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Table 2. Characteristic of AE and preceding epilepsy in the [a]present (cases 1, 2 and 3) and previously reported cases

Case no.	Age	Gender	*SCN1A* mutation	Antecedent infection	Other etiologic factors	Precedimg epliIpsy or seizure dieorder	AE	Seizure at acute stage of AE	Neuroimaging findings	Prognosis	Reference
1[a]	2 years 3 months	F	V982L/ missense	Upper respiratory infection	Theophylline	Partial epilepsy	AESD	Status, febrile, on day 1	CT/day 2, unremakable; CT/day 5, diffuse brain edema; follow-up MRI, central sparing	Spastic quadriplegia, severe MR	This study
2[a]	3 years	M	M1977L/ missense	Upper respiratory infection		GEFS+	Nonspecific AE	Cluster, febrile, on day 1	CT/day 2, unremakable	Complete recovery	This study
3[a]	0 year 9 months	M	R1575C/ missense	Acute gastroenteritis	Family history of AE	None	Mimiking ANE	Cluster, afebrile, on day 1	MRI/day 2, bilateral thalamic lesions	Complete recovery	This study
4	1 year 4 months	F	R1892X/ nonsense	Rotavirus gastroenteritis		Dravet syndrome	AESD (HH)	Status, febrile, on day 1	MR/day 6, left hemispheric edema	Mild MR (DQ = 71), right spastic heniplegia	Sakakibara et al., 2009
5	0 year 9 months	F	D43fs/ truncation	Fever of unknown etiology		Suspected Dravet syndrome	Atypical AESD	Status, febrile, on day 1	MRI/day ×3, diffuse high signal intesity in cortex and subcortical white matter	Spastic quadriplegia, severe MR	Takayanagi et al., 2010
6	6 years 5 months	M	R1575C/ missense	Fever of unknown etiology		Febrile seizure	AERRPS	Cluster and status, febrile on day 2	CT and MRI, unremarkable	Mild MR	Kobayashi et al., 2010

Cases 4, 5, and 6 were reported in references.

GEFS+, generalized epilepsy with febrile seizure plus; AESD, acute encephalopathy with biphasic seizures and late reduced diffusion; AE, acute encephalopathy; ANE, acute necrotizing encephalopathy; HH, hemiconvulsion-hemiplegia syndrome; AERRPS, acute encephalitis with refractory, repetitive partial seizures.

epilepsy and ANE, whereas case 6 reported previously had febrile seizure and AERRPS (Kobayashi et al., 2010). This difference suggests the involvement of factors other than *SCN1A* mutation in the pathogenesis of AE.

The family history of case 3 deserves attention. The younger sister of this patient also had the same type of AE, despite the absence of *R1575C* mutation, which strongly suggests the involvement of another, as yet unidentified factor in this familial ANE. Comparison between the siblings revealed a longer duration of status epilepticus in the brother (case 3), and a worse prognosis in the sister. Plausibly, the *SCN1A* mutation contributed more to the evolution of status epilepticus, and the unidentified factor more to the development of bithalamic lesions and the overall neurologic damage.

In summary, we found *SCN1A* mutations in 3 of 87 cases of AE, and identified them as a predisposing genetic factor of AE. As for both epilepsy and AE, clinical phenotypes were variable among patients with *SCN1A* mutations. This variability, together with the family history of one patient (case 3), suggested that factors other than *SCN1A* mutations are also involved in the pathogenesis of AE.

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Disclosures

None of author has any conflict of interest to disclosure.

