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Correlation of blood biomarkers and biomarker panels with traumatic findings on computed tomography after traumatic brain injury

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ABSTRACT

The aim of the study was to examine the ability of eight protein biomarkers and their combinations in discriminating CT-negative and CT-positive patients with TBI, utilizing highly sensitive immunoassays in a well-characterized cohort. Blood samples were obtained from 160 patients with acute TBI within 24h from admission. Levels of β-amyloid isoforms 1-40 (Aβ40) and 1-42 (Aβ42), glial fibrillary acidic protein (GFAP), heart fatty-acid binding protein (H-FABP), interleukin 10 (IL-10), neurofilament light (NF-L), S100 calcium-binding protein B (S100B) and tau were measured. Patients were divided into CT-negative (n=65) and CT-positive (n=95), and analyses were conducted separately for TBIs of all severities (Glasgow Coma Score 3-15) and mild TBIs (mTBI, Glasgow Coma Score 13-15). NF-L, GFAP and tau were the best in discriminating CT-negative and CT-positive patients, both in patients with mTBI and with all severities. In patients with all severities, area under the curve of the receiver operating characteristic (AUC) was 0.822, 0.817, and 0.781 for GFAP, NF-L and tau, respectively. In patients with mTBI, AUC was 0.720, 0.689 and 0.676, for GFAP, tau and NF-L, respectively. The best panel of three biomarkers for discriminating CT-negative and CT-positive patients in the group of all severities was a combination of GFAP+H-FABP+IL-10, with a sensitivity of 100% and specificity of 38.5%. In patients with mTBI, the best panel of three biomarkers was H-FABP+S100B+tau, with a sensitivity of 100% and specificity of 46.4%. Panels of biomarkers, sampled within 24 hours from the injury, outperform individual biomarkers in separating CT-negative and CT-positive patients. Panels consisted of mainly different biomarkers than those, which performed best as an individual biomarker.

Keywords: traumatic brain injury, biomarkers, computed tomography

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Introduction

Traumatic brain injury (TBI) is a major global health problems with more than 50 million new cases annually, and the incidence is rising among both young and elderly people.[1–3] Mild TBI (mTBI) represents 80-90% of all TBIs[4]. Some patients meeting the clinical diagnostic criteria for mTBI may have significant traumatic intracranial findings on head computed tomography (CT), requiring neurosurgery.[5] To determine the need for a head CT is sometimes challenging due to commonly occurring confounding factors.

The clinical significance of blood-based biomarkers in TBI for detecting patients with traumatic intracranial findings is still unclear. It is likely that instead of focusing on the use of a single biomarker, optimized combinations of biomarkers should be sought for different clinical questions, due to the complexity of the brain and heterogeneity of TBIs.

Guidelines when to perform a head CT scan have been introduced into clinical practice to help in screening patients who may have significant intracranial injuries.[6–8] Still, the majority of patients scanned following these recommendations show a negative CT.[9,10] Thus, improved regimens for decision making regarding CT scanning are warranted in order to decrease radiation load and costs. The Scandinavian guideline for management of mild head injury recommends the use of biomarker S100 calcium-binding protein B (S100B) in patients with mTBI who are admitted to hospital within six hours after the injury.[11] However, S100B is expressed in multiple extracerebral tissues and its levels increase e.g. after extracranial injuries [12] and physical exercise[13].

Recent research has found several novel protein biomarkers with more brain-specific origin, and which thus could be more suitable for assessing the need for a CT following TBI. Glial fibrillary acidic protein (GFAP), which is expressed in the cytoskeleton of glial cells[14] has been studied widely in detecting acute intracranial injuries after a TBI, with promising results[15–18]. Both S100B and the combination of GFAP and ubiquitin C-terminal hydrolase L1 (UCH-L1) have showed promise as biomarkers in screening for CT-positivity/negativity in patients with acute TBI[19–21]. Heart fatty-acid binding protein (H-FABP), a cytosolic trafficking protein[22], is expressed in the heart but also in the brain, and has been shown to predict TBI-related intracranial pathologies[23]. An anti-inflammatory mediator interleukin

10 (IL-10)[24] has also showed promise in differentiating CT-positive from CT-negative patients with mTBI.

Other brain-related protein biomarkers that have been studied in the diagnostics of TBI are β-amyloid isoforms 1-40 (Aβ40)[19, 20] and 1-42 (Aβ42)[17,25] reflecting amyloid precursor protein metabolism, neurofilament light chain (NF-L) being abundant in the long myelinated subcortical axons[26,27], and microtubule-associated protein tau located in the axonal cytoskeleton[17,28]. All these proteins have been mainly studied in the subacute/chronic stage of TBI, and their utility in predicting intracranial pathologies on acute CT after TBI is poorly known.

We investigated the ability of Aβ40 and Aβ42, GFAP, H-FABP, IL-10, NF-L, S100B and tau and their combinations in discriminating CT-negative (CT-) and CT-positive (CT+) patients with TBI, utilizing modern highly sensitive immunoassays[23,24,29] in a well-characterized study cohort[15,30–32].

