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## Brain injury markers in new-onset seizures in adults: A pilot study

Hanna Eriksson<sup>a,b,c</sup>, Rakesh Kumar Banote<sup>a,b</sup>, David Larsson<sup>a,b,c</sup>, Kaj Blennow<sup>d,e</sup>,  
Henrik Zetterberg<sup>d,e,f,g</sup>, Johan Zelano<sup>a,b,c,\*</sup>

<sup>a</sup> Department of Clinical Neuroscience, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

<sup>b</sup> Department of Neurology, Sahlgrenska University Hospital, Gothenburg, Sweden

<sup>c</sup> Wallenberg Center of Molecular and Translational Medicine, Gothenburg University, Gothenburg, Sweden

<sup>d</sup> Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden

<sup>e</sup> Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden

<sup>f</sup> Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London, UK

<sup>g</sup> UK Dementia Research Institute at UCL, London, UK

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## ABSTRACT

**Background:** Biochemical markers of brain pathology could potentially contribute to diagnosis and prediction in epilepsy. We describe levels of five brain injury markers in adults with new-onset seizures, and assess group differences in patients with a single seizure, epilepsy, and poststroke epilepsy.

**Methods:** In this prospective observational study, adults with new-onset seizures were recruited at Sahlgrenska University Hospital, Sweden, and concentrations of glial fibrillary acidic protein (GFAP), neurofilament light (NfL), microtubule-associated protein tau (tau), S100 calcium-binding protein (S100B), and neuron-specific enolase (NSE) were measured. Participants were categorized as epilepsy, poststroke epilepsy (PSE), or single seizure (no additional seizures). Patients were followed until a diagnosis of epilepsy or PSE, or for at least two years in single seizure cases.

**Results:** The cohort included 23 (37%) individuals with a single seizure, 24 (39%) with epilepsy, and 15 (24%) with PSE. The concentrations of S100B were higher in patients with epilepsy and PSE than in single seizures ( $p = 0.0023$  and  $p = 0.0162$ , respectively). The concentrations of NfL were higher in patients with PSE than in single seizures ( $p = 0.0027$ ). After age-normalization, levels of S100B were higher in patients with epilepsy and levels of NfL were higher in patients with PSE ( $p = 0.0021$  and  $p = 0.0180$ ).

**Conclusion:** Levels of S100B and NfL were higher in patients with epilepsy or PSE than patients with single seizures. Further studies are needed to investigate the biomarker potential of brain injury markers as predictors of epilepsy course or indicators of epileptogenesis.

## 1. Introduction

There is a need for biomarkers in epilepsy, especially markers that can be used to support diagnosis and prognostication early in the disease course. In patients with a first seizure, large structural brain lesions visible on brain imaging increase the risk of seizure recurrence [1]. Whether brain pathology capturable on a biochemical level has prognostic or diagnostic value is not known.

Biochemical brain injury markers are increasingly used in neurology. Prognostication of neurotrauma, dementia diagnosis, and intensive care monitoring are some applications [2,3]. For the purpose of this study, we selected brain injury markers in clinical use that reflect a range of

pathological processes in the CNS. Neurofilament light (NfL) reflects axonal injury [4]. Tau levels increase in response to amyloid plaque exposure [5], but also after stroke, hypoxia, or trauma [6]. Increased GFAP levels reflect astroglial activation or injury across a broad range of acute and chronic neurological diseases [7–11]. Neuron-specific enolase (NSE) is a cytoplasmic neuronal protein indicative of brain injury which is used to assess brain damage prognostication after cardiac arrest [12]. S100 calcium-binding protein (S100B) is an astrocyte marker used to assess head trauma [13] and blood–brain barrier dysfunction [14]. Brain injury markers have attracted some interest in epilepsy. NfL levels can increase after prolonged febrile seizures in children and in older individuals with drug-resistant epilepsy [15,16]. Children with new-onset

\* Corresponding author at: Department of Neurology, Sahlgrenska University Hospital, Blå stråket 7, 41345 Gothenburg, Sweden.

E-mail address: [johan.zelano@neuro.gu.se](mailto:johan.zelano@neuro.gu.se) (J. Zelano).

