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The Potential of Heart Fatty-Acid Binding Protein, Neurofilament Light, Interleukin-10 and S100 Calcium-Binding Protein B in the Acute Diagnostics and Severity Assessment of Traumatic Brain Injury

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Abstract

Background There is substantial interest in blood biomarkers as fast and objective diagnostic tools for traumatic brain injury (TBI) in the acute setting.

Methods Adult patients (≥18) with TBI of any severity and indications for CT scanning and orthopedic injury controls were prospectively recruited during 2011-2013 at Turku University Hospital, Finland. The severity of TBI was classified with GCS: GCS 13-15 was classified as mild (mTBI); GCS 9-12 as moderate (moTBI) and GCS 3-8 as severe (sTBI). Serum samples were collected within 24h of admission and biomarker levels analyzed with high-performance kits. The ability of biomarkers to distinguish between severity of TBI and CT positive and negative patients was assessed.

Results Among 189 patients recruited, neurofilament light (NF-L) was obtained from 175 TBI patients and 40 controls. S100 calcium-binding protein B (S100B), heart fatty-acid binding protein (H-FABP), and interleukin-10 (IL-10) were analyzed for 184 patients with TBI and 39 controls. There were statistically significant differences between levels of all biomarkers between the severity classes, but none of the biomarkers distinguished patients with moderate TBI (moTBI) from patients with severe TBI (sTBI). Patients with mTBI discharged from the emergency department had lower levels of IL-10 (0.26, IQR=0.21, 0.39 pg/mL), H-FABP (4.15, IQR=2.72, 5.83 ng/ml) and NF-L (8.6, IQR=6.35, 15.98 pg/ml) compared to those admitted to the neurosurgical ward, IL-10 (0.55, IQR=0.31, 1.42 pg/mL), H-FABP (6.022, IQR=4.19, 20.72 ng/ml) and NF-L (13.95, IQR=8.33, 19.93 pg/ml). We observed higher levels of H-FABP and NF-L in older patients with mTBI. None of the biomarkers or their combinations was able to distinguish computed tomography (CT)-positive (N=36) or CT-negative (N=58) patients with mTBI from controls.

Conclusions S100B, H-FABP, NF-L and IL-10 levels in patients with mTBI were significantly lower than in patients with moTBI and sTBI but alone or in combination, were unable to distinguish mTBI patients from orthopedic controls. This suggests these biomarkers cannot be used alone to diagnose mTBI in trauma patients in the acute setting.

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Keywords: traumatic brain injury, biomarkers, severity assessment, acute diagnostics

Key messages

- **What is already known on this subject**

  Traumatic brain injury (TBI), especially mild TBI, is still lacking objective, efficient and fast acute diagnostic tools. Blood-based biomarkers have been a target of interest as they could provide a fast and cost-efficient tool for diagnosis.

- **What this study adds**

  We studied S100B, H-FABP, NF-L and IL-10 in the acute diagnostics of TBI and found that the levels are significantly lower in mild TBI than in the more severe classes. None of these biomarkers or their combinations were able to distinguish patients with mild TBI from the orthopedic controls in this patient population.

Introduction

Traumatic brain injury (TBI) is diagnosed based on clinical and imaging findings. Mild TBI (mTBI) is challenging to diagnose and lacks objective, efficient and fast acute diagnostic tools. The diagnosis of moderate (moTBI) and severe TBI (sTBI) is easier as clinical signs are more reliable and patients have traumatic findings on head computed tomography (CT).[1] Blood-based biomarkers have been a target of interest as they could provide a fast and cost-efficient tool for diagnosis and aid in the referral to head CT scan.[2] TBI is a complex condition affecting several brain structures. Structural markers, S100 calcium-binding protein B (S100B), heart fatty-acid binding protein (H-FABP), neurofilament light (NF-L) and an inflammation marker interleukin-10 (IL-10) were studied.