Methods

Study population

This prospective study was part of the EU-funded TBIcare (Evidence-based Diagnostic and Treatment Planning Solution for Traumatic Brain Injuries) project, where we recruited patients with TBIs of all severities at the Turku University Hospital, Finland during November 2011 to October 2013 as described elsewhere[15]. All patients were treated according to local guidelines based on existing international guidelines and recommendations[33]. All patients were examined and classified for the presence of extracranial injuries using the Injury Severity Score (ISS).

Biomarker analyses

Blood samples for Aβ40, Aβ42, GFAP, H-FABP, IL-10, NF-L, S100B, and tau were obtained within 24 h from admission. Plasma H-FABP and IL-10 were analyzed using the K151HTD and K151QUD kits, respectively from Meso Scale (Meso Scale Diagnostics, Rockville, MD, USA) and S100B was measured using EZHS100B-33K kit from Millipore (Millipore, Billerica, MA, USA) according to the manufacturers' recommendations. For H-FABP, the lower limit

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of detection (LLoD) was 0.103 ng/mL and the calibration range was from 0.137-100 ng/mL. The test has not yet been fully validated by Meso Scale and therefore there is no established lower limit of quantification (LLoQ). For IL-10 the LLoD was 0.04 pg/mL, with the LLoQ at 0.298 pg/mL with a calibration range between 0.0774-317 pg/mL. For the S100B the LLoD was 2.7 pg/mL and the calibration range went from 2.7 to 2000 pg/mL. One patient was below detection range of the S100B and therefore the concentration of 1 pg/mL was attributed to this patient permitting statistical analysis. This applied concentration does not impact the statistics obtained. Plasma GFAP, NF-L and tau concentrations were measured using the Human Neurology 4-Plex A assay (N4PA) on an HD-1 Single molecule array (Simoa) instrument according to instructions from the manufacturer (Quanterix, Lexington, MA). For GFAP, the LLoD was 0.221 pg/mL, whilst the LLoQ was 0.467 pg/mL and the calibration range was 0.987 pg/mL to 725 pg/mL. The corresponding figures for NF-L were 0.104 pg/mL (LLoD), 0.241 pg/mL (LLoQ) and with a calibration range between 0.533 pg/mL to 453 pg/mL. The corresponding figures for tau were 0.024 pg/mL (LLoD), with a calibration range between 0.053 pg/mL (LLoQ) and 0.136 pg/mL to 112 pg/mL. Plasma Aβ40 and Aβ42 concentrations were measured using a duplex Simoa immunoassay (Quanterix, Lexington, MA, USA). For Aβ40, the LLoD was 0.045 pg/mL and the LLoQ was 0.142 pg/mL with a calibration range between 0 pg/mL to 90.0 pg/mL. For Aβ42, the LLoD was 0.142 pg/mL and the LLoQ was 0.69 pg/ml with a calibration range between 0 pg/mL to 11.0 pg/mL.

There were no samples below the LLoDs and LLoQs. The measurements were performed by board-certified laboratory technicians who were blinded to clinical data.

TBI severity and CT scan grading

TBI severity assessment was solely based on the lowest Glasgow Coma Scale (GCS) before intubation, either at the scene of accident or emergency department. A GCS value of 13-15 was considered mild, 9-12 moderate, and 3-8 severe TBI. For the analysis and taking into account clinical relevance, we analyzed the results in both the whole patient group, and in the mTBI group separately. In addition, the groups were further divided into non-isolated (i.e., concomitant extracranial injuries) and isolated TBI subgroups.

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Inclusion criteria were as follows: age ≥18 years, clinical diagnosis of TBI, and indications for acute head computed tomography according to the National Institute for Health and Care Excellence (NICE) criteria (http://www.nice.org.uk/guidance/cg176). Exclusion criteria were blast- induced or penetrating injury, chronic subdural hematoma, inability to live independently as a result of preexisting brain disease, TBI or suspected TBI not needing head CT, >2 weeks from the injury, not speaking the local language, and no consent obtained. CT scans were classified according to Marshall grading system[34]. Diffuse injury / grade I (no visual pathology) was considered CT-, whereas the other grades (II-VI) were regarded as CT+. Neuroradiologists at the Turku University Hospital and a senior neurosurgeon (JPP) double-read the CT scans.

Statistical analysis

Demographics of the subjects and time elapse from injury to blood sampling are presented as mean ± SD. Normality of distribution of biomarkers levels was assessed with the Kolmogorov-Smirnov test and by visually inspecting data histograms. The levels of biomarkers were not normally distributed, and data are presented as medians and 25th and 75th percentiles. The GCS scores were not normally distributed. Mann-Whitney U test was used to compare the biomarker levels between the groups and to compare GCS score between patient groups. The ability of biomarkers in discriminating CT+ and CT- patients is presented with area under receiver operating characteristic curve (AUC) and Youden's Index (J). J captures the maximum performance of a dichotomous diagnostic test when equal importance is given to sensitivity and specificity (J=sensitivity+specificity-1).[35] Partial AUC (pAUC) was used to compare only a portion of the biomarkers AUC curves, which here was set to the clinically relevant range 90-100% sensitivity. Panels were developed by the iterative combination of biomarkers and thresholds (ICBT) method using the toolbox Panelomix.[36] For each biomarker, several cut-offs are selected and the best combination of markers and threshold is selected to give the best panel performance. The size of panels was set to maximum three biomarkers and was evaluated when sensitivity was set at 90-100% and at 100%. The biomarker levels in different patient groups have been presented as medians and interquartile ranges. The correlations between the biomarker levels were analyzed with Pearson correlation coefficient.