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Original article

Serum and CSF biomarkers in acute pediatric neurological disorders

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Abstract

Background: There have been numerous reports regarding serum or cerebrospinal fluid (CSF) biomarkers in various disorders; however, the validities of such biomarkers for more precise diagnoses and prognosis estimates remain to be determined, especially in pediatric patients with neurological disorders. *Methods:* Serum/CSF S100B, neuron-specific enolase, and total tau (tTau) were measured in various acute pediatric neurological disorders, and their usefulness for diagnostic and prognostic predictions was validated using receiver operating characteristic curves and area under the curve (AUC) analysis. *Results:* A total of 336 serum and 200 CSF specimens from 313 patients were examined, and we identified statistically significant differences that were relevant from diagnostic and prognostic viewpoints. CSF and serum tTau levels could be good predictors for diagnosis (CSF tTau; AUC = 0.76) and prognosis (serum tTau; AUC = 0.78). *Conclusions:* Both CSF and serum tTau levels could be useful for precise diagnostic and prognostic estimations in acute pediatric neurological disorders. Further studies are needed to clarify the clinical significance of such biomarkers.

Keywords: S100B; Neuron-specific enolase; Total tau; Receiver operating characteristic curves; Area under the curve

1. Introduction

There are many kinds of pediatric neurological disorders with acute symptoms, such as headache, altered consciousness, seizures, paralysis, and ataxia [1]. Pediatricians or pediatric neurologists who treat these patients use various approaches, such as history taking, physical/neurological examinations, routine laboratory tests, conventional cerebrospinal fluid (CSF) examinations, electroencephalography, and brain imaging to identify the underlying cause. Due to limited time and resources, it would be useful to develop more precise diagnostic and prognostic predictions, which are sometimes difficult to attain, especially in the early stages of these disorders. For more than a decade, there have been reports about serum/CSF biomarkers that are useful in identifying various neurological disorders, at least in study settings [2–9]. We examined serum and CSF S100B, neuron-specific enolase (NSE), and total tau (tTau), which are glial, neuronal, and axonal damage markers, respectively, in patients with acute encephalopathy with biphasic seizures and late reduced diffusion. We found that all 3 biomarker levels were significantly increased and useful for diagnosis [3]. We hope to evaluate the usefulness of these markers as diagnostic and prognostic predictors in other diseases.

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2. Methods

2.1. Patients

From June 2007 to August 2012, patients were enrolled in the study mainly via mailing lists for Japanese pediatric neurologists or pediatricians, such as the Zao Seminar Mailing List (available at: https://sites.google.com/site/zaoseminar/) and the Japanese Pediatric Conferences Mailing List (available at: https://jpmlc.org/). Diagnoses were made by the attending physicians and later confirmed for the purpose of this study by examination of the available clinico–radiological information. We asked the attending physicians to provide each patient's clinical course and prognosis at the most recent visit. To evaluate the prognosis, the degree of disability was scored with the modified Rankin scale (mRS), which ranged from 1: no residual disability to 4: death (Table 1) [10]. Ethics approval was obtained from the Gunma Children's Medical Center institutional review board, and written informed consent was provided by the patients' parents.

2.2. Biomarker assays

Serum and CSF samples were obtained from each patient at any point during the disease and immediately stored at −80 °C until they were analyzed. Commercially available sandwich enzyme-linked immunosorbent assays (ELISA) for human S100B (BioVendor, Modrice, Czech Republic), NSE (Alpha Diagnostic International, Inc., San Antonio, Texas, USA), and tTau (Invitrogen Corp., Carlsbad, California, USA) were carried out according to the manufacturers' protocols [3]. The detectable range for each ELISA kit was 50–2000 pg/ml, 5–200 ng/ml, and 31.2–2000 pg/ml for S100B, NSE, and tTau protein, respectively.

