4	8	3.9
5	2	1.0
6	1	.5
No ASM	5	2.5

poor fit for tau ($R^2 = .032$, $p = .838$). Age was a significant predictor for NfL and GFAP, whereas sex and epilepsy duration were not significant predictors for any biomarker. Seizure status was a significant predictor of NfL levels, with seizures ≤2 months before inclusion being associated with higher levels compared to patients who were seizure-free >1 year ($B_{rel} = 37.9\%$, $p = .004$). Radiological result was also a significant predictor, with patients who had epileptogenic lesion presenting higher NfL levels ($B_{rel} = 35.0\%$, $p = .010$) and GFAP levels ($B_{rel} = 26.7\%$, $p = .038$) as compared to those with normal radiological findings. When further adjusting the model for number of ASMs (Tables S4–S6), the effect of seizure status on NfL was attenuated and no longer significant, given by $B_{rel} = 24.4\%$, for patients with seizures ≤2 months compared to patients who were seizure-free >1 year. Epileptogenic lesion on radiology was still significant for both NfL ($B_{rel} = 31.8\%$, $p = .015$) and GFAP ($B_{rel} = 25.3\%$, $p = .048$). Tau was not associated to any of the predictor variables.

3.7 | Anti-seizure medication

We finally analyzed whether a particular ASM was associated with high NfL levels (Table 2). Seventy-seven of 83 patients on lamotrigine (LTG) had below cutoff levels of NfL (92.8%, 95% CI 86–97, $p = .036$). Absolute levels of NfL

