Serum glial fibrillary acidic protein is a more specific biomarker than phosphorylated neurofilament heavy subunit, heart-fatty acidic protein, neuron specific enolase, and S100B protein for CT-positive mild-to-moderate traumatic brain injury

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Abstract

***Background*: Several biomarkers show diagnostic value for traumatic brain injury (TBI), especially in patients with severe TBI. In the present study, we examined whether glial fibrillary acidic protein (GFAP), phosphorylated neurofilament heavy subunit (pNF-H), heart-type fatty acid binding protein (H-FABP), neuron-specific enolase (NSE), and S100B protein (S100B) measured on admission to an emergency department showed diagnostic value in patients with mild-to-moderate TBI.**

***Methods*: A prospective study performed in our emergency department. After collecting informed consent, blood samples were obtained to measure GFAP, pNF-H, H-FABP, NSE, and S100B concentrations. All of the patients underwent head computed tomography (CT). The CT findings were considered positive if hemorrhagic brain injury was present. Receiver-operating characteristic (ROC) curve analysis was performed for each biomarker with positive head CT findings as the outcome variable.**

***Results*: Fifty-seven patients were included (39% male). Their median age was 70 years and the median Glasgow coma scale score was 15. Twelve patients (21%) had positive head CT findings (CT-positive group). The area under the ROC curves for GFAP, pNF-H, H-FABP, NSE, and S100B were 0.845, 0.569, 0.518, 0.744, and 0.753, respectively, and were statistically significant for GFAP, NSE, and S100B ($p<0.001$, $p=0.013$, and $p=0.010$, respectively). The area under the ROC curve was greater for GFAP than those for the other biomarkers.**

***Conclusions*: Our results suggest that serum GFAP measured shortly after emergency department admission shows greater diagnostic potential for head CT-positive TBI as compared with pNF-H, H-FABP, NSE, and S100B.**

Key words: GFAP, pNF-H, H-FABP, NSE, S100B.

Introduction

Many studies have evaluated whether serum biomarkers could be used in the diagnosis of traumatic brain injury (TBI); for example, to reduce the need for head computed tomography (CT) and/or to help diagnose CT-negative brain injury. Therefore, the concentrations of several biomarkers have been measured in several studies of patients with mild-to-moderate TBI, to determine the sensitivity and specificity of each biomarker. In our previous study, we measured the serum concentrations of glial fibrillary acidic protein (GFAP), S100B protein (S100B), and neuron-specific enolase (NSE) in patients with severe TBI and evaluated the diagnostic value of these biomarkers for predicting CT-positive brain injury. (1) In the present study, we measured the serum levels of these three biomarkers in patients with mild-to-moderate TBI, and also measured two additional biomarkers, phosphorylated neurofilament heavy subunit (pNF-H) and heart-type fatty acid

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binding protein (H-FABP).

GFAP is a monomeric intermediate-filament component of the astrocytic cytoskeleton that is frequently measured in studies of TBI, and shows excellent sensitivity and specificity for CT-positive and -negative TBI. (2,3) Moreover, GFAP might be superior to S100B and NSE for this purpose. (1) S100B is a calcium-binding protein localized to the cytoplasm of perivascular astrocytes. NSE is a glycolytic enzyme expressed in neuronal calls. S100B and NSE are among the most widely studied proteins in the field of TBI. (4) In addition, pNF-H and H-FABP have been proposed as candidate biomarkers for TBI. (5-7) pNF-H is a structural protein localized to axon fibers and is not normally detectable in blood. Although H-FABP is primarily expressed in the heart, it is also expressed in the brain, and its expression is elevated in patients with stroke or TBI.

In the present study, we compared the diagnostic value of GFAP with that of other established (NSE and S100B) and candidate (pNF-H, H-FABP) biomarkers for the diagnosis of mild-to-moderate TBI in patients admitted to an emergency department.

Materials and methods

Study design, patients, and sample collection

We conducted a prospective observational study of patients with mild-to-moderate TBI admitted to our emergency department, using blood samples obtained upon admission. The protocol and consent procedures were approved by the Institutional Review Board of Kumamoto Medical Center, Japan. The study was performed between May 2014 and June 2016 in the emergency department at Kumamoto Medical Center. The inclusion criteria were as follows: admission to the emergency department of Kumamoto Medical Center, single blunt head trauma, mild-to-moderate TBI with Glasgow coma scale score of 9-15, and head computed tomography (CT) scheduled before collecting informed consent. Pregnant women were excluded. A total of 57 patients were enrolled in this study. Informed consent was obtained from the next of kin for all patients.