2.3. Statistical analysis

Data were expressed as the median and interquartile range (IQR) unless otherwise specified. Statistical analysis was performed with the statistical package R (version 2.15.2, available as a free download from http://www.r-project.org). Comparisons were performed between numerical variables with the Wilcoxon rank-sum test and between proportions with the proportion test. For multiple comparisons between numerical variables or proportions, the Kruskal–Wallis rank sum test or the proportion test were performed, then, if there were significant differences, the pairwise Wilcoxon rank-sum tests or the pairwise proportion tests were performed, adjusted with Holm's method. A P-value < 0.05 was considered statistically significant. As a measure of binary decision performance, the receiver operating characteristic (ROC) curves were assessed, using the area under the curve (AUC) with Bootstrap method [11]. An optimal threshold value (cutoff point) was selected as the situation maximizing the Youden index (Youden index = sensitivity + specificity − 1) [12].

3. Results

We collected 497 serum and 274 CSF specimens from 372 patients with various disorders. To evaluate the usefulness of serum/CSF biomarkers as diagnostic or prognostic predictors in the early phase of acute pediatric neurological disorders, we only used specimens taken within 5 days of illness (DOI; the first day of neurological symptoms was regarded as DOI 0) and only investigated diagnostic categories with specimens from more than 5 patients. Thus, 336 serum and 200 CSF specimens from 313 patients were available for evaluation (median age, 2 years; IQR, 1–5 years; male: female ratio, 160:153). Diagnostic categories were as follows (in alphabetical order); acute encephalitis/encephalopathy (AEE), aseptic meningitis (AM), afebrile seizures (AS), controls (CTR), febrile seizures (FS), and septic meningitis (SM). AEE comprised various types of acute encephalitis and encephalopathy, such as acute disseminated encephalomyelitis, acute encephalopathy with biphasic seizures and late reduced diffusion, and acute encephalitis with refractory repetitive partial seizures [13,14]. AS included epileptic seizures or gastroenteritis-related convulsions [15]. CTR included patients who were suspected to have a neurological disorder or involvement, but testing revealed that they did not, such as extra-cerebral infections, Kawasaki disease, and blood disorders. The study

Table 1
Modified Rankin scale.

Score	Description
1	No residual disability; the child attends regular education and does not need remedial teaching[a]
2	Mild residual disability; the child is able to attend regular education but needs remedial teaching because of mild motor disturbances, mild learning disability, or both
3	Severe residual disability; the child has a severe motor deficit (needs braces or wheelchair), severe learning disability, or both, attends a school for special education or is confined to a daily care centre
4	Death

[a] For patient who has underlying condition and disability, the score is determined as 1, unless the disability is worsened after the event.

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Table 2
Characteristics of study populations.

Diagnosis	Number	Age (year)	Gender (M:F)	Sampling time (DOI)	mRS
AEE	88	3 (1–6)	37:51	1 (0–3)	2 (1–3)
AM	15	4 (2.5–8.5)	10:5	0 (0–1)	1 (1–1)
AS	52	2 (0.75–4)	20:32	0 (0–0)	1 (1–1)
CTR	85	3.5 (1–8)	40:45	Not available	1 (1–1)
FS	51	1 (1–3)	40:11	0 (0 – 1)	1 (1–1)
SM	22	1 (0–2.5)	13:9	1 (0–2)	1 (1–1)

AEE, Acute encephalitis/encephalopathy; AM, aseptic meningitis; AS, Afebrile seizures; CTR, control; FS, Febrile seizures; SM, septic meningitis; DOI, day of illness, mRS, modified Rankin scale.
Numerical variables are expressed as median (inter quartile range). There were statistically significant differences in age (between AEE and SM*), mRS (between AEE and AM**, AS**, CTR**, FS**, SM*; SM and CTR*), Gender (between FS and AEE**, AS**, CTR**), and sampling time (between AEE and AS**, FS**; SM and AS**) (* denoting P-value less than 0.05, ** less than 0.01).

population characteristics were summarized, and we found statistically significant differences in age, mRS, gender, and sampling time between diagnostic groups (Table 2).

