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ORIGINAL ARTICLE

Serum and cerebrospinal fluid brain damage markers neurofilament light and glial fibrillary acidic protein correlate with tick-borne encephalitis disease severity—a multicentre study on Lithuanian and Swedish patients

Malin Veje[1,2] | Vytautas Griška[3] | Jolita Pakalnienė[3] | Auksė Mickienė[3] | Daniel Bremell[1,2] | Henrik Zetterberg[4,5,6,7,8,9] | Kaj Blennow[4,5] | Lars Lindquist[10] | Marie Studahl[1,2]

[1]Institute of Biomedicine, Department of Infectious Diseases, Sahlgrenska Academy at the Gothenburg University, Gothenburg, Sweden

[2]Region Västra Götaland, Department of Infectious Diseases, Sahlgrenska University Hospital, Gothenburg, Sweden

[3]Department of Infectious Diseases, Lithuanian University of Health Sciences, Kaunas, Lithuania

[4]Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden

[5]Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden

[6]Department of Neurodegenerative Disease, UCL Institute of Neurology, London, UK

[7]UK Dementia Research Institute at UCL, London, UK

[8]Hong Kong Center for Neurodegenerative Diseases, Hong Kong, China

[9]Wisconsin Alzheimer's Disease Research Center, University of Wisconsin School of Medicine and Public Health, University of Wisconsin-Madison, Madison, Wisconsin, USA

[10]Department of Medicine, Karolinska Institute, Stockholm, Sweden

Correspondence
Malin Veje, Institute of Biomedicine, Department of Infectious Diseases, Sahlgrenska Academy at the Gothenburg University, Journalvägen 10, Gothenburg S-416 50, Sweden.
Email: malin.veje@gu.se

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Abstract

Background and purpose: Our aim was to examine the correlation between biomarkers of neuronal and glial cell damage and severity of disease in patients with tick-borne encephalitis.

Methods: One hundred and fifteen patients with tick-borne encephalitis diagnosed in Lithuania and Sweden were prospectively included, and cerebrospinal fluid (CSF) and serum samples were obtained shortly after hospitalization. Using pre-defined criteria, cases were classified as mild, moderate or severe tick-borne encephalitis. Additionally, the presence of spinal nerve paralysis (myelitis) and/or cranial nerve affection were noted. Concentrations of the brain cell biomarkers glial fibrillary acidic protein (GFAP), YKL-40, S100B, neurogranin, neurofilament light (NfL) and tau were analysed in CSF and, in addition, NfL, GFAP and S100B levels were measured in serum. The Jonckheere-Terpstra test was used for group comparisons of continuous variables and Spearman's partial correlation test was used to adjust for age.

Results: Cerebrospinal fluid and serum concentrations of GFAP and NfL correlated with disease severity, independent of age, and with the presence of nerve paralysis. The markers neurogranin, YKL-40, tau and S100B in CSF and S100B in serum were detected, but their concentrations did not correlate with disease severity.

Conclusions: Neuronal cell damage and astroglial cell activation with increased NfL and GFAP in CSF and serum were associated with a more severe disease, independent of age. Increased GFAP and NfL concentrations in CSF and NfL in serum were also indicative of spinal and/or cranial nerve damage. NfL and GFAP are promising prognostic biomarkers in tick-borne encephalitis, and future studies should focus on determining the association between these biomarkers and long-term sequelae.

KEYWORDS
central nervous system, neurofilament, neuroglia, tick-borne encephalitis, viral encephalitis

INTRODUCTION

Tick-borne encephalitis (TBE) is the most common central nervous system (CNS) viral disease in parts of Europe and Asia. It is highly endemic in Lithuania, and the incidence in Sweden is increasing [1]. No antiviral treatment against TBE is available. The clinical presentation varies substantially, and more severe symptoms in the acute phase have been linked to a larger proportion of long-term sequelae [2].