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epilepsy have higher levels of S100B than controls [17].

Blood biomarkers that can assist in early identification of epilepsy would be very useful. We recruited adults with new-onset seizures, and evaluated levels of five brain injury markers. The objective of the study was to investigate if levels of brain injury markers differed between patients with single seizures and epilepsy.

## 2. Methods

### 2.1. Study cohort

This was a prospective longitudinal study. Patients with a new-onset seizure referred to the Department of Neurology at Sahlgrenska University Hospital in Gothenburg between June 2016 and June 2019 were included. The inclusion criteria were >25 years of age, and an unprovoked new-onset seizure. A new-onset seizure was defined as a seizure for which the patients sought medical care for the first time. Exclusion criteria were inability to give informed consent or progressive structural cerebral disease. Recruitment was not consecutive, but opportunistic.

### 2.2. Study procedures and classification

A total of 73 patients were included. Blood samples were obtained at the inclusion visit and the participants were followed yearly by visits or telephone calls in addition to medical chart review. Five patients had uncertain basis for the diagnosis preventing certain classification, and six patients had insufficient follow-up, leaving 62 patients for the final analysis.

Patients were prospectively followed until a diagnosis of epilepsy (EP), poststroke epilepsy (PSE), or single seizures (SS) could be made. Classification of single seizure required at least two years of seizure-free follow-up. PSE was separated from other epilepsy because a first unprovoked seizure after stroke indicates epilepsy according to the ILAE [18], and because of the distinct structural brain injury which may itself cause elevation of brain injury markers [19]. PSE was diagnosed in cases of at least one single seizure >7 days after a stroke. Stroke or old infarcts were assessed by computed tomography (CT) as part of the routine examination after a first asymptomatic seizure. Epilepsy was diagnosed based on two or more unprovoked seizures occurring more than 24 h apart [18]. Patients with acute symptomatic seizures were not included.

### 2.3. Blood samples

Blood samples were obtained at the first visit in ethylenediaminetetraacetic acid (EDTA) tubes for plasma, and gel tubes for serum. Both EDTA tubes and serum tubes were centrifuged. The EDTA tubes were centrifuged for 10 minutes in room temperature in order to collect plasma. The samples were stored at -80°C after aliquotation.

### 2.4. Measurements of markers

Serum S100 calcium-binding protein B (S100B) and NSE concentrations were measured using ElectroChemiluminescence Immunoassay (ECLIA) on the Elecsys platform (Roche Diagnostics, Penzberg, Germany). Intra-assay coefficients of variation were 2.6% or lower. Plasma NfL, tau and GFAP concentrations were measured using commercially available kits on a Single molecule array (Simoa) HD-1 Analyzer, according to instructions provided by the manufacturer (Quanterix, Billerica, MA). Intra-assay coefficients of variation were 7.3% or lower. The biomarker measurements were performed in one round of experiments by board-certified laboratory technicians who were blinded to clinical data.

### 2.5. Statistical analyses

Data were analyzed using IBM SPSS Statistics version 26.0 for

Windows or GraphPad Prism®. All tests were 2-sided and significance was set at  $p \leq 0.05$ . Correlation analyses were performed using Pearson correlation coefficient test. Group differences were assessed between patients with single seizures and the epilepsy or PSE group, respectively, by non-parametric Kruskal-Wallis test with Benjamini-Hochberg adjustment for multiple comparisons. The Kruskal-Wallis statistical method function with outliers allows comparisons of ordinal groups (SS, EP and PSE). Comparison of brain injury marker levels in participants with and without ASM was performed with unpaired two-sample *t*-test.

The levels of some brain injury markers increase with age [19–21]. We therefore performed age-adjusted analyses, in which concentrations were normalized to the single seizure group-mean for the corresponding age group.

## 3. Results

The study cohort consisted of 62 patients, which were followed prospectively and classified as single seizure ( $n = 23$ ), epilepsy ( $n = 24$ ), or PSE ( $n = 15$ ). Epilepsy was either diagnosed during follow-up, or at the initial visit if the history revealed previous seizures that had not received medical attention. Seventeen (71%) of the patients in the epilepsy group had their first-ever seizure in the six months before the blood test.