3.1. Serum and CSF biomarkers between each diagnostic group

Initially, the serum and CSF levels of S100B, NSE, and tTau were compared between each diagnostic group (Fig. 1). There were statistically significant differences in serum NSE between AEE (median, 14.5 ng/ml; IQR, 6.0–35.8) and AS (median, 5.0 ng/ml; IQR, 5.0–8.0; $P < 0.001$), AEE and CTR (median, 8.0 ng/ml; IQR, 5.0–13.1; $P = 0.011$), AEE and FS (median, 5.9 ng/ml; IQR, 5.0–11.2; $P = 0.001$), AS and CTR ($P = 0.030$), and AS and SM (median, 9.0 ng/ml; IQR, 5.5–18.0; $P = 0.017$). Significant difference were also observed from serum tTau: AEE (median, 31.2 pg/ml; IQR, 31.2–292.5) and AS (median, 31.2 pg/ml; IQR, 31.2–31.2; $P < 0.001$), AEE and CTR (median, 31.2 pg/ml; IQR, 31.2–31.2; $P < 0.001$), AEE and FS (median, 31.2 pg/ml; IQR 31.2–50.0; $P = 0.006$), AS and SM (median, 40.0 pg/ml; IQR, 31.2–122.5; $P = 0.009$), CTR and SM ($P < 0.001$). For CSF S100B, we observed significant differences between AEE (median, 90.0 pg/ml; IQR 58.7–300.0) and CTR (median, 50.0 pg/ml; IQR, 50.0–50.0; $P < 0.001$), AEE and FS (median, 51.5 pg/ml; IQR, 50.0–77.4; $P = 0.011$), CTR and SM (median, 130.0 pg/ml; IQR, 79.6–272.2; $P < 0.001$), and FS and SM ($P = 0.006$). For CSF tTau, we found significant differences between AEE (median, 230.0 pg/ml; IQR, 116.0–800.0) and FS (median, 100.0 pg/ml; IQR, 50.0–141.8; $P = 0.002$).

3.2. Serum and CSF biomarkers between patients with good and poor prognoses

Next, the serum and CSF levels of S100B, NSE, and tTau were compared between patients with good and poor prognoses (defined as mRS of 1–2 and 3–4, respectively) (Fig. 2). The levels of all the measured biomarkers were significantly higher in patients with poor prognosis than in those with good prognosis, i.e., serum S100B, good (median, 50.0 pg/ml; IQR 50.0–70.4) vs. poor (median, 66.6 pg/ml; IQR 50.0–581.0; $P < 0.001$); serum NSE, good (median, 7.5 ng/ml; IQR, 5.0–15.0) vs. poor (median, 16.3 ng/ml; IQR, 7.3–132.9; $P < 0.001$); serum tTau, good (median, 31.2 pg/ml; IQR 31.2–40.0) vs. poor (median, 227.7 pg/ml; IQR, 31.2–1603.0; $P < 0.001$); CSF S100B, good (median, 60.0 pg/ml; IQR, 50.0–120.0) vs. poor (median, 182.8 pg/ml; IQR, 81.7–340.0; $P < 0.001$); CSF NSE, good (median, 5.0 ng/ml; IQR 5.0–5.0) vs. poor (median, 5.0 ng/ml; IQR, 5.0–7.2; $P = 0.020$); and CSF tTau, good (median, 118.7 pg/ml; IQR, 50.0–294.2) vs. poor (median, 319.3 pg/ml; IQR, 120.8–1900.0; $P = 0.002$).

