Hindawi
Acta Neurologica Scandinavica
Volume 2023, Article ID 7246373, 5 pages
https://doi.org/10.1155/2023/7246373

Research Article

Serum Markers of Neuronal Damage and Astrocyte Activity in Patients with Chronic Epilepsy: Elevated Levels of Glial Fibrillary Acidic Protein

Monika Mochol,[1,2,3] **Erik Taubøll**,[2,3] **Pål Aukrust**,[3,4,5] **Thor Ueland**,[3,5,6] **Ole A. Andreassen**,[7] **and Sigrid Svalheim**[2]

[1]*Department of Neurology, Østfold Hospital Trust, Norway*
[2]*ERGO-Epilepsy Research Group of Oslo, Department of Neurology, Oslo University Hospital, Norway*
[3]*Institute of Clinical Medicine, University of Oslo, Norway*
[4]*Section of Clinical Immunology and Infectious diseases, Oslo University Hospital, Norway*
[5]*Research Institute of Internal Medicine, Oslo University Hospital, Norway*
[6]*K.G. Jebsen Thrombosis Research and Expertise Center, University of Tromsø, Norway*
[7]*NORMENT Centre, Institute of Clinical Medicine, University of Oslo and Division of Mental Health and Addiction, Oslo University Hospital, Norway*

Correspondence should be addressed to Monika Mochol; monikamochol@hotmail.com

Received 31 August 2022; Revised 8 November 2022; Accepted 26 December 2022; Published 7 February 2023

Academic Editor: Elinor Ben-Menachem

Copyright © 2023 Monika Mochol et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Objectives. Blood-brain barrier (BBB) dysfunction is one of the key pathogenic mechanisms in the development of epilepsy. There is therefore an increasing need to identify BBB biomarkers as these will have prognostic and therapeutic implications. The purpose of this study was to assess the levels of the BBB permeability markers, glial fibrillary acidic protein (GFAP), neuron-specific enolase (NSE), S100B, and furin in patients with stable epilepsy compared with the levels in healthy controls. *Materials and Methods*. This cross-sectional study included 119 epilepsy patients and 80 healthy controls. Circulating levels of GFAP, NSE, S100B, and furin were measured and questionnaires regarding epilepsy, use of drugs, and comorbidities were completed by all participants. *Results*. GFAP levels were higher in epilepsy patients after adjustment for potential confounders (sex, age, and BMI) in linear regression ($p = 0.042$). No significant differences were found in levels of S100B, NSE, or furin. None of the markers were significantly associated with epilepsy duration, seizure type or severity, or seizures in the preceding six months. The majority of the patients (79.7%) did not report seizures within the last 6 months. *Conclusion*. Our main finding is elevated serum levels of GFAP in epilepsy patients. The results may suggest the presence of astrocyte activation in our patient population with stable epilepsy. Future prospective studies focusing on the longitudinal relationship between epilepsy debut, seizures, and time of blood sampling for BBB markers, also within CSF, could provide valuable knowledge including regarding novel treatment options. The study registration number is 2011/1096, 2018/1437.

1. Introduction

The blood-brain barrier (BBB) plays an important role in homeostasis and protection of the brain against various molecules that may otherwise gain entry. Impaired function of BBB is considered as one of the mechanisms underlying epileptogenesis. Studies in animal models and humans have shown BBB disruption during both acute epileptic seizures and in chronic epilepsy [1, 2]. BBB dysfunction allows passage of brain-specific proteins into peripheral circulation following gradient concentration, and also allows extravasation of serum albumin, as well immune cells and their

inflammatory products, into the brain. This could promote hyperexcitability and changes that contributes to epileptic seizure development [1, 3].