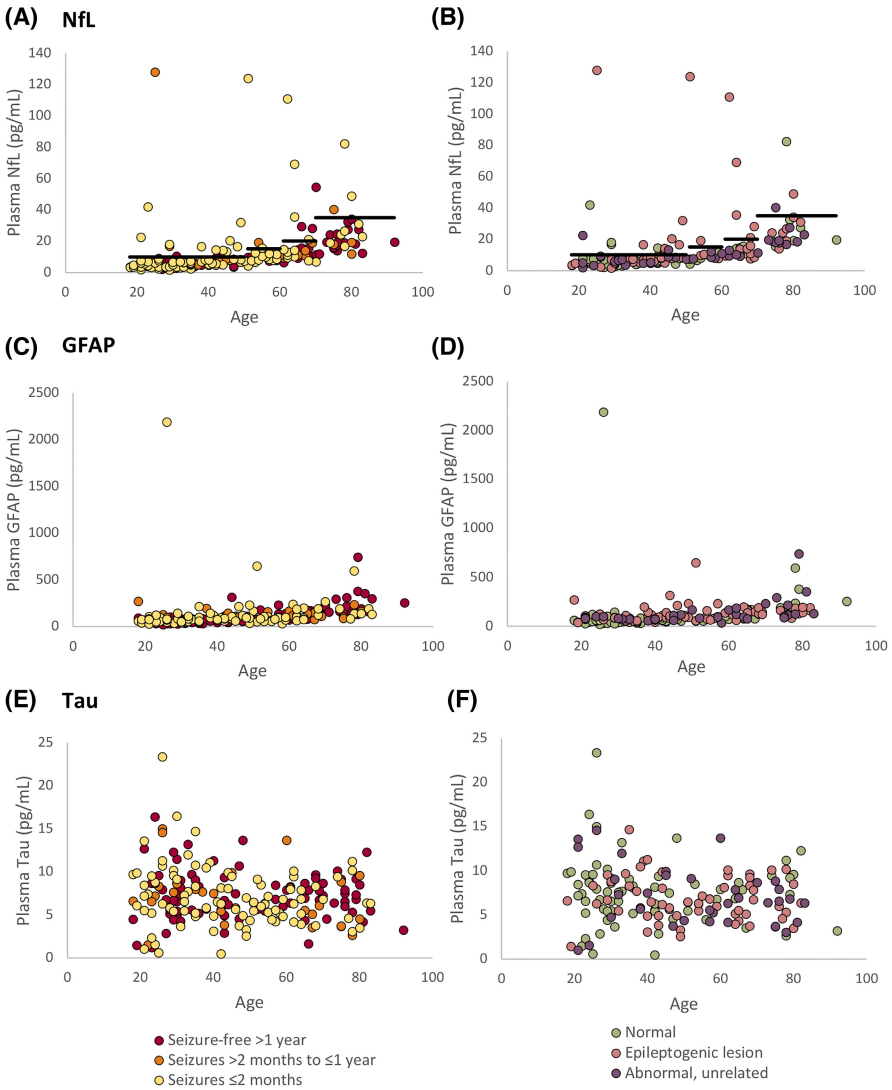

FIGURE 2 (A,C,E) Distribution of marker values across all ages according to seizure status. (B,D,F) Distribution of marker values across all ages according to radiology result. Black lines for NfL represent age-adjusted threshold values from Simrén et al.[22] (18–50 years: 10 pg/mL, 51–60: 15 pg/mL, 61–70: 20 pg/mL, >70: 35 pg/mL).

were also significantly lower in patients (<65 years) on LTG (mean 8.5 pg/mL vs 12 pg/mL, median 6.0 pg/mL vs 7.3 pg/mL, $p = .004$). The significance remained also when stratifying for patients exclusively taking LTG without any other ASM ($p = .011$). No differences in absolute levels were found for GFAP or tau. We also assessed if patients on VPA had higher levels of NfL, as VPA has been associated with cases of progressive brain atrophy. Of 11 patients on VPA, we found only 2 with levels above the threshold for NfL.

4 | DISCUSSION

Blood biomarkers of brain injury offer unprecedented possibilities for tracking brain health in vivo. In this cross-sectional investigation of established brain injury markers in a broad epilepsy cohort, our main finding is that only a few patients with epilepsy have NfL levels similar to those seen in degenerative or destructive disorders of the nervous system. This is particularly reassuring given our use of a cohort of patients recruited at neurology appointments, which selects toward more difficult-to-treat or complicated epilepsy. Our findings support the current notion that epilepsy and seizures, in most cases, do not cause brain damage comparable to that seen in neurodegenerative disorders.

Of interest, a small proportion of patients did have abnormally high biomarker levels for NfL. Most patients with abnormal biomarker levels had a previous stroke, in agreement with our earlier report of high NfL levels in poststroke epilepsy.[23] Persistent high levels after the stroke or ongoing cerebrovascular disease could explain the higher level in these cases. Previous stroke is common in epilepsy clinics, and more research is needed on blood biomarker levels specifically in poststroke epilepsy.

Aside from stroke, abnormal biomarker levels also seemed linked to seizures. More than 80% of patients with high NfL levels were not seizure-free. This fits well with previous reports of higher levels of NfL in adults with drug-resistant epilepsy,[20] and indicates that in cases where

TABLE 2 Number of patients with NfL levels above and below cutoff based on clinical characteristics.