In the context of TBI, serum S100B represents astrocyte damage.[3] S100B is also expressed in other tissues and its levels increase after polytrauma and exercise.[4] S100B can be used to rule out intracranial lesions in selected patients with mTBI.[5] H-FABP is expressed in the heart and predominantly in the neuronal cell bodies in the brain.[6] H-FABP has shown promise in the diagnosis of mTBI.[7] However, as it is also a marker for cardiac injury, its performance as a specific marker of brain injury remains undetermined.[8] NF-L is a marker of myelinated axonal injury[9] and possibly identifies patients requiring acute brain imaging following TBI.[10] IL-10 is an anti-inflammatory cytokine expressed in response to brain injury. Although the correlation of IL-10 with GCS in patients with TBI remain conflicting [11] [12], it seems to distinguish between CT-positive and CT-negative patients with mTBI. [11]

TBI is a heterogenous condition and diagnostics based on a single biomarker is perhaps not adequately sensitive and specific.[13] Accordingly, biomarker panels have been studied and combined biomarkers indicating different kinds of structural injuries are likely to be more efficient in diagnostics than single biomarkers.[13]

The first aim of this study was to evaluate how the biomarkers of different cellular origins correlate with the severity of TBI. The second aim was to assess if the biomarkers or their combinations could distinguish patients with mTBI - with or without positive CT findings - from orthopedic controls.

Methods

Study Population

This prospective study was part of the EU-funded TBIcare project (Evidence-based Diagnostic and Treatment Planning Solution for Traumatic Brain Injuries). Patients were recruited (from 8 a.m. to 10 p.m., convenience sampling) at Turku University Hospital between November 2011 and October 2013. Biomarkers were available for 189 patients with all severity of TBI and 40 orthopedic controls.

Inclusion criteria for the TBI group were: age ≥18 years, clinical diagnosis of TBI with indications for acute head CT according to National Institute for Health and Care Excellence criteria.[14] Exclusion criteria were head injury without an indication for CT, blast-induced or penetrating injuries, prior neurological disease causing inability to live independently, more than two weeks from the injury, chronic subdural hematoma, inability to speak Finnish or no consent obtained. The orthopedic controls were ≥18 years old and had acute non-trivial orthopedic injuries to the extremities or trunk. Exclusion criteria were any suspicion of earlier TBI or degenerative neurological disease, polytrauma needing intensive care, or trivial injuries not needing acute interventions or follow-up. All patients or their proxies were given oral and written information about the study and written consent was obtained. Southwest Finland Hospital District Research Ethics Committee (decision 68/180/2011) approved the study.

Traumatic brain injury severity classes and head computed tomography classifications

The severity of TBI was based on the lowest GCS before possible intubation, either at the scene of accident or emergency department (ED). GCS 13-15 was classified as mTBI; GCS 9-12 moTBI and GCS 3-8 sTBI.

CT scans were classified according to Marshall grading system.[15] Neuroradiologists at the Turku University Hospital and a senior neurosurgeon (JPP) double-read the CT scans.

Biomarker analyses

Blood samples for NF-L, H-FABP, IL-10 and S100B were obtained within 24 hours from admission. NF-L levels were measured using the Human Neurology 4-Plex A assay (N4PA) on an HD-1 Single molecule array (Simoa) from Quanterix (Quanterix, Lexington, MA). LLoD (lower limit of detection) for NF-L was 0.104 pg/mL, LLoQ (lower limit of quantification) 0.241 pg/mL, calibration ranging from 0.533 pg/mL to 453 pg/mL. The K151HTD kit was used to analyze H-FABP and K151QUD for IL-10, both from Meso Scale (Meso Scale Diagnostics, Rockville, MD, USA). LLoD for H-FABP was 0.103 ng/mL with calibration range of 0.137-100 ng/mL. LLoQ had not been established as the test has not been fully validated yet. LLoD for IL-10 was 0.04 pg/mL with LLoQ being 0.298 pg/mL, calibration rate being 0.0774-317 pg/mL. S100B was measured using EZHS100B-33K kit from Millipore (Millipore, Billerica, MA, USA). LLoD was 2.7 pg/mL with calibration ranging from 2.7 to 2000 pg/mL. There were no samples below the LLoDs and LLoQs. All the kits were used according to the manufacturers' recommendations. The measurements were performed by board-certified laboratory technicians blinded to clinical data using one batch of reagents in one round of experiments. Intra-assay coefficients of variation monitored using high and low QC samples that were common across plates, were below 10% for all analytes.