Patients in the PSE group were older than patients in the single-seizure or epilepsy groups (Table 1). The median time from the first seizure to the blood test was just over one month in the single seizure group and just over two months in the PSE group. The stroke date was available for 14 PSE cases; with a median time from stroke to blood test of 429 days (IQR: 245). In some cases, brain imaging by CT or MRI showed abnormalities of uncertain clinical relevance; in the PSE group there was one case of frontal encephalomalacia, in the epilepsy group, arachnoidal cyst, focal cortical dysplasia, and possible posttraumatic findings, and in the single seizure group asymmetry of the temporal lobe, mycotic aneurysm, and frontal calcification.

### 3.1. Brain injury marker levels

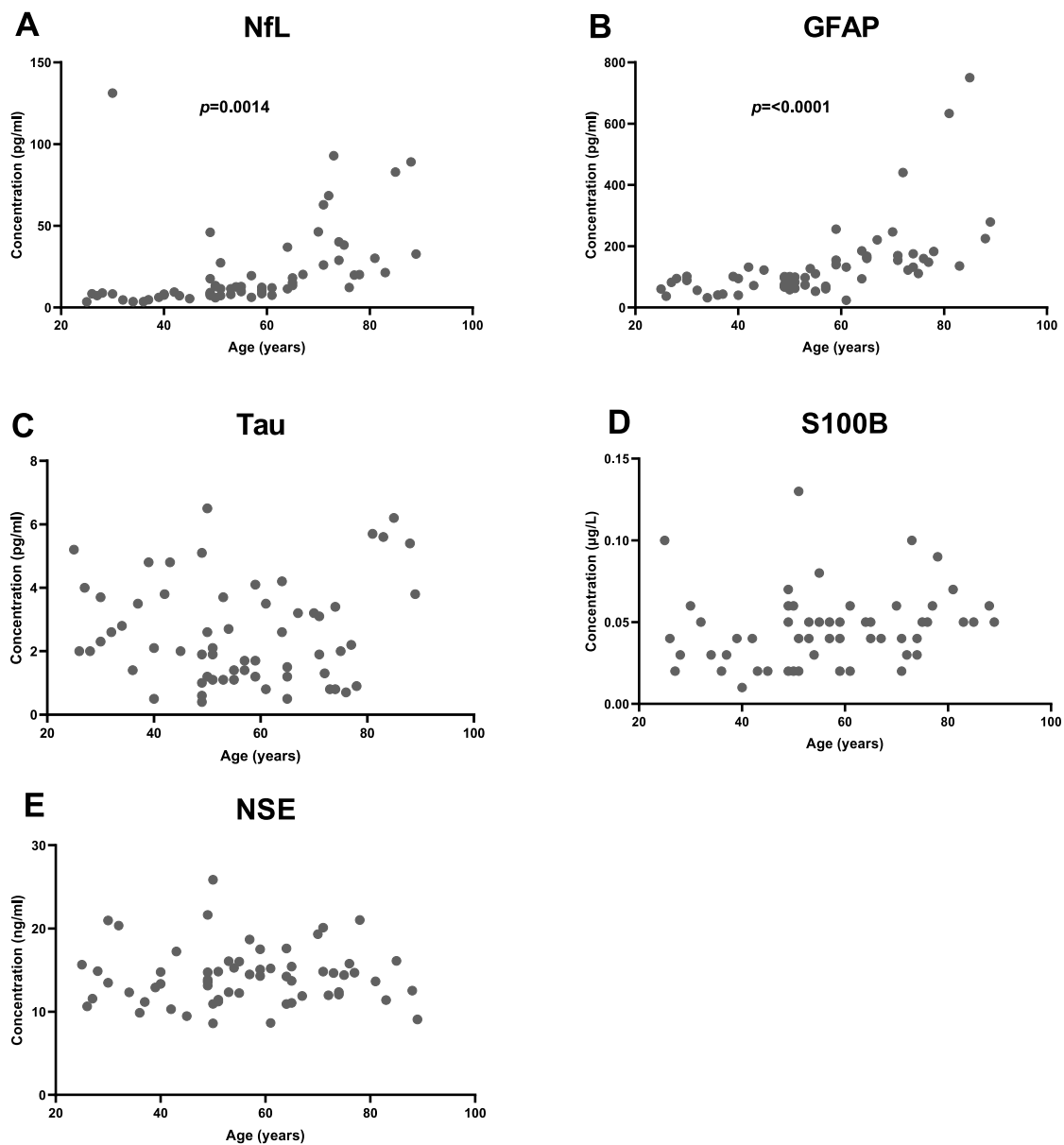
Because of the age differences between groups, we assessed associations between marker levels and age (Fig. 1). NfL and GFAP levels increased significantly with age ( $p = 0.0014$  and  $p \leq 0.0001$  respectively) (Fig. 1 A,B).