Neurons in several parts of the CNS can be infected by TBE virus (TBEV), mainly the medulla oblongata, pons, nucleus dentatus, basal ganglia, anterior horns of the spinal cord, and cerebellum [3]. To what extent the neurological damage is caused by direct viral infection or by inflammation has not been completely clarified [4]. TBEV RNA is rarely detected in cerebrospinal fluid (CSF), and viral RNA levels cannot be used to determine either disease severity or prognosis [5]. Magnetic resonance imaging, which has been investigated in a few studies, has hitherto not been shown sensitive enough to predict long-term prognosis [6].

From a prognostic perspective, a variety of different brain damage markers have been described in CNS disorders, including infectious diseases [7–10]. Upon cell damage, these markers leak into the CSF and blood and their detection reflects impairment of different cells in the CNS.

Neurofilament light (NfL) in serum and CSF is a well-established marker of neuroaxonal damage in other acute neurological conditions, such as traumatic brain injury and stroke. Serum NfL levels can be used to predict the extent of neurological damage and prognosis after ischaemic stroke [9, 11]. In herpes simplex encephalitis (HSE) patients, higher CSF NfL seems to correlate with the presence of long-term sequelae [10].

Glial fibrillary acidic protein (GFAP) is part of the cytoskeletal protein of astrocytes and reflects astrocyte function. GFAP can be detected both in serum and CSF and concentrations rise early after stroke [12].

YKL-40, a glycoprotein involved in inflammation and produced by several cell types in the CNS, when measured in CSF is considered a marker of astroglial activation and neurodegeneration [13]. The intracellular protein S100B is actively secreted by astrocytes in response to stress and has been used for prognostic prediction after traumatic brain injury [8]. Tau protein is considered a marker of neurodegeneration and neurogranin, a protein reflecting synaptic degeneration [14].

The above-mentioned biomarkers are well established in several neurological conditions. However, apart from the study on HSE [10], they have been sparsely examined in CNS infections. A report on patients infected with West Nile virus, a flavivirus related to TBEV, described elevated concentrations of both neurofilament heavy protein and astrocyte markers GFAP and S100B [15].

In TBE patients, few studies have investigated these markers. In a recent report from Czechia, concentrations of phosphorylated neurofilament heavy subunit measured in the CSF were higher in TBE patients than in healthy blood donors [16]. A Polish group found higher CSF concentrations of tau protein and neuron-specific enolase in TBE patients compared with controls [17, 18]. An earlier study conducted in Sweden concluded that GFAP concentration was elevated in TBE patients compared with controls, but at significantly lower levels than in HSE patients [19].

There is a need for biomarkers predicting prognosis and possibly for monitoring effect during future treatment studies in TBE. In the present study, the aim was to investigate the use for markers of brain damage detected in serum and CSF samples from prospectively included TBE patients in Lithuania and Sweden.

MATERIALS AND METHODS

Patients

Two prospectively included cohorts form the basis for our study. At the Department of Infectious Diseases at Lithuanian University of Health Sciences, patients with TBE were included prospectively between June 2018 and May 2019. Starting in 2014, a prospective study on TBE patients has been conducted at the Department of Infectious Diseases, Sahlgrenska University Hospital, in Gothenburg,

Sweden. During 2019, patients were also included at the Department of Infectious Diseases, Skaraborg Hospital in Skövde. Inclusion criteria were age ≥18 years and confirmed TBE diagnosis, according to the European Centre for Disease Prevention and Control definition [1]. Serum and CSF were collected. In all cases, the first available samples were analysed. All samples were drawn during the first 2 weeks after hospitalization (range −3 to 12 days, with median day 0 after admission).

During the acute phase, all patients were categorized as having mild, moderate or severe disease, in line with previous studies. Mild disease was defined as causing primarily meningeal symptoms (fever, headache, neck rigidity, nausea, vomiting). Moderate disease included the presence of monofocal encephalitic symptoms (ataxia, dysphasia, tremor, single cranial nerve paralysis) and/or moderate diffuse brain dysfunction (Glasgow Coma Scale score 14–10). Cases with multifocal encephalitic symptoms and/or Glasgow Coma Scale ≤9 were defined as severe [2]. Additionally, data on spinal and/or cranial nerve impairment were collected.