Glial fibrillary acidic protein (GFAP) is a highly brain-specific astrocyte cytoskeletal protein and is released during reactive astrogliosis. This is often seen as a consequence of neuronal injury induced by seizures [4]. S100B is a Ca^{2+}-binding protein, mainly concentrated in astrocytes, and is recognized as a biomarker of neuronal distress [5]. An elevated serum concentration of S100B is also associated with epileptic seizures [6]. Neuron-specific enolase (NSE) originates from the neuronal cytoplasm and neuroendocrine cells and is released into peripheral blood during neuronal damage. Elevated serum levels of NSE have been reported shortly after epileptic seizures and status epilepticus (SE) as well as in patients with cerebrovascular disorders [7–10]. GFAP, S100B, and NSE are considered as biomarkers of neuronal and astrocytic injury. Impaired BBB integrity will allow transportation of those proteins into to the blood. Increased protein blood concentrations are already used as clinical markers for BBB disruption, indicating neuronal damage and increased astrocyte activity [11]. Recently, the protease enzyme furin was described as a potential biomarker of neuronal injury. Furin is localized in brain tissue, mainly in neurons. Studies in animal models of chronic epilepsy have indicated a role in seizure susceptibility [12]. Other studies have described furin having an impact on hypoxia-induced BBB permeability and blocking of furin improves BBB stability [13].

Identifying the serum levels of biomarkers that indicate BBB disruption and neuronal damage has been of great clinical interest in recent years, and a simple quantitative method will have both diagnostic and prognostic value.

Most of the research studying inflammation biomarkers is conducted in the acute phase shortly after seizures, SE, or in highly drug-resistant epilepsy patients. The purpose of the current study was to investigate whether circulating levels of GFAP, S100B, NSE, and furin were dysregulated in patients with chronic and rather stable epilepsy compared with healthy controls. We find this patient population of special interest as aggravation of epilepsy and increasing seizure frequency is observed also in some patients with previously stable epilepsy. We further evaluated whether the concentrations of BBB markers correlated with seizure type and frequency, epilepsy type, epilepsy debut, and use of antiseizure medications (ASMs).

2. Materials and Methods

2.1. Study Population. The study population has been described in detail in Mochol et al. [14]. Adult patients with either focal or generalized epilepsy, along with healthy controls, were included in this cross-sectional study. The 119 patients were recruited from neurological departments attending hospitals in and around Oslo, Norway. None of the participants had autoimmune or infectious disorders or malignancies. All patients had been treated with either carbamazepine (CBZ), lamotrigine (LTG), or levetiracetam (LEV) in monotherapy for at least six months before inclusion. The 80 participants in the control group were recruited from among students, hospital staff, and the general population of Oslo, Norway.

The study was approved by the Regional Committee for Medical and Health Research Ethics (REC Norway–2018/1437).

2.2. Data Collection and Clinical Characteristics. All patients were assessed by neurologists. The participants completed standardized questionnaires regarding their demographic and clinical characteristics. Patients were grouped according to their use of ASM (LEV, CBZ, or LTG monotherapy). Further division into subgroups was based on seizure type (generalized or focal), total number of seizures during lifetime, and occurrence of seizures during the previous six months. Those patients with fewer than five epileptic seizures in total were classified as "low-seizure frequency during the lifetime," patients with between five and ten seizures as "moderate-seizure frequency," and those with more than ten seizures during lifetime were classified as "high-seizure frequency".

2.3. Measurement of Cytokines. Venous blood samples were collected in the morning, and the plasma was immediately isolated and stored at -80°C before analysis. Concentrations of GFAP, S100B, NSE, and furin levels were determined in duplicate serum samples using antibodies from RnDsystems (Stillwater, MN) in a 384-well format, using a combination of a SELMA (Jena, Germany) pipetting robot and a BioTek (Winooski, VT) dispenser/washer. Absorptions were read at 450 nm, with wavelength correction set to 540 nm, using an ELISA plate reader (BioTek). Intra- and interassay coefficients were <10%.