Next, we assessed absolute markers levels in all three groups (Fig. 2). The concentrations of S100B were higher in patients with epilepsy and PSE than in single seizures [mean concentration (ug/L): SS 0.033, EP 0.052 and PSE 0.051,  $p$  values: SS vs EP  $p = 0.0023$  and SS vs PSE  $p = 0.0162$ ] (Fig. 2 D). The concentrations of NfL were higher in patients

**Table 1**  
Patient characteristics by single seizure, epilepsy and poststroke epilepsy (PSE).

	Single seizure ( $n=23$ )	Epilepsy ( $n=24$ )	PSE ( $n=15$ )
Age at test Median (IQR)	53 (21)	51 (30)	72 (23)
Female sex (%)	11 (48)	16 (67)	5 (33)
<b>Clinical characteristics</b>			
Days from first-ever seizure	38 (30)	87 (286)	43 (64)
Median (IQR)			
<b>Imaging</b>			
Hemorrhagic stroke (%)			5 (33)
Ischemic stroke (%)			10 (67)
Other (%)	3 (13)	3 (13)	1 (7)

PSE = poststroke epilepsy. IQR = interquartile range. Days are from first ever seizure until first inclusion visit. Imaging was done with computerized tomography alone or in combination with magnetic resonance imaging. Other = possible epileptogenic findings ( $n = 1$  per finding); SS: asymmetry of temporal horns, mycotic aneurysms, and frontal calcification. EP: arachnoidal cyst, possible posttraumatic changes, and focal cortical dysplasia. PSE: possible frontal encephalomalacia.



**Fig. 1.** Correlation between biomarkers and age in the cohort by Pearson correlation ( $n = 62$ ). NfL and GFAP showed a significant correlation with age. Tau, S100B and NSE levels were not associated with age.

with PSE than in single seizures [mean concentration (pg/mL): SS 14.08, EP 19.12 and PSE 38.95,  $p$  value: SS vs PSE  $p = 0.0027$ ] (Fig. 2 A). Similarly, the concentration of GFAP was higher in patients with PSE than in single seizures [mean concentration (pg/mL): SS 122.32, EP 115.75 and PSE 197.47,  $p$  value: SS vs PSE  $p = 0.0243$ ] (Fig. 2 C). We found no significant differences in absolute concentrations for Tau or NSE between the groups (Fig. 2 B,E).