After obtaining informed consent, the remainder of the blood sample taken upon emergency department admission was stored at -80 °C.

Positive head CT findings were defined as intracranial hemorrhagic findings. And the patients' clinical information was obtained from the patient's clinical chart.

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Biomarker assays

The serum biomarker concentrations were measured using enzyme-linked immunosorbent assays (GFAP, pNF-H: BioVendor - Laboratorni Medicina a.s., Brno, Czech Republic; NSE: Roche - Diagnostic Company, Tokyo, Japan; S100-B: Biovendor Laboratory Medicine, Modrice, Czech Republic; H-FABP: DS Pharma Biomedical Company, Osaka, Japan).

Statistical analysis

The patients were divided into two groups depending on whether the head CT findings were positive or negative for hemorrhagic brain injury. The two groups were compared by univariate analysis, with the Mann-Whitney U test for continuous variables and Fisher's exact probability test for categorical variables. The sensitivity and specificity of each biomarker were assessed by receiver-operating characteristic (ROC) curve analysis. The threshold of significance was set at $p<0.05$. Statistical analyses were performed using SPSS software version 23.0 (SPSS Inc., Chicago, IL, USA).

Results

Table 1 shows the characteristics of all 57 patients included in the study. Their median age was 70 years old and 39% were male. The CT head findings were positive in 12 patients (21%). The median biomarker concentrations were as follows: GFAP, 0.11 ng/ml; pNF-H, 53.9 pg/ml; H-FABP, 5.2 ng/ml; NSE, 12.6 ng/ml; and S100B, 7.2 pg/ml.

Table 2 compares the patient characteristics and biomarker concentrations, between the head CT-positive and -negative groups. The Glasgow coma scale score was significantly different between the two groups, with median values of 14 and 15 in the head CT-positive and -negative groups, respectively ($p=0.001$). The concentrations of GFAP, NSE, and S100B ($p<0.001$, $p=0.012$, and $p=0.007$, respectively) were significantly different between the two groups.

Figure 1 and **Table 3** show the results of the ROC curve analysis using the serum biomarkers to predict head CT-positive findings. The area under the ROC curves for GFAP, pNF-H, H-FABP, NSE, and S100B were 0.845, 0.569, 0.518, 0.744, and 0.753, respectively. The area under the ROC curves were statistically significant for GFAP, NSE, and S100B ($p<0.001$, $p=0.013$, and $p=0.010$, respectively).

Discussion

We previously reported that serum GFAP is a more specific biomarker for severe TBI as compared with NSE and S100B in severe TBI patients. (1) In this study using blood samples obtained within 3 hours of admission, the area under the ROC curve were 0.983, 0.670, and 0.658 for the biomarkers GFAP, NSE, and S100B for predicting positive head CT findings. Notably, the ROC curves for NSE and S100B were consistently below the ROC curve for GFAP.

In the present study, serum GFAP could be superior to other biomarkers (NSE, S100B, pNF-H, and H-FABP) for predicting positive head CT findings in patients with mild-to-moderate TBI because the ROC curve for GFAP was consistently above the curves for the other biomarkers. However, the area under the ROC for positive head CT of GFAP, NSE, and S100B were 0.845, 0.744, and 0.753, respectively. Considering that the value for GFAP was less than that observed in our earlier study in severe TBI, it is possible that serum GFAP might be correlated with the severity TBI and head CT findings. In fact, Pelinka et al reported that serum GFAP reflected the head CT classification and intracranial hypertension among patients with TBI. (8) Therefore, GFAP might show weaker diagnostic value for positive head CT findings in patients with mild-to-moderate than in patients with severe TBI.

Although both pNF-H and H-FABP were expected to show sufficient diagnostic value for detecting positive head CT, the area under the ROC curves for both biomarkers were inferior to those of the established biomarkers NSE and S100B. Although pNF-H and H-FABP were reported as prognostic biomarkers, they might be unsuitable for predicting the presence of mild-to-moderate TBI. (5-7)

NSE and S100B are well-established biomarkers for TBI and several studies have documented associations between these biomarkers and the severity of TBI. (4,9-11) In the present study, although the area under the ROC curves for NSE and S100B were >0.7 in mild-to-moderate TBI, both ROC curves were below the curve for GFAP.