The study complied with the Declaration of Helsinki. All patients gave oral and written consent, and the study protocols were approved by the respective regional ethics committees (Gothenburg regional ethics committee, 994-14 and 229-14; Kaunas regional bioethics committee, BE-2-45).

Detection of brain damage markers

Cerebrospinal fluid GFAP and NfL concentrations were measured with in-house sandwich enzyme-linked immunosorbent assays (ELISAs), as previously described [20, 21]. For quantification of NfL in serum, an in-house digital ELISA on a single molecule array (Simoa) platform (Quanterix, Billerica, MA, USA) was utilized, as previously described [22]. Serum GFAP concentration was measured using the GFAP Discovery Kit for Simoa (Quanterix). CSF tau concentration was measured using the commercially available INNOTEST ELISA (Fujirebio, Ghent, Belgium). S100B concentration in serum and CSF was measured on the modular system using the S100 reagent kit (Roche Diagnostics, Basel, Switzerland). For CSF YKL-40 detection, a commercial ELISA (R&D Systems, Minneapolis, MN, USA) was used. CSF neurogranin concentration was measured with a previously described in-house ELISA [23]. All analyses were performed by board-certified laboratory technicians in one round of analyses using one batch of reagents. Intra-assay coefficients of variation were below 10%.

Statistical analyses

For comparison between groups, the Mantel–Haenszel chi-squared test was used for ordinal categorical variables and the Jonckheere-Terpstra test for continuous variables. The association between two continuous variables was tested with the Spearman correlation. Adjustment for age was made with Spearman's partial correlation. Data analyses were performed with SAS, version 9.4 (SAS Institute, Cary, NC, USA) and Prism version 9.5.0 (GraphPad Software Inc., La Jolla, CA, USA).

RESULTS

In total, 115 TBE patients (60% male, 40% female) were included in the study, 98 from Lithuania and 17 from Sweden (15 from Gothenburg and two from Skövde). There was no mortality. In summary, 56 patients (49%) had mild disease, 48 patients (42%) moderate and 11 patients (9.6%) severe disease. Twenty-seven patients (23.5%) suffered from either spinal ($n = 18$) or cranial ($n = 15$) nerve impairment, whereas six patients (5.2%) had both. The most frequent spinal nerve affection was monoparesis of the upper extremities ($n = 12$), followed by paraparesis of the lower extremities ($n = 2$). One patient each suffered from monoparesis of the lower extremities, hemiparesis, tetraparesis and erectile dysfunction. The most frequently affected cranial nerve was *n. oculomotorius* ($n = 6$), followed by *n. vestibulocochlearis* ($n = 4$), *n. facialis* ($n = 3$) and *n. hypoglossus* ($n = 1$). One patient suffered from unilateral vocal cord paresis (*n. vagus*); however, it could not be determined whether this was caused by infection or intubation. Disease severity differed between the two cohorts, where the proportions of mild/moderate/severe disease were 56%/37%/7.1% in the Lithuanian patients and 5.9%/71%/24% amongst the Swedish patients. 83/115 cases (72%) had a biphasic disease pattern, and the percentage of monophasic disease was higher in patients with more severe disease ($p < 0.05$). Disease severity correlated with increasing age. The proportion of men was higher in the mild disease group (Table 1).

Detection of brain damage markers and their relation to disease severity

All tested biomarkers could be detected in the patient samples (Table S1). The concentrations of CSF biomarkers NfL, GFAP and YKL-40, as well as NfL and GFAP in serum, were significantly higher in patients with moderate or severe disease compared with patients with a mild clinical presentation ($p < 0.05$, Figure 1a–e). CSF

TABLE 1 Age and sex distribution and proportion of tick-borne encephalitis cases with monophasic disease and nerve impairment in relation to disease severity.