3.3. Evaluations of diagnostic and prognostic validities, using ROC curve analyses

Finally, in order to evaluate diagnostic and prognostic validities, we applied ROC curve analyses. To qualify the diagnostic and prognostic validities, we analyzed each biomarker's ability to distinguish AEE from FS and between poor and good prognoses. Then we drew the ROC curves and calculated each AUC (Fig. 3). AUCs for diagnosis were as follows: serum S100B, 0.58, 95% confidence interval (CI) 0.50–0.66; serum NSE, 0.71, 95% CI 0.62–0.79; serum tTau, 0.68, 95% CI 0.60–0.76; CSF S100B, 0.72, 95% CI 0.61–0.82; CSF NSE, 0.62, 95% CI 0.56–0.68; and CSF tTau, 0.76, 95% CI 0.64–0.86. AUCs for prognosis were as follows: serum S100B, 0.64, 95% CI 0.56–0.73; serum NSE, 0.71, 95% CI 0.60–0.81; serum tTau, 0.78, 95% CI 0.70–0.86; CSF S100B, 0.72, 95% CI 0.59–0.82; CSF NSE, 0.61, 95% CI 0.49–0.73; and CSF tTau, 0.72, 95% CI 0.60–0.83. Furthermore, optimal threshold values were calculated for biomarkers with AUC > 0.75. The values for CSF tTau (AUC = 0.76) to distinguish AEE from FS were 156.7 pg/ml (Youden index 0.59), sensitivity

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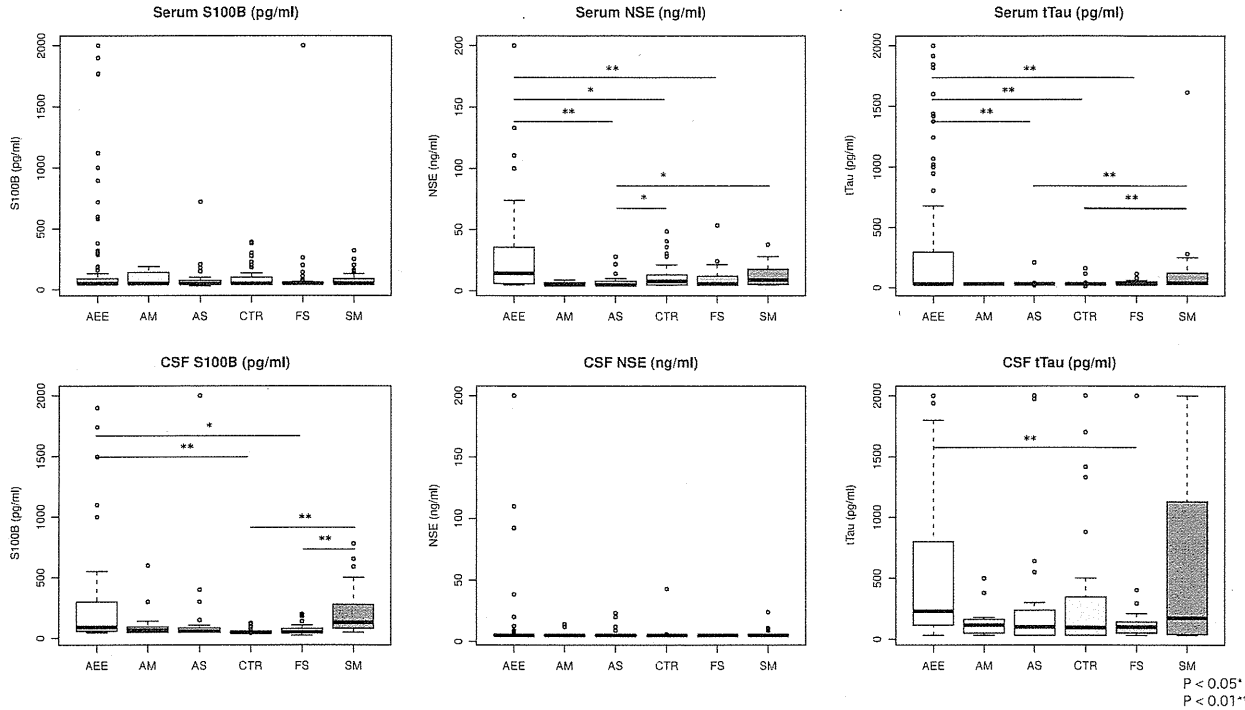

Fig. 1. Boxplot of serum (top) and CSF (bottom) levels of S100B (left), NSE (middle), and tTau (right) from patients with acute encephalitis/encephalopathy, AEE; aseptic meningitis, AM; afebrile seizures, AS; control, CTR; febrile seizures, FS; and septic meningitis, SM. Center lines denote medians, boxes denote 25–75% percentiles, and whiskers denote minimum and maximum values (white circles denote outliers). Parameters with statistically significant differences are noted with asterisks ($P < 0.05$) or double asterisks ($P < 0.01$).