Our findings suggest that serum GFAP is superior than NSE, S100B, pNF-H, and H-FABP for predicting positive head CT findings in mild-to-moderate TBI. A recent systematic review of blood-based biomarkers for the diagnosis of intracranial lesions on head CT (12) suggested the possibility that S100B could help decision-making in emergency departments. However, the authors concluded there is still insufficient evidence for S100B, GFAP, NSE, ubiquitin C-terminal hydrolase-L1 (UCH- L1), and tau protein.

Recent studies have indicated that GFAP and UCH-L1 are both remarkable diagnostic marker for mild-to-moderate TBI, including brain concussion. (13,14) We provide further evidence showing the superiority of GFAP to other biomarkers. Unfortunately, UCH-L1 was not measured in this study. Therefore, we suggest that the diagnostic value of UCH-L1 should be examined in future studies.

Based on our findings and results reported elsewhere, we believe that GFAP is an excellent candidate biomarker for the diagnosis of mild-to-moderate TBI, as well as severe TBI as reported elsewhere, as compared with NSE, S100B, pNF-H, and H-FABP. We think that there are several possible applications for these biomarkers that should be considered in the future, including: 1. avoiding unnecessary head CT scans, and 2. developing a diagnostic tool combined with head CT for TBI. Indeed, acute coronary syndrome can be diagnosed using a combination of symptoms, electrocardiography, and cardiac biomarkers. Prospective studies are needed to evaluate these possible applications.

This study had some limitations. First, although we conducted a prospective study, the investigators involved in obtaining informed consent and data collection were not available on a full-time, round-the-clock basis. Therefore, we could not include all patients admitted to our emergency department, which weakens the strength of our findings. Second, the study outcome was defined as positive head CT findings; brain concussion was not included in this outcome. Third, we used blood samples taken within 3 hours after admission; we did not evaluate the diagnostic value of follow-up biomarkers.

Conclusions

In conclusion, the results of the present study suggest that GFAP measured soon after admission to an emergency department is an excellent biomarker for diagnosis of positive head CT findings in patients with mild-to-moderate TBI. GFAP may be superior then other established (NSE and S100B) and candidate (pNF-H and H-FABP) biomarkers for the diagnosis of positive head CT findings in patients with mild-to-moderate TBI.

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Table 1. Patient characteristics and biomarker concentrations in all patients (n=57)

Variable	Value
Age (years)	70 (57-81)
Males	22 (39%)
Glasgow coma scale score	15 (14-15)
Systolic blood pressure (mmHg)	149 (126-165)
Diastolic blood pressure (mmHg)	79 (69-93)
Heart rate (beats/min)	84 (78-98)
Respiratory rate (breaths/min)	19 (17-24)
Serum biomarkers	
- GFAP (ng/mL)	0.11 (0.00-0.22)
- pNF-H (pg/mL)	53.9 (0.0-265.4)
- H-FABP (ng/mL)	5.2 (3.1-9.6)
- NSE (ng/mL)	12.6 (7.9-15.6)
- S100B (pg/mL)	7.2 (0.0-48.7)
Positive head CT findings	12 (21%)
- Traumatic SAH	4
- SDH	7
- Brain contusion	1

Legend: Values are expressed as n (%) or median (range). GFAP=glial fibrillary acidic protein; pNF-H=phosphorylated neurofilament heavy subunit; H-FABP=heart-type fatty acid binding protein; NSE=neuron-specific enolase; S100B=S100B protein; CT=computed tomography; SAH=subarachnoid hemorrhage; SDH=subdural hematoma.

Table 2. Comparison of patient characteristics and biomarker concentrations between the CT-positive and CT-negative groups

Variable	CT positive (n=12)	CT negative (n=45)	p value
Age (years)	69 (61-81)	70 (56-82)	0.922
Males	3 (25%)	19 (42%)	0.335
Glasgow coma scale score	14 (11-15)	15 (15-15)	**0.001**
Systolic blood pressure (mmHg)	140 (124-150)	155 (127-171)	0.117
Diastolic blood pressure (mmHg)	78 (68-85)	79 (69-97)	0.493
Heart rate (beats/min)	89 (83-96)	83 (78-99)	0.348
Respiratory rate (breaths/min)	23 (17-27)	19 (17-24)	0.164
Serum biomarkers			
- GFAP (ng/mL)	0.25 (0.17-2.42)	0.08 (0.00-0.16)	<**0.001**
- pNF-H (pg/mL)	89.1 (14.3-216.4)	43.1 (0.00-270.7)	0.521
- H-FABP (ng/mL)	7.3 (3.1-12.9)	5.1 (3.2-9.5)	0.768
- NSE (ng/mL)	18.2 (12.9-27.2)	11.3 (7.8-14.0)	**0.012**
- S100B (pg/mL)	94.0 (9.4-137.0)	0.2 (0.00-25.9)	**0.007**