Statistical Analysis

All available data was used without a priori sample size estimation. Data were analyzed using IBM SPSS Statistics version 24 (IMB Corporation, Armonk, New York, USA). Demographics of the patients are presented as mean ± SD. Normality of the biomarkers was assessed by Kolmogorov-Smirnov test. The biomarker levels were not normally distributed and nonparametric tests were used, the results presented as medians (IQR). Correlations of biomarker levels with gender and age for all severities of patients with TBI were analyzed with Spearman's rank correlation and Pearson's correlation, respectively. Mann-Whitney U test was used to compare the levels of biomarkers between the severities of TBI and between the patients with mTBI who were admitted to hospital vs

discharged from the ED. P<0.05 was considered statistically significant. Correction for multiple testing was not done.

mTBI patients' neurological symptoms may be vague and not fulfill the criteria for a head CT. Therefore, diagnostic ability of the biomarkers in differentiating between orthopedic controls and all patients with mTBI and patients with mTBI with or without CT findings was evaluated with the area under the receiver operating characteristic (ROC) (pROC package for S+ version 8.1 (TIBCO, Software Inc.))[16] curve (AUC). AUC of 0.8-1.0 was considered good, AUC of 0.7-0.8 adequate, and AUC < 0.7 poor. All tests were two-tailed. Partial AUC (pAUC) was used to compare only a clinically significant portion of the AUC curves (sensitivity range of 90–100%). Its value summarizes a pre-specified range of interest of the ROC curve excluding regions with low levels of sensitivity or specificity.

Combinations of biomarkers were obtained using PanelomiX,[17] which uses iterative permutation-response calculations. The cut-off values of each molecule were changed iteratively by 2% increment quantiles. After each iteration the specificity (SP) was calculated using a sensitivity (SE) set between 90%–100% in order to minimize the false negative cases of mTBI patients.

A maximum number of three biomarkers or clinical parameters in each model were investigated. Cross validation and ROC analysis were used to evaluate the performance of the model. When evaluating a combination of biomarkers, only patients with all tested parameters were included in the analysis. All the patients with missing data were excluded from the panel testing. The index test results were cross-tabulated against the results using the threshold calculated by setting the sensitivity above 90%.

Patient and Public Involvement

No patient involved.

Results

The mean age was 49±20 and 52±19 years in patients with TBI and orthopedic controls, respectively. Most patients with TBI were male 135/189 (71%) whereas most orthopedic controls were female 22/40 (55%). mTBI was diagnosed in 108/189 (57%), moTBI in 48/189 (25%) and sTBI in 33/189 (18%) of the patients. CT was negative (Marshall I) in 77/189 (41%) patients and positive (Marshall II-VI) in 112/189 (59%) patients. Table 1 demonstrates patient characteristics.

Table 1 Demographic and Clinical Characteristics of patients with TBI and orthopedic controls

	TBI (n=189)	Controls	P-value	TBI CT+ (n=112)	TBI CT- (n=77)	P-value
Age (years)	49 ± 20	52 ± 19	0.351[a]	53±20	42±18	**P<001[a]**
Sex, n (%)						
Male	135 (71.4)	18 (45)	0.431[b]	86 (76.8)	49 (63.6)	**0.049[b]**
Female	54 (28.6)	22 (55)		26 (23.2)	28 (36.4)	
Severity, n (%)						
Mild (GCS 13-15)	108 (57.1)			41 (36.6)	67 (87)	
Moderate (GCS 9-12)	48 (25.4)			42 (37.5)	6 (7.8)	
Severe (GCS 3-8)	33 (17.5)			29 (25.9)	4 (5.2)	
Injury Severity Score[18] (median [IQR])	13 (16)			17 (16)	6 (11)	**P<0.001[c]**
Cause of injury, n (%)						
Incidental fall	105 (55.6)			70 (62.5)	35 (45.5)	**0.038[b]**
Road traffic crash	55 (29.1)			29 (25.9)	26 (33.8)	
Violence/assault	18 (9.4)			8 (7.1)	10 (13)	
Other non-intentional injury	4 (2.1)			0 (0)	4 (5.2)	
Suicide attempt	2 (1.1)			1 (0.9)	1 (1.3)	
Other	5 (2.6)			4 (3.6)	1 (1.3)	
CT findings (Marshall Grade), n (%)						
No visible pathology	77 (40.7)			0 (0)	77 (100)	
Diffuse injury	37 (19.6)			37 (33)		
Diffuse injury with swelling	6 (3.2)			6 (5.4)		
Diffuse injury with shift	2 (1.1)			2 (1.8)		
Evacuated mass lesions	37 (19.6)			37 (33)		
Unevacuated mass lesions	30 (15.9)			30 (26.8)		
Pupil reactivity, n (%)						
Unreactive	17 (9)			15 (13.4)	2 (2.6)	**0.009[b]**
Sluggish	6 (3.2)			5 (4.5)	1 (1.3)	
Reactive	154 (81.5)			82 (73.2)	72 (93.5)	
Missing data	12 (6.3)			10 (8.9)	2 (2.6)	
Total	189			112	77	