2.4. Statistical Analyses. Demographic data, clinical characteristics, and subgroup analysis are shown using descriptive, statistics including frequency and proportions for categorical variables; means with standard deviations (SD) or medians with ranges were used for continuous variables. Groups were compared by Pearson's chi-squared test, Student's t-test, and Mann–Whitney U test, as appropriate. Coefficients of correlation were calculated by Spearman's rank test for comparison of two continuous variables. Binary and multinomial logistic regression with correction for age and sex were used to investigate association between dichotomous and continuous variables. Analyses, including correction for potential confounders, were done by linear regression on log or Box-Cox transformed values of S100B, GFAP, NSE, and furin. Probability values (two-sided) were considered significant at $p < 0.05$. All calculations were performed with SPSS for Windows statistical software (version 28.0; SPSS Inc., Chicago, IL).

3. Results

3.1. Clinical Characteristics. A total of 119 patients with epilepsy and 80 healthy controls were included in the study. Results from demographic data have been detailed in our previous paper [14]. The epilepsy patients had a higher percentage of men ($p = 0.003$), were slightly older ($p = 0.004$),

Table 1: Serum levels of GFAP, NSE, S100, and furin in healthy control group and patients with epilepsy.

	Mann–Whitney U test			Mann–Whitney U test		
	Controls (range) $N = 80$	Patients (range) $N = 119$	p value*	CBZ (range) $N = 55$	LTG (range) $N = 49$	LEV (range) $N = 15$
GFAP (pg/ml)	170 (60-15000)	190 (70-15000)	**0.042**	184 (77-12335)	176 (70-6809)	275 (90-1500)
NSE (ng/ml)	4.1 (1.9-33.7)	4.4 (2.0-12.4)	0.587	4.6 (2.1-12.4)	4.4 (2.2-12.4)	4.4 (2.0-10.9)
S100B (ng/ml)	130 (89-1962)	119 (80-4129)	0.112	117 (84-4129)	126 (80-914)	115 (98-248)
Furin (ng/ml)	0.31 (0.15-0.85)	0.28 (0.4-5.00)	0.596	0.28 (0.13-5.0)	0.34 (0.13-5.00)	0.25 (0.11-0.47)

Significant value is achieved at the $p < 0.05$. GFAP: glial fibrillary acidic protein; NSE: neuron-specific enolase; S100B: S100 Ca^{2+}-binding protein B; CBZ: carbamazepine; LTG: lamotrigine; LEV: levetiracetam; N: number. *adjusted for age, sex, and BMI.

and had a higher BMI ($p < 0.001$) than healthy controls. Accordingly, age, sex, and BMI were included as covariates when comparing patients and controls. We collected information about epilepsy type and seizure types from 113 participants. Most patients (69%) had focal epilepsy. Seizure frequency was classified as "low" in 57 patients (50.9%), "moderate" in 19 (17%) patients, and "high" in 36 (32.1%) subjects. Generalized tonic-clonic seizures, including both primary generalized and focal to bilateral tonic-clonic seizures, occurred in 70 patients (61.9%). Ninety-four (79.7%) reported no seizures for the last 6 months.

Fifty-five patients (46%) were treated with CBZ, 49 (41%) with LTG, and 15 (12%) with LEV.

Mean age at seizure onset was 20.5 years, with a mean epilepsy duration of 11.9 years. Age, sex, and BMI were included as covariates when comparing patients and controls due to significant differences between groups.

3.2. Serum Levels of S100B, GFAP, NSE, and Furin. Descriptive statistic revealed skewed distribution of biomarkers serum concentration. However, the values were successfully normalized after accordantly log or Box-Cox transformation. As shown in Table 1, after adjustment for potential demographic confounders (sex, age, and BMI), patients had significantly higher serum levels of GFAP ($p = 0.042$) than controls. Initially, Mann–Whitney U tests show significant difference in S100B concentration between groups. However, after adjustments for demographic data in linear regression, we found no significant differences in serum levels of S100B neither NSE or furin between the patients with epilepsy and healthy controls (Table 1).