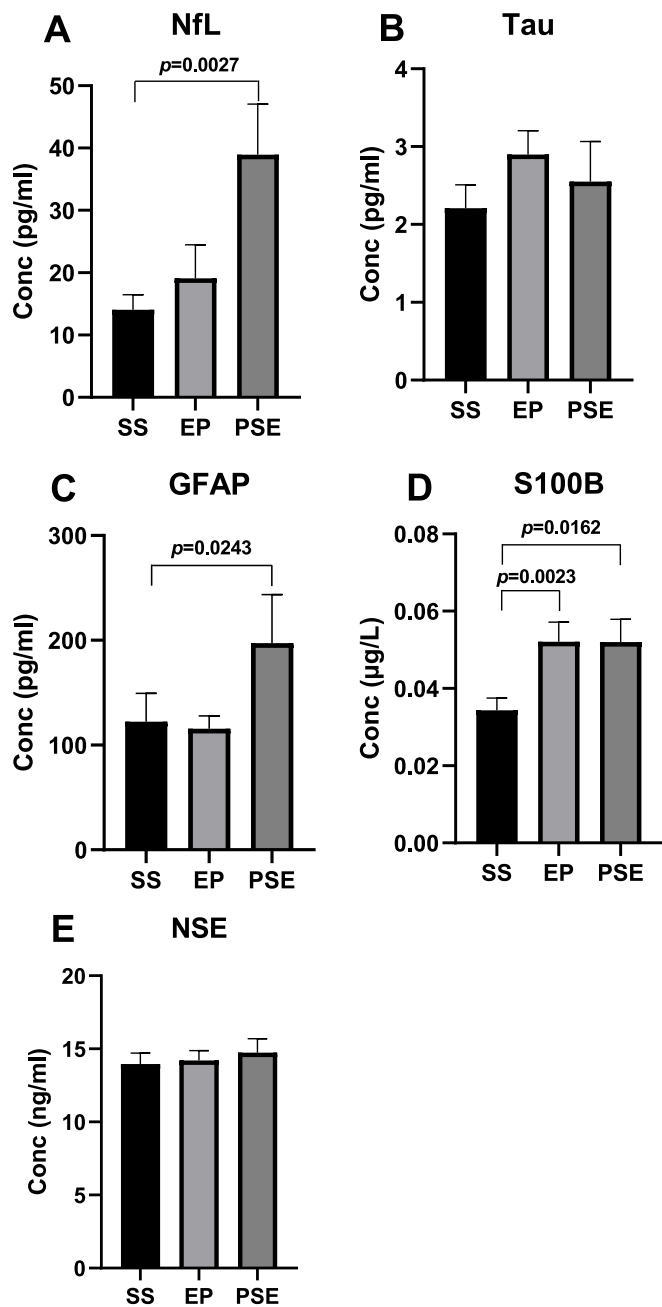
To address potential influence by age, we divided the participants into three similarly sized age groups (25-49, 50-61 or 64-89 years) and normalized the concentrations to the single seizure group. Similarly to the analysis of absolute values, a significant difference was seen for S100B between epilepsy and single seizures ( $p = 0.0021$ ) (Fig. 3 D), and for NfL between PSE and single seizure ( $p = 0.0180$ ) (Fig. 3 A).

Some patients had antiseizure medication (ASM) at the time when the brain injury markers were collected; 9 (38 %) with EP, 14 (93 %) with PSE, and none from the SS group. There were no significant differences in the level of any brain injury marker between patients with or without ASM in the EP group.

#### 4. Discussion

We describe levels of a panel of brain injury markers in adults with new-onset seizures. Our main finding is that S100B concentrations were higher in patients with epilepsy, both in absolute and age-adjusted analyses. Additionally, levels of NfL were higher in patients with PSE, but the likely contribution of the stroke makes the biomarker potential of NfL with regard to epilepsy more difficult to assess. Similarly, absolute plasma GFAP was increased in PSE patients compared to patients with single seizures. Larger studies are needed. Nonetheless, our findings suggest that biochemical brain injury markers could be clinically useful for identification of early epilepsy, and indicate a need for more studies of their use in new-onset seizures.