[a] Student t-test significance; [b] Chi-squared test significance; [c] Mann-Whitney U test significance.

Marshall grade I = CT-negative (no visible pathology), Marshall grade II-VI = CT-positive (pathological findings in CT)

Blood samples were obtained within 24h from admission and analyzed in two laboratories. Unfortunately, some patients did not have enough frozen serum tubes to be sent to both laboratories explaining the lack of biomarker data for those patients. Time elapse from injury to blood sampling was 15.6±12.4 hours in patients whose exact time of injury was known (N=84). The exact time of injury was unavailable for 105 patients and 33 controls and was estimated using the best available

information. Out of these, 40 patients and 21 controls were sampled within 24h and 65 patients and 12 controls more than 24h from the injury. For seven controls no estimate was possible as no injury time was available at all.

Single biomarkers

The results for single biomarkers for all severities of TBI are reported in Table 2 and Figure 1. For all individual biomarkers, there were significant differences between patients with mild TBI vs moderate TBI and mild TBI vs severe TBI (all p<0.001 or 0<0.0001), but no significant difference between patients with moderate TBI vs severe TBI.

Table 2 Levels of single biomarkers in patients with TBI.

	Mild n=104*	**Moderate n=47***	**Severe n=33***
IL-10) median (IQR) pg/ml	0.436 (0.25, 0.89)	1.41 (0.67, 5.36)	1.38 (0.62, 4.33)
H-FABP median (IQR) ng/ml	5.17 (3.78, 10.41)	8.67 (5.47, 21.25)	12.66 (8.37, 46.11)
S100B median (IQR) pg/ml	78.05 (44.36, 114.39)	168.24 (63.14, 278.95)	184.45 (69.02, 498.87)
NFL median (IQR) pg/ml	12.35 (7.52, 19.02)	70.95 (49.75, 154.70)	79.4 (41.7, 179)

- ** For NFL, n in mTBI = 98, n in moTBI = 46; n in sTBI = 31*

Orthopedic controls vs mTBI

In orthopedic controls the median S100B (N=39), H-FABP (N=39), NF-L (N=40) and IL-10 (N=39) were 85.1 (IQR 42.4, 137.5) pg/ml, 7.1 (IQR 5.0, 11.1) ng/ml, 10.7 (IQR 6.8, 20.7) pg/ml and 0.51 (IQR 0.27, 0.92) pg/ml, respectively (Figure 1). Analyses were performed choosing a high sensitivity cut off from the ROC curve in attempt to find the true mTBI patients from the orthopedic controls. As shown in Table 3 none of the biomarkers were able to distinguish patients with mTBI from orthopedic controls, nor were they able to distinguish the patients with mTBI with or without CT findings from the orthopedic controls (Supplemental Tables 1, 2). Frequencies of the below and above thresholds

of measured biomarkers in patients with mTBI and controls are represented in Supplemental Table 3.

Table 3 Ability of the individual biomarkers in discriminating between all patients with mTBI (n=94, CT-positive and CT-negative) and orthopedic controls (n=39) with sensitivity set to >90%.