Disease severity	Mild ($n = 56$)	Moderate ($n = 48$)	Severe ($n = 11$)
Female (%)	33.9	45.9	45.5
Mean age (years)	47.9	55.0	61.1
Monophasic disease (%)	14.3	37.5	54.5
Spinal nerve impairment (%)	7.1	16.7	54.5
Cranial nerve impairment (%)	8.9	12.5	36.3

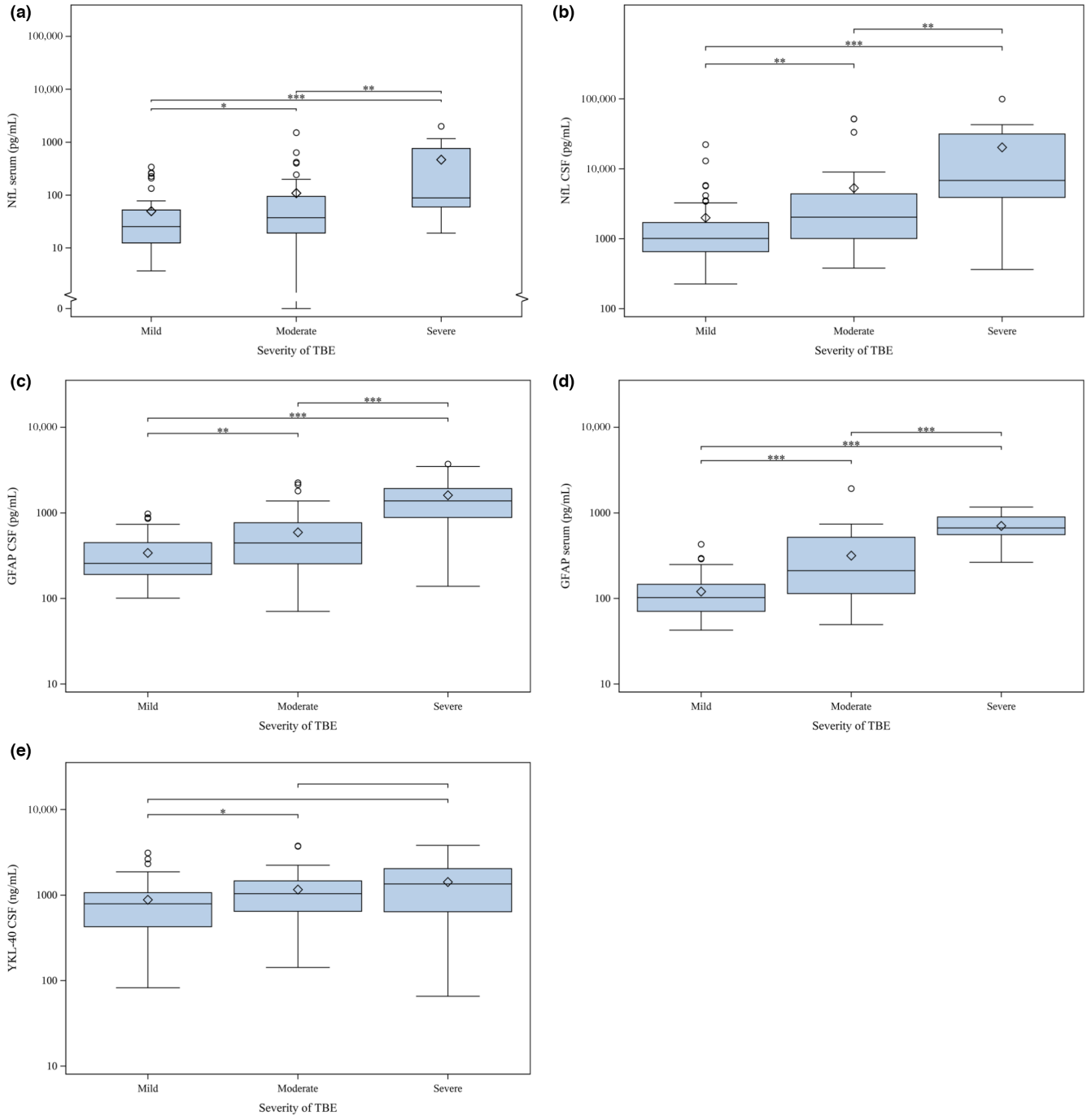

FIGURE 1 Concentrations of brain damage markers in cerebrospinal fluid and serum in patients with tick-borne encephalitis in relation to disease severity. $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.