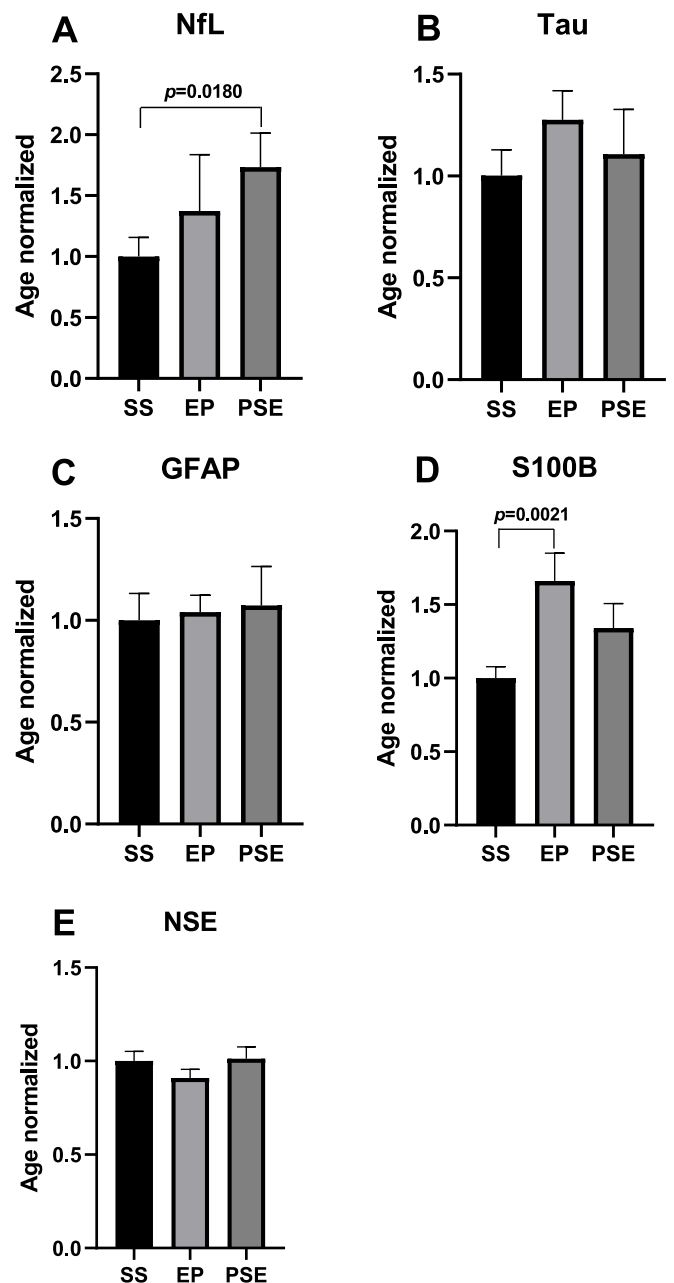
The biomarkers investigated in our study could reflect different pathological processes and temporal dynamics in epileptogenesis, so our pragmatic study design may not have captured all intricacies. NfL has a slow temporal course; the biomarker peaks approximately two weeks after acute injury with an apparent half-life of 2-3 months [8]. Tau and GFAP, on the other hand, show a faster increase, which becomes



**Fig. 2.** Absolute levels of NfL, tau, GFAP, S100B and NSE in single seizure, epilepsy and poststroke epilepsy (PSE). A significant difference was seen in the absolute levels of NfL and S100B in patients with PSE compared to patients with single seizures. Also, S100B levels were found to be significantly higher in epilepsy patients compared to single seizures. No significant difference was seen in GFAP, tau and NSE when comparing single seizure with other groups.

apparent already a few hours post-injury in acute neurological diseases, but the half-lives are around 10-20 hours [3]. In our data, there was variability in NfL and tau levels in the epilepsy group, indicating a need for more detailed studies. Unlike NfL, tau levels in CSF and blood are not well correlated, for unknown reasons [22], but possibly because tau protein also is produced in peripheral tissues. Tau and NSE were very similar in all groups in our study, perhaps indicating less promise for these biomarkers in early epilepsy. Epilepsy has been associated with accumulation of hyperphosphorylated tau in the brain [23,24], but our measurements of total tau blood levels in the new-onset seizure setting were not able to capture any such process.

Several investigators have demonstrated elevated brain injury



**Fig. 3.** Age-normalized values of NfL, tau, GFAP, S100B and NSE in single seizure, epilepsy and poststroke epilepsy (PSE). NfL was significantly higher in the PSE group compared to the single seizure group. S100B was significantly elevated in the epilepsy group compared to the single-seizure group.

marker levels in epilepsy, but there is not much data on levels in adults with new-onset seizures. Our results are well in line with previous studies of elevated S100B levels in epilepsy [25–27], which indicate that this marker should be investigated further for biomarker potential early in the disease course and perhaps as a marker of seizure burden. Pathophysiologically, increased levels of S100B could result from disruption of the blood brain barrier [14,28,29]. Bargerstock *et al.* found increased levels of S100B in patients at seizure onset, and the authors suggest that the immunomodulation by released S100B could contribute to epileptogenesis [30]. Our material was too small to allow analysis of timing in relation to the last seizure and controls without any seizure at all would have been an interesting comparator for the SS group, but more studies on S100B in epilepsy seem motivated.