	AUC (95% CI)	**pAUC (95% CI)**	**Threshold**	**SE (%) (95% CI)**	**SP (%) (95% CI)**
H-FABP (ng/ml)	0.592 (0.495-0.688)	0.2 (0.0-0.8)	53.31	98.9 (96.8-100.0)	2.6 (0.0-7.7)
IL-10 (pg/ml)	0.544 (0.438-0.649)	0.3 (0.0-1.2)	83.70	100.0 (100.0-100.0)	2.6 (0.0-7.7)
S100B (pg/ml)	0.527 (0.413-0.642)	0.7 (0.1-1.7)	244.90	94.7 (89.5-98.9)	10.3 (2.6-20.5)
NF-L (pg/ml)	0.526 (0.416-0.636)	0.4 (0.0-1.4)	4.2	97.9 (94.7-100.0)	2.6 (0.0-7.7)

When the SE is set to > 90%, the examination area of the ROC curve covers only the range established between 90 to 100% SE. According to that, pAUC values that are displayed in this manuscript moves from 1 to 10%, being 10 a perfect partial ROC curve and 5 a non-relevant discrimination. SE = sensitivity, SP = specificity, Threshold = Biomarker concentration.

Patients with mTBI discharged from the ED had lower levels of IL-10, H-FABP and NF-L compared to those admitted to neurosurgical ward (Table 4).

The effect of age or gender were studied with all severities of TBI. They did not have any correlation with S100B or IL-10. Levels of H-FABP (r=0.300, p=0.002) and NF-L (r=0.315, p=0.002) correlated positively with age only in mTBI. Males had higher levels of NF-L than females, 14.40 (IQR 8.5, 19.95) vs 8.80 (IQR 6.7, 15.75) (p=0.04) also in mTBI only, whereas gender did not have any effect with H-FABP.

Table 4 Demographics of the discharged (n=30) vs admitted (78) patients with mTBI.

	Home	Ward	P-value
Age (years)	39 ± 18	46 ± 19	**0.093[a]**
Sex, n (%)			
Male	16 (53.3)	55 (70.5)	**0.092[b]**
Female	14 (46.7)	23 (29.5)	
Injury Severity Score (median [IQR])	3 (4.5)	12 (13)	
No of patients with GCS 13-15			
15	24 (80.0)	53 (67.9)	
14	6 (20.0)	19 (24.4)	
13	0	6 (7.7)	
IL-10 median (IQR) pg/ml	0.26 (0.21, 0.39)	0.55 (0.31, 1.42)	<0.001
H-FABP median (IQR) ng/ml	4.15 (2.72, 5.83)	6.02 (4.19, 20.72)	<0.001
NF-L median (IQR) pg/ml	8.6 (6.35, 15.98)	13.95 (8.33, 19.93)	0.018

[a] Student t-test significance; [b] Chi-squared test significance.

7 patients with mTBI lacked all biomarkers due to the insufficient amount of blood sample drawn.

Combination of biomarkers

PanelomiX was used to assess if combinations of biomarkers could distinguish patients with mTBI from orthopedic controls, or patients with mTBI with or without CT findings from orthopedic controls. When sensitivity was set to >90%, none of the single biomarkers (Table 3, Supplemental Tables 1,2) or their combinations (Table 5) was able to distinguish patients with mTBI (all or those with or without CT findings) from orthopedic controls. Supplemental Table 4 presents the index test results from the biomarker panels cross-tabulated against the outcome (mTBI vs orthopedic controls) shown in Table 5.

Table 5 PanelomiX: Panels of the best biomarker combinations in discriminating patients with mTBI (CT-positive and CT-negative) and orthopedic controls with sensitivity set to > 90% (n(mTBI)=94, n(mTBI, CT-negative)=58, n(mTBI, CT-positive)=36, n(orthopedic controls)=39).