concentrations of tau, S100B or neurogranin and serum concentrations of S100B did not differ significantly according to disease severity (Table S1).

The correlation between brain damage marker concentrations and disease severity was independent of age at admission (Table S2).

Levels of NfL and GFAP in serum and CSF were statistically significantly higher amongst the patients with both spinal and cranial nerve paralysis, whereas tau concentrations were higher in patients with cranial nerve paralysis. Concentrations of S100B, YKL-40 and neurogranin did not differ significantly between the groups with or without nerve paralysis (data not shown).

Concentrations of both NfL and GFAP in serum and CSF correlated with disease severity, regardless of whether patients with pareses were excluded from the analysis (Table S3).

Concentrations of GFAP and S100B in serum, but not CSF, were statistically higher in female patients than in males, whereas the opposite was seen for serum and CSF albumin. All measured brain damage marker concentrations except for tau and neurogranin

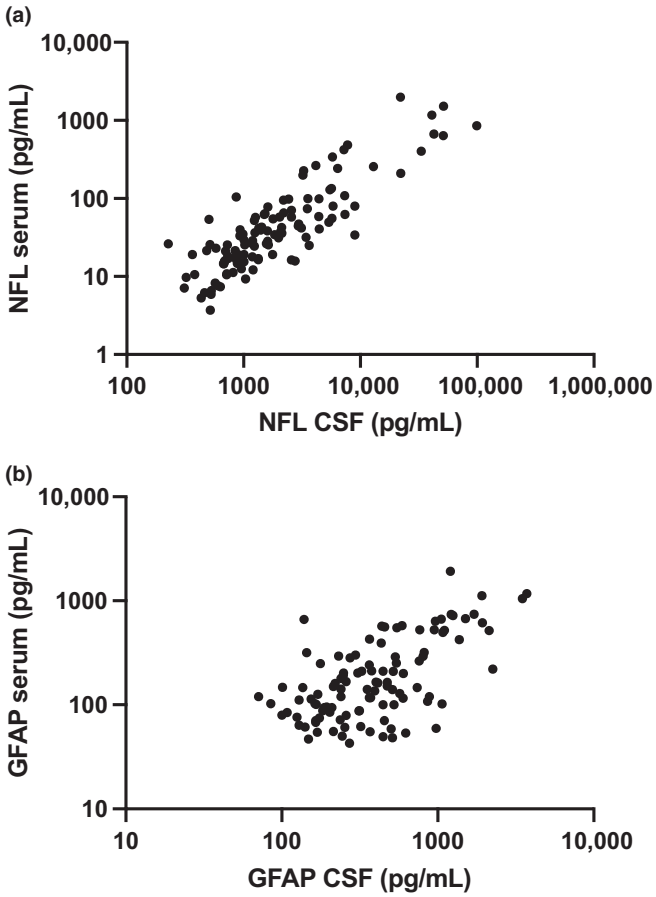

FIGURE 2 (a) Correlation between CSF and serum NfL in 114 patients with tick-borne encephalitis ($r = 0.82$, $p < 0.0001$). (b) Correlation between CSF and serum GFAP in 107 patients with tick-borne encephalitis ($r = 0.54$, $p < 0.0001$). CSF, cerebrospinal fluid; GFAP, glial fibrillary acidic protein; NfL, neurofilament light.

correlated with age (Table S2). Furthermore, a statistical correlation between serum and CSF concentrations of both NfL and GFAP was noted (Figure 2a,b).