No significant association was found between serum levels of the four markers related to epilepsy, such as epilepsy duration, epilepsy or seizure types, numbers of seizures, presence of seizures in the last six months, or ASM used.

4. Discussion

The main finding in our study is significantly higher serum levels of GFAP in epilepsy patients than in healthy controls. This may indicate activation of astrocytes in our patient population. Elevated levels of GFAP after generalized tonic-clonic seizures (GTCS) in children have been described by Elhady et al. [4]. Simani et al. reported significantly enhanced concentrations of GFAP in serum after epileptic seizures compared to serum concentrations in patients with psychogenic nonepileptic attacks (PNES) and healthy controls [15]. GFAP was therefore suggested as a marker to differentiate between epileptic seizure and PNES [15]. It has also been reported that GFAP levels in the peripheral circulation remain elevated in children with epilepsy several months after seizures [16]. Our results indicate that levels of GFAP remain elevated in this specific patient population with stable epilepsy which is the largest patient group seen in clinical practice. Further, the findings may suggest that the increased concentration of GFAP is related to epilepsy per se, independent of ongoing or recent seizures. The results may support that chronic astrocyte activation is present also in a relatively stable phase of epilepsy.

We did not find any significant alteration in levels of S100B in epilepsy patients. Several previous studies have investigated the presence of S100B in patients with epilepsy. Griffin et al. showed that in sections of surgically resected temporal lobe tissue from patients with drug-resistant temporal lobe epilepsy, the numbers of S100B immunoreactive astrocytes were three times higher than in controls [17]. Regarding S100B levels in peripheral blood in epilepsy patients, various studies have reported both enhanced or normal levels of this protein in different types of epilepsy [18–20]. This may reflect differences in time from seizure occurrence to blood sampling, an issue that has not been systematically studied in larger studies.

We did not find any increase in levels of NSE in epilepsy patients. Significantly elevated serum levels of NSE have been reported in patients with focal seizures and after single GTC seizures, as well in status epilepticus [7, 21, 22]. Maiti et al. observed that concentrations of NSE decreased significantly within 4 weeks after introduction of ASMs like CBZ and oxcarbazepine [7]. We did not find the same correlation in our patients. However, we did note slightly lower levels of NSE in the LEV and LTG treatment groups. Considering also the low levels of S100B, we speculate whether ASMs can affect the levels of some or all of these proteins. This should be investigated by further prospective studies that should also adjust for relevant confounders.

In the current study, no difference between furin levels in epilepsy patients and controls could be identified (Table 1). Furin has been the subject of fewer studies than the proteins discussed above. It has been reported that furin increases susceptibility to epileptic seizures due to its involvement in regulation of Notch signalling [12, 23, 24]. Baumann et al. revealed that blocking of furin reduces BBB permeability

after hypoxic injury [13]. This is an interesting observation which could be a new therapeutic option for improving BBB function after events compromising BBB and could be a valuable avenue for further exploration.

After examination of four biomarkers, only one was significantly higher in the epilepsy patient group. In contrast to NSE and furin which originate form neurons, GFAP is a specific marker for astrocyte activation. Based on our results, one might speculate that astrocyte activation is perhaps more prominent than neuronal activation in chronic epilepsy, although this definitely has to explored further.

We could not find any correlation between epilepsy duration, seizure or epilepsy types, total numbers of seizures, presence of seizures in the last six months, or ASM and the studied biomarkers. Thus, based on the modestly elevated levels of GFAP, and similar levels of the other BBB markers, our study suggests no marked dysregulation of BBB integrity, as reflected by circulating BBB markers, in patients with stable epilepsy. However, we cannot rule out that the levels of markers could be higher in patients with drug-resistant epilepsy or be elevated in the acute phase, immediately after seizures or SE.