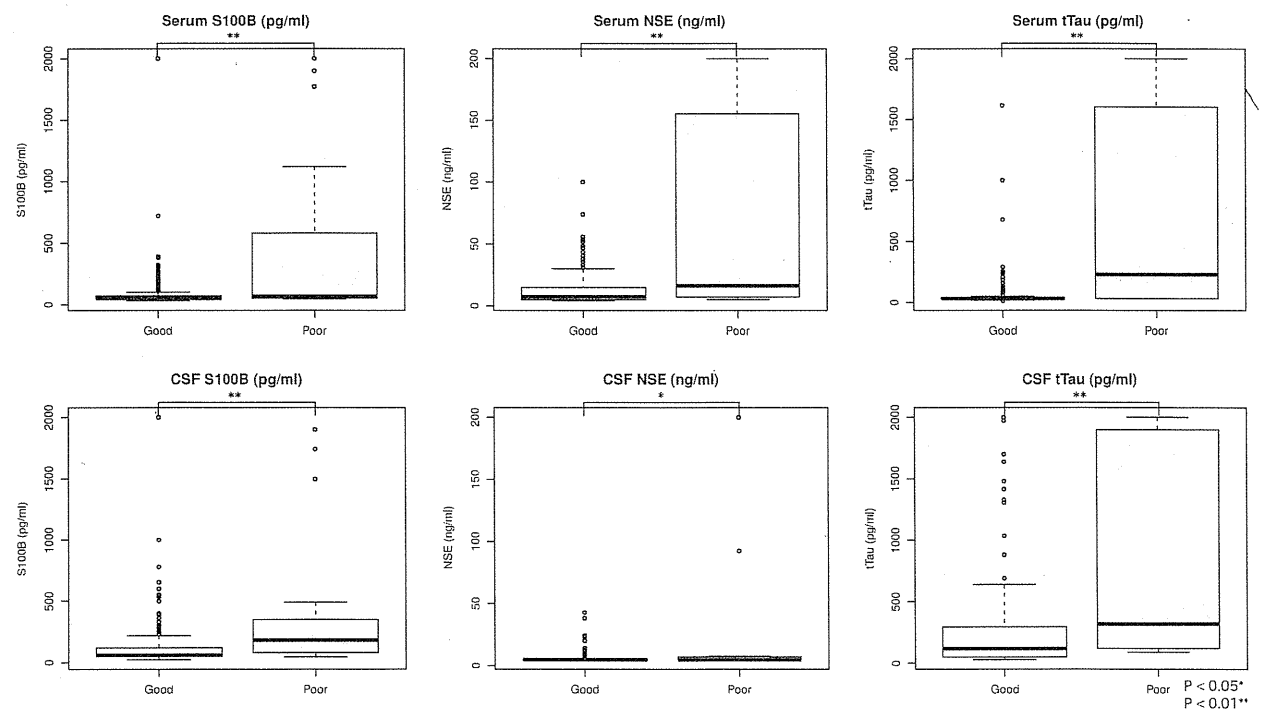

Fig. 2. Boxplot of serum (top) and CSF (bottom) levels of S100B (left), NSE (middle), and tTau (right) from patients with good prognosis (mRS 1–2) and poor prognosis (mRS 3–4). The centerlines denote medians, boxes denote 25–75% percentiles, and whiskers denote minimum and maximum values (white circles denote outliers). Parameters with statistically significant differences are noted with asterisks ($P < 0.05$) or double asterisks ($P < 0.01$).