	Number of biomarkers	**(pg/ml)**	**Biomarkers H-FABP(ng/ml) S100B(pg/ml)**	**(pg/ml)**	**No of biomarkers needed to be +**	**Sensitivity(%) (95%CI)**	**Specificity(%) (95%CI)**	**pAUC (%) (95% CI)**	**p**
mTBI vs controls	3	IL-10 (<0.359)	H-FABP (<4.66)	NF-L (>11.8)	1	90.4 (84.0-95.7)	33.3 (20.5-48.7)	1.7 (0.8-3.2)	0.1494
mTBI (CT-) vs controls	3	IL-10 (<0.274)	H-FABP (<4.06)	NF-L (>10)	1	91.4 (82.8-98.3)	30.8 (17.9-46.2)	1.8 (0.7-3.5)	0.32993
mTBI (CT+) vs controls	3	IL-10 (<0.269)	S100B (<47.9)	NF-L (>12)	1	91.7 (80.6-100.0)	33.3 (17.9-48.7)	2.0 (0.7-3.9)	0.52813

When the SE is set to > 90%, the examination area of the ROC curve covers only the range established between 90 to 100% SE. According to that, pAUC values that are displayed in this manuscript moves from 1 to 10%, being 10 a perfect partial ROC curve and 5 a non-relevant discrimination. CT- = CT-negative, CT+ = CT-positive.

Discussion

Our first aim was to evaluate how the biomarkers correlated with the severity of TBI. The second purpose was to assess if the biomarkers or their combinations could distinguish patients with mTBI––with or without traumatic intracranial findings—from orthopedic control patients without TBI.

All studied biomarkers showed significantly lower levels in patients with mTBI than in cases with moTBI and sTBI. There were no statistically significant differences in the biomarkers between the patients with moTBI and sTBI. None of the single biomarkers or biomarker panels were able to distinguish patients with mTBI (all or those with or without traumatic CT findings) from the orthopedic controls. The level of IL-10 was significantly higher in patients with mTBI who were admitted to ward than in patients who were discharged.

Significantly higher levels of S100B have been found in CT-positive patients with mTBI than in CT-negative patients with mTBI.[13] Scandinavian guidelines suggest using S100B obtained ≤ 6 hours after the trauma in decision-making for a head CT in patients with mTBI[19]. The suggestion has been validated in an external cohort.[5] We found that S100B did not differentiate head trauma patients with or without abnormal CT findings, nor did it distinguish head trauma patients from controls, findings which are consistent with other studies [20] as the likely reason for this is that S100B is not entirely brain-specific and our sampling time in most of our TBI patients exceeded the cut-off of six hours. Also, our choice of Millipore assay instead of Elecsys may have influenced the results. Notably, the Scandinavian guidelines suggest performing a head CT past six hours and when extracranial injuries are present.

Significantly higher levels of H-FABP have been found in CT-positive than in CT-negative patients with mTBI.[10 13] We did not find any difference between the patients with mTBI with or without CT findings and orthopedic patients. The kinetics of H-FABP seems to be fast[21] and would require blood sampling within a few hours of the injury, and the sampling time exceeded this in most of our patients. H-FABP however is not brain specific as higher levels of H-FABP are observed in patients with polytrauma compared with patients with isolated TBI.[22] This is in line with our finding that H-FABP is

also released to bloodstream in orthopedic trauma. The combination of TBI and orthopedic trauma may cause an additive increase in the biomarker level.

Significantly higher levels of NF-L have been found in CT-positive than in CT-negative patients with mTBI.[10] There were no difference in NF-L levels between the patients with mTBI with or without CT findings and orthopedic patients. There are few studies on serial sampling of NF-L in TBI. The level of NF-L increases slowly. The half-life time is very long and is not yet known properly[23] indicating that NF-L might perform better if blood samples were collected at later time points.

We found that patients who were clinically in better condition and were discharged had significantly lower levels of IL-10 compared to those admitted to a ward, suggesting that IL-10 may reflect the severity of isolated TBI. There were no difference between the biomarker levels of the mTBI patients with or without abnormal CT findings and the orthopedic controls, suggesting that IL-10 increases also in orthopedic trauma.

Interestingly, the levels of IL-10 in moTBI were higher than in sTBI. IL-10 seems to increase rapidly after TBI and stay elevated for several days.[12] The finding of higher levels of IL-10 in moTBI than in sTBI is somewhat contradictory to the finding of higher level of NF-L in sTBI than in moTBI as the peak time of NF-L appears later. Again our sampling time varied substantially, causing a possible confounding factor. However, these findings also contribute to the existing debate about distinguishing moderate and severe TBI. The clinical classification of moTBI and sTBI at acute phase are based on GCS and CT findings. In our study the biomarkers could not distinguish between moTBI and sTBI, supporting the assumption that the severity of TBI diagnosed at acute phase by GCS is artificial and that moderate and severe TBI have overlapping features.