Some study limitations are acknowledged. The cross-sectional design makes it difficult to observe direct changes shortly after seizures. The information regarding number of seizures was based on self-reporting questionnaires with its challenges with accuracy. Furthermore, serum levels may not necessarily reflect levels of GFAP, S100B, NSE, and furin in CNS, although previous studies have shown positive correlations between elevated serum and cerebrospinal fluid levels of NSE, and possibly S100B, after SE and traumatic brain injury [8, 25]. It should also be mentioned that several of these markers are also expressed to varying degrees outside the CNS.

5. Conclusion

In conclusion, our study shows a slightly higher level of GFAP in patients with stable epilepsy. This may indicate chronic activation of astrocytes However, most of the BBB markers showed normal levels in this group of patients. This might suggest that BBB may be affected during seizures, but generally normalizes in a stable phase without seizures. Future prospective studies of the longitudinal relationship between epilepsy debut, seizures, current ASMs, and time of blood sampling for biomarkers are recommended.

Data Availability

The data is not publicly available due to privacy policy.

Conflicts of Interest

Ole A. Andreassen has received consultant honorarium from HealthyLytix. None other authors have any conflict of interest to declare.

References

[1] E. A. van Vliet, A. S. da Costa, S. Redeker, R. van Schaik, E. Aronica, and J. A. Gorter, "Blood-brain barrier leakage may lead to progression of temporal lobe epilepsy," *Brain*, vol. 130, no. 2, pp. 521–534, 2007.

[2] N. Marchi, L. Angelov, T. Masaryk et al., "Seizure-promoting effect of blood–brain barrier disruption," *Epilepsia*, vol. 48, no. 4, pp. 732–742, 2007.

[3] Q. P. Verhoog, L. Holtman, E. Aronica, and E. A. van Vliet, "Astrocytes as guardians of neuronal excitability: mechanisms underlying epileptogenesis," *Frontiers in Neurology*, vol. 11, article 591690, 2020.

[4] M. Elhady, E. R. Youness, M. M. AbuShady et al., "Circulating glial fibrillary acidic protein and ubiquitin carboxy-terminal hydrolase-L1 as markers of neuronal damage in children with epileptic seizures," *Child's Nervous System: ChNS: Official Journal of the International Society for Pediatric Neurosurgery*, vol. 37, no. 3, pp. 879–884, 2021.

[5] F. Michetti, N. D'Ambrosi, A. Toesca et al., "The S100B story: from biomarker to active factor in neural injury," *Journal of Neurochemistry*, vol. 148, no. 2, pp. 168–187, 2019.

[6] K. G. Liang, R. Z. Mu, Y. Liu, D. Jiang, T. T. Jia, and Y. J. Huang, "Increased serum S100B levels in patients with epilepsy: a systematic review and meta-analysis study," *Frontiers in Neuroscience*, vol. 13, p. 456, 2019.

[7] R. Maiti, B. R. Mishra, S. Sanyal, D. Mohapatra, S. Parida, and A. Mishra, "Effect of carbamazepine and oxcarbazepine on serum neuron-specific enolase in focal seizures: a randomized controlled trial," *Epilepsy Research*, vol. 138, pp. 5–10, 2017.

[8] J. Correale, A. L. Rabinowicz, C. N. Heck, T. D. Smith, W. J. Loskota, and C. M. Degiorgio, "Status epilepticus increases CSF levels of neuron-specific enolase and alters the blood-brain barrier," *Neurology*, vol. 50, no. 5, pp. 1388–1391, 1998.

[9] C. M. DeGiorgio, J. D. Correale, P. S. Gott et al., "Serum neuron-specific enolase in human status epilepticus," *Neurology*, vol. 45, no. 6, pp. 1134–1137, 1995.

[10] A. Bharosay, V. V. Bharosay, M. Varma, K. Saxena, A. Sodani, and R. Saxena, "Correlation of brain biomarker neuron specific enolase (NSE) with degree of disability and neurological worsening in cerebrovascular stroke," *Indian Journal of Clinical Biochemistry: IJCB*, vol. 27, no. 2, pp. 186–190, 2012.