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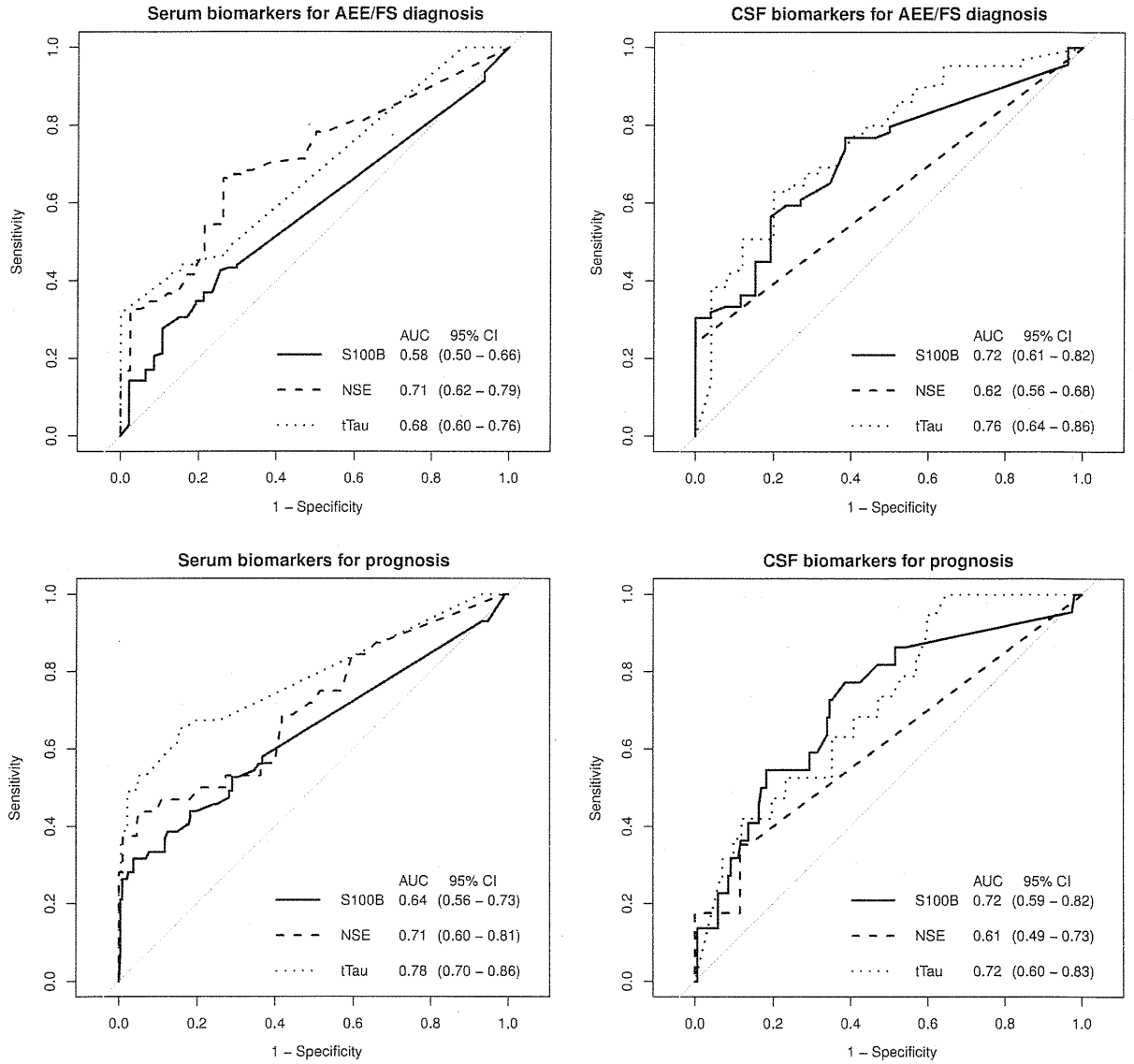

Fig. 3. ROC curves for diagnosis (top) and prognosis (bottom) with serum (left) and CSF (right) biomarkers (solid line, S100B; dashed line, NSE; and dotted line, tTau). AUCs are noted in the right lower corner with 95% CIs.