DISCUSSION

In this study of 115 prospectively included TBE cases, elevated serum and CSF concentrations of both NfL and GFAP were found, indicating neuronal cell damage and astroglial activation. It was also shown that the concentrations of these well-established brain damage markers correlate with disease severity.

The clinical characteristics of our patient material correlate well with previous publications. Compared with other studies, the proportions of different disease severities in the total cohort were similar, and the percentage of male patients slightly higher. The proportion of spinal and/or cranial nerve paresis was 23% compared with up to 15% in other studies. There was no mortality, in line with publications from Sweden and Lithuania reporting a case fatality rate of 0.75%–1.1% [2, 24].

Previously considered less relevant, astrocytes are now seen as important for a large variety of processes occurring in the brain, involving neurotransmission, modulation of inflammatory response and structural support for neurons. Astrocytes, unlike microglia, cannot migrate to the lesion, but change their phenotype and increase in number in response to infection and inflammation [25]. In post-mortem studies on TBE patients, neuronal damage as well as astroglial and microglial proliferation have been detected [3], corresponding with our findings of elevated concentrations of NfL and GFAP in TBE patients with more severe clinical manifestations. TBE patients vary extensively in clinical presentation, from a very mild meningitis to a severe encephalomyelitis requiring intensive care and long rehabilitation [26]. Most patients in our cohort who were classified with mild disease had meningitis. Encephalitis, with its inflammatory involvement of brain parenchyma, gives rise to higher NfL and GFAP levels, which were found in the patients with moderate and severe disease. The finding of increased GFAP in CSF also confirms the results from the previous, smaller Swedish study [19]. In patients with both spinal and cranial nerve damage, GFAP and NfL concentrations correlated with disease severity. However, the number of patients with paresis was small ($n = 27$). NfL, GFAP, YKL-40 and S100B concentrations correlated with age, whereas neurogranin and tau levels did not. Several other studies have reported that NfL levels increase with age [7].

In our cohort, CSF YKL-40 levels correlated with a more severe disease. The only previous publication on YKL-40 in TBE patients also found elevated YKL-40 in CSF samples from 32 TBE patients. Of note, YKL-40 concentrations were higher in TBE patients with meningitis compared with those with meningoencephalitis [27]. In contrast, YKL-40 levels in our patients increased with disease severity. Cohort size and assay differences could potentially explain the different results.

Tau protein and S100B have previously been detected in TBE patients at higher concentrations than among controls. In our cohort, these proteins were detected but did not correlate with disease severity, the same as for neurogranin. In the Polish study, CSF levels of tau did not differ from the control group in the sample drawn at hospital admission, but the concentrations were significantly higher in the second sample, drawn after 14 days [18]. All samples in our study were drawn within 2 weeks from hospital admission, in median on the day of hospital admission. The kinetics of these markers in CNS infections have not been extensively studied, and their concentrations may vary over time, as shown for NfL in HSE patients where peak NfL levels in CSF were detected at around 2 weeks after admission [10, 19].

The correlation between NfL concentrations in serum and CSF is well established [11]. One of our major findings is the correlation between concentrations in serum and CSF for both NfL and GFAP. Advantages with future prognostic biomarkers detectable in serum instead of CSF are obvious; blood sampling is much less invasive and logistically more feasible.

The limitations of our study are that it cannot be excluded that the freeze–thaw procedure might have affected CSF GFAP concentration [28], but probably not the concentrations of the other markers [29], although all samples were transported frozen and analysed together. Further, the disease severity definition is rather rough, but on the other hand has been repeatedly used in previous publications. It is noted that the Swedish patients had a higher proportion of moderate and severe disease compared to the Lithuanian cohort. However, these differences in disease severity between Lithuania and Sweden are most probably due to chance, considering the difference in group size. Previous data from Sweden show that 14% of TBE patients are diagnosed in primary healthcare [24], and our study only included hospitalized participants. One could speculate whether the difference in disease severity between the two countries would be due to the presence of different virus subtypes. In Sweden, only the European TBEV subtype has been detected [30]. There are limited data on subtypes circulating in Lithuania, but in Estonia all three major virus subtypes have been detected [31].