	Plasma NfL	
	Below ($n=177$)	Above ($n=27$)
	n (%)	*n* (%)
Gender		
Male	89 (50.3)	17 (63.0)
Female	88 (49.7)	10 (37.0)
Seizure status		
Seizures ≤2 months	71 (40.1)	18 (66.7)[a]
Seizures >2 months to ≤1 year	26 (14.7)	4 (14.8)
No seizures for >1 year	80 (45.2)[a]	5 (18.5)
Seizure frequency		
Range (median)	0–98 (0)	0–50 (1)
25th Percentile (Q1)	0	0
75th Percentile (Q3)	2	3
Days since last seizure (last seizure to last clinic visit)		
25th Percentile (Q1)	23	5
75th Percentile (Q3)	1778	102
Median	318	40
NfL level (pg/mL)		
Mean (SD)	9.2 (6.5)	39.2 (34.5)
Median	7.3	22.5
Epilepsy duration (years)		
Range (median)	<1–73 (8)	<1–47 (9)
25th Percentile (Q1)	2	<1
75th Percentile (Q3)	20	22
Epilepsy diagnosis		
Focal	110 (62.1)	21 (77.8)
Generalized	33 (18.6)	4 (14.8)
Unknown	34 (19.2)	2 (7.4)
Tonic–clonic seizures	86 (48.6)	15 (55.6)
Radiological finding		
Normal	66 (37.3)	5 (18.5)
Epileptogenic lesion	43 (24.3)	15 (55.6)[a]
Abnormal, unrelated	31 (17.5)	3 (11.1)
No imaging	37 (20.9)	4 (14.8)
EEG Result		
Normal	46 (26.0)	3 (11.1)
Epileptiform activity	59 (33.3)	8 (29.6)
Slowing	31 (17.5)	7 (25.9)
No EEG	41 (23.2)	9 (33.3)
Etiology		
Stroke	12 (6.8)	8 (29.6)
Trauma	6 (3.4)	1 (3.7)
Infection	2 (1.1)	0 (0)
Tumor	4 (2.3)	0 (0)
Other	22 (12.4)	5 (18.5)
Unknown	131 (74.0)	13 (48.1)
ASM type		
Carbamazepine (CBZ)	23 (13.0)	7 (25.9)
Lamotrigine (LTG)	77 (43.5)[a]	6 (22.2)
Lacosamide (LCM)	18(10.2)	3 (11.1)
Topiramate (TPM)	19 (10.7)	3 (11.1)
Valproate (VPA)	9 (5.1)	2 (7.4)
Levetiracetam (LEV)	62 (35.0)	11 (40.7)

[a]Indicates a significant proportion of patients with marker levels either above or below NfL cutoff.

biochemical evidence of brain injury exists in epilepsy, so do seizures. Higher levels of GFAP have also been reported after seizures.[24,25] In our data, GFAP levels were higher in young patients with recent seizures and epileptogenic lesion. In addition, there was a group difference in NfL and GFAP levels between patients with and those without recent seizures. We had limited information about the nature of the recent seizures but tried to compare patients with focal seizures only to those who had ever experienced tonic–clonic seizures. We did not find higher biomarker levels in patients with epilepsy including tonic–clonic seizures than in patients with focal seizures only, suggesting that the extent of the epileptogenic network is not very important for biomarker levels. Future studies should collect more information about the nature of all recent seizures and attempt to correlate marker levels to seizure types. At lower levels, one can speculate whether the markers may not necessarily reflect minor brain damage but instead network reorganization or plasticity.

The PREDICT biobank is a regional study with one blood sample collected at inclusion. The use of a regional cohort enabled the analysis of biomarker levels in a group of patients whose age and sex closely resemble that of all patients seen for epilepsy in the health care region. Our population was representative of different epilepsy types and radiological findings; we, therefore, could also investigate possible associations of biomarker levels with various clinical characteristics. The cross-sectional design does not offer longitudinal analysis, making it challenging to interpret the correlations of NfL and GFAP with seizure number or recency. Measurement errors most