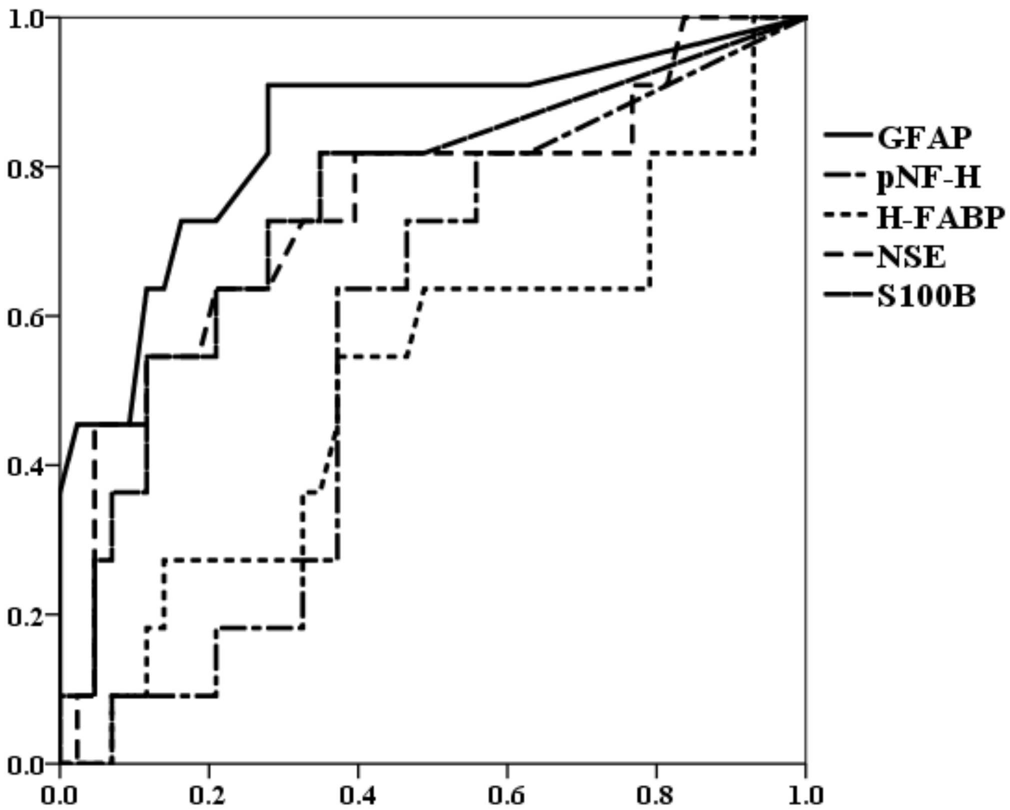
Legend: Values are expressed as n (%) or median (range). Bold font indicates significant difference at p<0.05. CT=computed tomography; GFAP=glial fibrillary acidic protein; pNF-H=phosphorylated neurofilament heavy subunit; H-FABP=heart-type fatty acid binding protein; NSE=neuron-specific enolase; S100B=S100B protein.

Table 3. Area under the receiver-operating characteristic curves analysis of each biomarker

Biomarker	AUC curve (95% CI)	p value
GFAP	0.845 (0.698-0.991)	<**0.001**
pNF-H	0.569 (0.398-0.739)	0.485
H-FABP	0.518 (0.315-0.721)	0.855
NSE	0.744 (0.565-0.923)	**0.013**
S100B	0.753 (0.582-0.924)	**0.010**

Legend: GFAP=glial fibrillary acidic protein; pNF-H=phosphorylated neurofilament heavy subunit; H-FABP=heart-type fatty acid binding protein; NSE=neuron-specific enolase; S100B=S100B protein; AUC curve=area under the characteristic curve; CI=confidence interval.

Figure 1. Receiver-operating characteristic curves for serum biomarkers for the prediction of positive head computed tomography findings



Legend: GFAP=glial fibrillary acidic protein; pNF-H=phosphorylated neurofilament heavy subunit; H-FABP=heart-type fatty acid binding protein; NSE=neuron-specific enolase; S100B=S100B protein.

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