63.0% (95% CI 52.3–73.9%), and specificity 80.0% (95% CI 64.0–96.0%). The values for serum tTau (AUC 0.78) for distinguishing between poor and good prognoses were 68.8 pg/ml (Youden index 0.50), sensitivity 65.3% (95% CI 51.0–77.6%), and specificity 84.8% (95% CI 79.6–89.5%).

4. Discussion

There have been a many reports regarding the use of serum or CSF biomarkers to monitor various disorders, such as acute ischemic stroke, cerebral hemorrhage, traumatic brain injury, hypoxic ischemic encephalopathy, encephalitis, and meningitis [2–9]. Because different biomarkers were employed across studies and for different disorders, it was challenging to compare findings and establish firm conclusion; however, many biomarkers seemed to be increased in more severe disorders. Alterations in various biomarkers might reflect each central nervous system (CNS) cell damage rather than disease-specific changes. Here, we employed S100B, NSE, and tTau, as astrocytic, neuronal, and axonal damage markers in various acute pediatric neurological disorders to clarify their utility in making more precise diagnostic or prognostic predictions.

There were significant differences in serum NSE, serum tTau, CSF S100B, and CSF tTau (Fig. 1). As a whole, there tended to be the higher levels of the assessed biomarkers levels in AEE, which could reflect

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greater CNS damage than other more benign disorders. The level of serum NSE was higher in CTR than AS, however, their CSF NSE levels were not different. The patients in CTR were not healthy controls and NSE is also secreted outside CNS [3,16]. Thus the increased level of serum NSE in CTR must be reflected their extra CNS pathologies. As a prognostic evaluation, all biomarker levels were higher in patients with poor prognoses than in those with good prognoses (Fig. 2).

AEE can resemble FS, especially in an early stage of disease, in terms of fever, seizure, and consciousness disturbance. Therefore, we performed ROC curve analyses not only to differentiate between diagnoses of AEE and FS, but also to distinguish between poor and good prognoses. When AUC is higher than 0.75, the discriminative performance is thought to be good, and when AUC is higher than 0.90, it is thought to be excellent [11,12]. We found that CSF tTau was useful for discriminating AEE from FS (AUC = 0.76), and Serum tTau could differentiate between poor and good prognoses (AUC = 0.78). tTau was originally examined in CSF and was found to be increased in various neurological disorders; later, serum tTau was demonstrated as a good prognostic predictor [2–4,17–21]. Our results emphasize the usefulness of both CSF and serum tTau levels. tTau is considered more CNS specific than S100B and NSE, which corresponds to our findings [22].

Because they have relative low sensitivities and high specificities, as well as optimal threshold values, serum and CSF tTau could be useful for "ruling in" conditions, i.e., if serum or CSF tTau is higher than a threshold value, the patient is likely to have the target state, more severe disorder, or a more grim prognosis [23].

In this study, we did not employ a strict protocol for sampling timing or frequency. Therefore, it was not possible to do longitudinal analyses with serial specimens. Because CSF sampling is more invasive, serial CSF sampling is impractical. However, blood sampling is less invasive, so serial sampling for serum tTau examinations in various disorders could be a good strategy for further research. Finally, we would like to mention that an obstacle for clinical utilization of these biomarkers was that we used ELISA kits, which would not be suitable for a clinical setting, especially for emergencies in a patient-by-patient manner. Thus, a more convenient way for measuring potentially useful biomarkers is clearly needed.

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