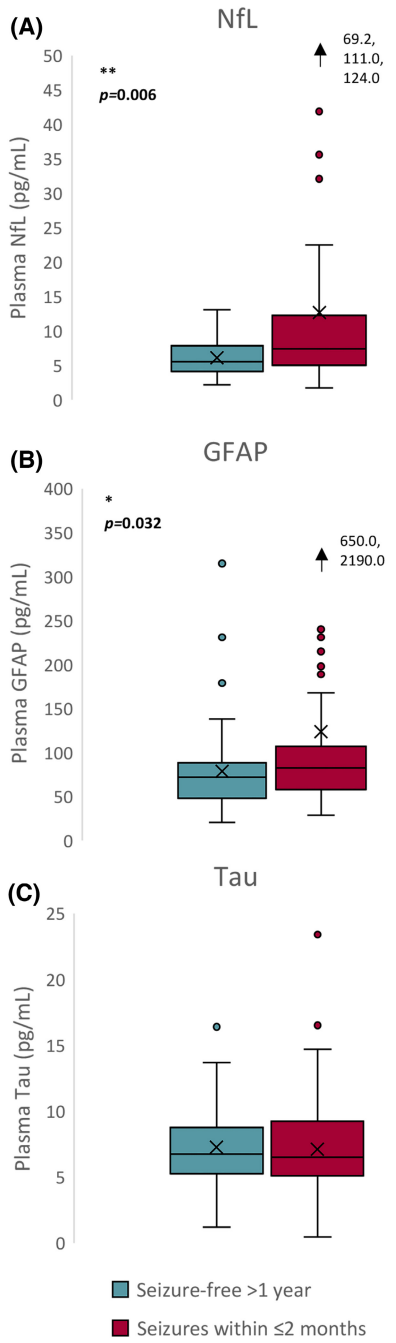

FIGURE 3 Comparison between marker values in patients <65 years using Mann–Whitney *U* test (significance set at $p \leq .05$). Significant differences were observed for neurofilament-light (NfL) ($p = .006$) and glial fibrillary acidic protein (GFAP) ($p = .032$) between seizure-free patients (no seizures >1 year) and patients with recent seizures (seizures ≤2 months).

likely exist; it is difficult for patients to recall and report the number of seizures. Similarly, we have no estimate of subclinical seizure activity. Body mass index (BMI) was not collected in our data set, so the possible association between plasma NfL and BMI was not accounted for. The ASM analyses should also be interpreted with caution. First, some patients were taking multiple ASMs simultaneously making it difficult to uncover the specific effects of each individual medication. The analyses may also be confounded by factors such as epilepsy severity and epilepsy duration, which influence the type and number of medications prescribed.

It is important to note that the correlations between NfL and GFAP with the number of recent seizures or time since last seizures were weak. Our interpretation is that we are unlikely to find a specific cutoff for any of the investigated markers indicating seizures that is useful for most patients with epilepsy. Rather than such cutoffs for these biochemical markers, individual dynamics in relation to seizures may prove informative. A definite answer to this question will require repeat blood sampling. Our results suggest that NfL should be the biomarker of choice for such analyses; the marker was identified as elevated in most patients, and closely correlated to GFAP. Differences between biomarker levels in patients taking different ASMs also need further study; our finding regarding LTG could for instance represent age or duration of epilepsy. In this regard, we plan to extend our study by re-analyzing the same biomarkers on a larger cohort of patients, allowing us to perform additional analyses, such as syndrome-specific analyses for epilepsies such as TLE.

5 | CONCLUSION

Our main finding is that most patients with epilepsy do not have biochemical evidence of extensive brain injury, in line with the current understanding of epilepsy as a dysfunctional connectivity and/or dynamic of neuronal networks in the brain. Higher levels were associated with symptomatic etiology and future studies should assess whether biochemical markers could be useful, for instance, as indicators of a need for radiological vigilance or additional ASM treatment. Although the biomarker levels were not necessarily comparable to those observed in neurodegenerative disorders, our findings still suggest the possibility of less severe neurodegenerative changes in a subset of people with epilepsy that needs more investigation. Notably, we observed an association between NfL and GFAP with recent seizures, and an interesting question is whether this could be prevented by improved seizure control. The relationship between seizures and NfL is intriguing, although our study indicates that clear cutoffs indicating recent seizures may not be attainable. Instead, individual tracking of NfL could be a way forward, or a search for more-specific markers of seizures.

AUTHOR CONTRIBUTIONS

S.A.: manuscript drafting, data analyses, and interpretation. F.A.: recruitment and clinical data collection. R.B.:

biomarker analyses. M.A.: data analyses and interpretation. H.Z.: biomarker analyses. J.Z.: study conceptualization, recruitment, clinical data collection, manuscript drafting, data analyses, and interpretation. All authors revised the manuscript for intellectual content.

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CONFLICT OF INTEREST STATEMENT

H.Z. has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave; has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Rochel and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). J.Z. has received consultancy fee from the Swedish Medical Products Agency; speaker honoraria from UCB and Eisai for non-branded education events; and as employee of Sahlgrenska University Hospital is or has been an investigator/sub investigator in clinical trials sponsored by GW Pharma, SK life science, UCB, and Bial (no personal compensation). F.A. has received speaker honoraria from Angelini Pharma Nordics for non-branded education events. The remaining authors have no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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