We also analyzed patients diagnosed with PSE, which constitute a

distinct group because of the presence of structural brain lesions and the known high recurrence risk allowing epilepsy to be diagnosed already after a first seizure [18]. Somewhat unsurprisingly, because of the previous stroke, we found higher levels of NfL and S100B in patients with PSE than in patients with single seizures. Since epilepsy is diagnosed already at the first seizure in this patient group, the prognostic value of biomarkers in this group is limited, but the levels may provide neurobiological insight. Interestingly, a study by Abaira *et al.* found that decreased levels of S100B six hours after stroke was independently associated with the development of poststroke epilepsy [31]. The increased levels detected in our study could therefore perhaps be specifically related to subsequent epileptogenesis. Alternatively, the high levels of S100B may simply reflect the higher age of PSE patients or a later response to the previous stroke; we have previously described peak S100B levels at 48–72 hours in stroke patients that later developed PSE [32]. The elevated levels of NfL and GFAP long after the stroke in the PSE group, despite the relatively short half-life of these markers, are interesting. This finding could perhaps reflect more severe brain injury, a prolonged injury response, or other processes related to epileptogenesis. More studies are needed on the capacity of brain injury markers to predict poststroke epilepsy.

There are a number of limitations to our study. First, the number of patients included was quite small and for practical reasons consecutive recruitment was not possible. Second, it could be of value to measure the markers at different time points after a new-onset seizure. We included patients that were referred for a first assessment of seizures, but in many instances in the epilepsy group, clinicians identified previous seizures – resulting in a heterogenous epilepsy duration and seizure frequency before the blood test. Most patients in the EP and SS groups had normal imaging, but it is possible that larger or differently designed studies could identify different marker levels in patients with abnormal imaging due to different underlying brain pathologies. Thus, the study is of a pilot nature – but indicates a need for more and larger studies on more homogenous groups tailored to the research question: single seizure patients for detection of epileptogenesis and epilepsy patients with different seizure frequency for studies of prognosis and seizure burden quantification.

## 5. Conclusion

We conclude that S100B levels are higher in patients with new-onset epilepsy compared to patients with a single seizure. Future studies on brain injury markers should aim to identify biomarker profiles indicative of different phases of epileptogenesis.

## Declaration of Competing Interest

JZ 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).

KB has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu, Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, all outside the work presented in this paper.

HZ has served at scientific advisory boards for Eisai, Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics, Nervgen, AZTherapies and CogRx, has given lectures in symposia sponsored by Cellectricron, Fujirebio, Alzecure and Biogen, 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).

The funding source had no involvement in the process of designing

the study, data analysis, interpretation of data, writing process or submission of the manuscript.

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KB is supported by the Swedish Research Council (#2017-00915), the Alzheimer Drug Discovery Foundation (ADDF), USA (#RDAPB-201809-2016615), the Swedish Alzheimer Foundation (#AF-742881), Hjärtfonden, Sweden (#FO2017-0243), the Swedish state under the agreement between the Swedish government and the County Councils, the ALF-agreement (#ALFGBG-715986), the European Union Joint Program for Neurodegenerative Disorders (JPND2019-466-236), and the National Institute of Health (NIH), USA, (grant #1R01AG068398-01).

## Availability of data and material

Anonymized data are available for research upon request.

## Code availability

N/A

## Authors contributions

HE: analyses, manuscript draft. RB: analyses, manuscript draft. DL: clinical data collection HZ and KB: biomarker analyses. JZ: study conceptualization, recruitment, clinical data collection, analyses, manuscript draft. In addition, all authors revised the manuscript for intellectual content.

## Ethics approval

The regional boards of ethics in Gothenburg (approval number 844-15) approved the study which was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

## Consent to participate/publication

All patients provided written informed consent prior inclusion to the study, including consent to publication in scientific journals.

## Acknowledgement

N/A.

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