Our results indicate neuronal cell damage and astroglial activation in TBE, and the concentrations of NfL and GFAP in serum and CSF and YKL-40 in CSF correlate with disease severity in the acute phase. TBE pathogenesis is not fully understood and severe disease in the acute phase has repeatedly been correlated with more sequelae [2, 26]. Interestingly, our findings confirm those from the 2022 Czech study on phosphorylated neurofilament heavy subunit, where a correlation with increased disease severity (duration of hospitalization, admission to the intensive care unit) was found [16]. Neurofilament heavy protein seems to correlate with NfL levels, at least for CSF samples from patients with neurodegenerative diseases [32].

To date, no prognostic biomarkers for TBE exist. Evaluation of rehabilitation efforts as well as clinical antiviral drug trials will be facilitated by surrogate markers for disease severity and prognosis. In this context, especially the serum markers NfL and GFAP might prove valuable for estimation of brain damage. Future studies should explore the time-point of peak concentrations of NfL and GFAP in TBEV infection and correlate the biomarkers with clinical data on long-term outcome.

AUTHOR CONTRIBUTIONS

Conceptualization: M.V., V.G., A.M., M.S. Methodology: M.V., V.G., A.M., D.B., M.S. Formal analysis: M.V., V.G., A.M., D.B., H.Z., K.B., M.S. Resources: M.V., V.G., A.M., D.B., M.S. Writing—original draft: M.V., V.G., A.M., M.S. Writing—review and editing: M.V., V.G., J.P., A.M., D.B., H.Z., K.B., L.L., M.S. Visualization: M.V., D.B., M.S. Funding acquisition: A.M., M.S. Parts of the preliminary results of this study have previously been published at the 31st ECCMID online conference, abstract no. 04895, title: 'Brain damage biomarkers in patients with tick-borne encephalitis in correlation with disease severity' (first author and presenter V.G.). M.V., A.M., D.B. and M.S. are members of the ESGIB, the ESCMID study group for infectious diseases of the brain.

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CONFLICT OF INTEREST STATEMENT

M.V. has received payments for lectures on symposia sponsored by Pfizer Inc. and Bavarian Nordic and transport fees for speakers for the ESCMID 3rd postgraduate encephalitis course and 2nd zoonosis course. V.G. has received the Pfizer Independent Research Grant ID#53233947, fees for expert opinions from State Health Care Accreditation Agency under the Ministry of Health, Lithuanian Ministry of Health and the State Forensic Medicine Service, transport fees for speakers from ESCMID and sponsored participation in the 8th ESWI Influenza Conference 2021. J.P. has received the Pfizer Independent Research Grant ID#53233947. A.M. received grants for the Independent Investigator Initiated Research (Project Code/PO/Tracking Number WI236259; Grant ID#53233947); Pfizer R&D Investigator-Initiated Research program (https://www.pfizer.com/science/collaboration/investigator-initiated-research) for the scientific project 'A prospective study on the long-term outcome and pathogenesis of tick-borne encephalitis'. D.B. reports no conflicts of interest. 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 Roche, 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). K.B. reported personal fees from Abcam, Axon, Biogen, JOMDD/Shimadzu, Lilly, MagQu, Novartis, Roche Diagnostics and Siemens outside the submitted work and being cofounder of Brain Biomarker Solutions in Gothenburg AB, which is a part of the GU Ventures Incubator Program. L.L. is affiliated to the Stockholm County Committee of Health Care but has received no payments. M.S. is co-author of the Swedish national guidelines for viral CNS infections and has received an ESCMID study group grant in 2020.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Malin Veje https://orcid.org/0000-0001-5487-0616
Jolita Pakalnienė https://orcid.org/0000-0002-3252-3901
Daniel Bremell https://orcid.org/0000-0002-6137-911X

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SUPPORTING INFORMATION

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