As biomarkers represent injuries in different structures of the brain, combining them in a diagnostic panel could provide better precision than any biomarker alone. Biomarker panels have been shown to discriminate CT-negative and CT-positive mTBIs[10 13] as well as TBIs of all severities.[10] In our previous work,[10] the best biomarker panel to discriminate CT-positive patients with mTBI from CT-negative was H-FABP, S100B and tau whereas a combination of GFAP, H-FABP and IL-10

discriminated best CT-positive patients with TBI from CT-negative in the group including all severities.[10] In the current study, we did not use the panels to distinguish the CT-positive and CT-negative patients with mTBI from each other. We were interested in finding a panel to distinguish the orthopedic trauma patients without TBI from the patients with mTBI (CT- or CT+) in the acute phase. However, in the current study, none of the single biomarkers or their combinations distinguished patients with mTBI from orthopedic controls. One explanation could be that almost 70% of our patients with mTBI had GCS of 15, indicating a minimal head injury. Future studies should assess if biomarkers could distinguish mTBI patients with GCS of 13-14 in need of a head CT.

Age did not affect the levels of S100B in our study which is discordant with another study on patients with mTBI over 65 years of age.[24] That study used a cutoff point at 65 years, whereas we did not have any specific cutoff point. Neurodegenerative diseases or brain aging per se might have an effect on the results.[24] We observed higher levels of H-FABP and NF-L in older patients with mTBI, supporting previous finding that the levels of NF-L are age dependent.[25] Age-related cut-offs for elevated levels will probably be needed for some TBI biomarkers. Gender affected only the levels of NF-L with males having higher levels than females in mTBI.

Our study has limitations. The severity assessment of TBI based on GCS is artificial and defined at a single point in time does not represent the biological seriousness of the trauma well. The time of the accident was not known in all cases causing variable delays to the blood sampling and variability on the levels of biomarkers. However, in clinical reality different delays after the injury will always remain a problem. The initial blood test represents only a narrow window on the dynamic pathophysiological processes of TBI. Several samples at standard timepoints would be more informative. Finally, our recruitment logistic favored patients with mTBI admitted to the ward and the percentage of patients with mTBI was thus smaller than in many other studies. Therefore, our results are not necessarily applicable to the mildest patients with mTBI who are discharged from the ED, many without a head CT scan.

In conclusion, studied biomarkers showed significantly lower levels in patients with mTBI compared to more severe TBIs, but were not able to distinguish moTBI from sTBI reliably. None of the studied biomarkers or panels of biomarkers helped in distinguishing patients with mTBI from orthopedic controls or aid in decision making for CT scanning. Our study highlights the need to assess the reliability and usability of different diagnostic biomarkers at various time points and in various patient populations after a TBI.

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Contributorship statement

PK, JPP and RSKT conceived and designed the current study. JPP, RSKT, AJK, HRM, JT and OT recruited the patients. JPP, RSKT, AJK, MM, IH, HRM, JT, PK and OT collected and curated the data. MM, LA and LL conducted the statistical analyses. HZ, KB and JS supervised the biomarker analyses. PH, DKM, VFJN and OT supervised the TBIcare study. PK drafted the manuscript with critical contributions from JPP and RSKT. All authors substantially contributed to the revision of the manuscript.

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Competing interests

Professor Menon reports grants from European Union, during the conduct of the study; grants, personal fees and non-financial support from GlaxoSmithKline, personal fees and non-financial support from Pfizer Ltd, personal fees from NeuroTrauma Sciences, personal fees from Calico Ltd, grants and personal fees from PressuraNeuro Ltd, grants and personal fees from Integra Neurosciences, grants and personal fees from Lantmannen AB, outside the submitted work; Dr. Newcombe reports grants from Grant from Roche, outside the submitted work; Professor Zetterberg reports that he has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics and CogRx, has given lectures in symposia sponsored by 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; Dr. Blennow reports that he 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 the other authors have nothing to disclose.

Data availability statement

Data are available upon reasonable request. De-identified clinical, imaging, and biochemical data not published within the article can be shared with a qualified investigator by request.

Disclosures

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