[11] N. Marchi, M. Cavaglia, V. Fazio, S. Bhudia, K. Hallene, and D. Janigro, "Peripheral markers of blood-brain barrier damage," *Clinica Chimica Acta; International Journal of Clinical Chemistry*, vol. 342, no. 1-2, pp. 1–12, 2004.

[12] Y. Yang, M. He, X. Tian et al., "Transgenic overexpression of furin increases epileptic susceptibility," *Cell Death & Disease*, vol. 9, no. 11, p. 1058, 2018.

[13] J. Baumann, S. F. Huang, M. Gassmann, C. C. Tsao, and O. O. Ogunshola, "Furin inhibition prevents hypoxic and TGFβ-mediated blood-brain barrier disruption," *Experimental Cell Research*, vol. 383, no. 2, article 111503, 2019.

[14] M. Mochol, E. Taubøll, P. Aukrust, T. Ueland, O. A. Andreassen, and S. Svalheim, "Interleukin 18 (IL-18) and its binding protein (IL-18BP) are increased in patients with epilepsy suggesting low-grade systemic inflammation," *Seizure*, vol. 80, pp. 221–225, 2020.

[15] L. Simani, M. Elmi, and M. Asadollahi, "Serum GFAP level: a novel adjunctive diagnostic test in differentiate epileptic seizures from psychogenic attacks," *Seizure*, vol. 61, pp. 41–44, 2018.

[16] M. Zhu, J. Chen, H. Guo, L. Ding, Y. Zhang, and Y. Xu, "High mobility group protein B1 (HMGB1) and interleukin-1β as prognostic biomarkers of epilepsy in children," *Journal of Child Neurology*, vol. 33, no. 14, pp. 909–917, 2018.

[17] W. S. T. Griffin, O. Yeralan, J. G. Sheng et al., "Overexpression of the neurotrophic cytokine S100β in human temporal lobe epilepsy," *Journal of Neurochemistry*, vol. 65, no. 1, pp. 228–233, 1995.

[18] J. Palmio, J. Peltola, P. Vuorinen, S. Laine, J. Suhonen, and T. Keränen, "Normal CSF neuron-specific enolase and S-100 protein levels in patients with recent non-complicated tonic-clonic seizures," *Journal of the Neurological Sciences*, vol. 183, no. 1, pp. 27–31, 2001.

[19] L. V. C. Portela, A. B. L. Tort, R. Walz et al., "Interictal serum S100B levels in chronic neurocysticercosis and idiopathic epilepsy," *Acta Neurologica Scandinavica*, vol. 108, no. 6, pp. 424–427, 2003.

[20] M. Calik, M. Abuhandan, H. Kandemir et al., "Interictal serum S-100B protein levels in intractable epilepsy: a case-control study," *Neuroscience Letters*, vol. 558, pp. 58–61, 2014.

[21] C. M. DeGiorgio, C. N. Heck, A. L. Rabinowicz, P. S. Gott, T. Smith, and J. Correale, "Serum neuron-specific enolase in the major subtypes of status epilepticus," *Neurology*, vol. 52, no. 4, p. 746, 1999.

[22] T. Büttner, B. Lack, M. Jäger et al., "Serum levels of neuron-specific enolase and s-100 protein after single tonic-clonic seizures," *Journal of Neurology*, vol. 246, no. 6, pp. 459–461, 1999.

[23] L. Sha, X. Wu, Y. Yao et al., "Notch signaling activation promotes seizure activity in temporal lobe epilepsy," *Molecular Neurobiology*, vol. 49, no. 2, pp. 633–644, 2014.

[24] F. Logeat, C. Bessia, C. Brou et al., "The notch 1 receptor is cleaved constitutively by a furin-like convertase," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 14, pp. 8108–8112, 1998.

[25] A. Dadas, J. Washington, R. Diaz-Arrastia, and D. Janigro, "Biomarkers in traumatic brain injury (TBI): a review," *Neuropsychiatric Disease and Treatment*, vol. 14, pp. 2989–3000, 2018.