Data Availability Statement

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

Results

One-hundred-sixty (160) patients were enrolled. There were 117 males (73.1%) and 43 females (26.9%) with a mean age of 47.2±19.6 years. In 94 patients (58.7%) the TBI was isolated, and 66 patients (41.3%) had TBI with other concomitant extracranial injuries. There were 93 patients with mTBI. Among all patients, a negative CT was found in 65 (40.6%) patients and a positive CT in 95 (59.4%). Demographic data are presented in Table 1. Blood samples of all patients were obtained within 24 h from admission. In those patients for whom the exact time of injury was available, the time elapse from injury to blood sampling was 15.2±11.5 h (n=70). Among those patients in whom the exact injury time was unavailable, 34 patients were sampled within 24 h and 56 patients were sampled after 24 h from the injury.

As the need for CT was an inclusion criterion and the imaging was done rapidly after deciding the need for CT, the blood samples were drawn after the CT scan with few exceptions. The ability of individual biomarkers to distinguish CT- from CT+ patients is shown in Figures 1 & 2 and Tables 2A & 2B (patients with mTBI and TBIs of all severities), as well as in Figures 3 & 4 and Tables 2C & 2D (isolated mTBIs and isolated TBIs of all severities). Combinations of biomarkers for discriminating CT- and CT+ patients are presented in Tables 3A & 3B (patients with mTBI and TBIs of all severities), and in Tables 3C and 3D (isolated mTBIs and isolated TBIs of all severities). The biomarker levels in different patient groups are presented in Table 4.

In patients with both mTBI and TBIs of all severities, there were no significant differences in the GCS scores between patients with isolated vs. non-isolated TBI (Table 5A). A comparison of biomarker levels between isolated and non-isolated TBIs was made both for all patients and patients with mTBI. Non-isolated TBI patients with all severities showed higher levels of H-FABP than isolated TBI in the CT+ group of all severities (p=0.023), and CT+ patients with non-isolated mTBI had higher levels of IL-10 (p=0.014), S100B (p=0.019)

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and tau (p=0.005). The biomarker levels and their differences in different patient groups are presented in Table 5B. Table 6 shows the correlations between the various biomarkers used in this study.

Individual biomarkers in CT- and CT+ patients with TBIs of all severities

In patients with TBIs of all severities (Table 2A), the biomarker levels in CT+ and CT- groups were significantly ($p<0.001$) different for all other studied biomarkers, except Aβ42 and S100B. The AUC values varied from 0.584 to 0.822, with GFAP and NF-L showing the highest values (0.822 and 0.817, respectively) (Figure 1, Table 2A). Js varied from 0.20 to 0.55, again with NF-L and GFAP having the best indices (0.55 and 0.52, respectively). When sensitivity was set to 100%, all biomarkers showed poor specificities, ranging from 0 to 13.8%; the best ones shown by GFAP and NF-L (13.8% and 6.2%, respectively). If the pAUC in the range of 90-100% sensitivity or the best specificity at 90-100% sensitivity was used to compare the biomarkers, GFAP and NF-L were again the best (Table 2A).

In patients with isolated TBIs of all severities, all biomarkers, except for Aβ42 and S100B, were significantly ($p\leq0.001$) different between the CT+ and CT- groups. The AUC values were slightly higher in isolated TBIs of all severities, ranging from 0.466 to 0.859, with NF-L and GFAP having the best AUCs (0.848 and 0.859, respectively). Also, the Js and pAUCs were higher in the isolated TBIs of all severities, with NF-L and GFAP showing the best values (NF-L: 0.59 and 3.29 and GFAP: 0.57 and 4.03, respectively). When sensitivity was set to 100%, GFAP and Aβ40 showed the best specificities (17.9% and 12.8%, respectively) (Table 2C).

Individual biomarkers in CT- and CT+ patients with mTBI

In cases with mTBI (Table 2B), the biomarker levels in CT+ and CT- groups were significantly different for tau, GFAP, NF-L and H-FABP ($p<0.05$). The AUC values varied from 0.557 to 0.720, with GFAP and tau showing the highest values (Figure 2, Table 2B). The Js varied from 0.17 to 0.37, with tau and GFAP having the best values (0.37 and 0.33, respectively). At 100% sensitivity, the specificity varied from 0 to 16.1%, with GFAP, Aβ40 and tau having the highest specificities (16.1%, 14.3% and 14.3%, respectively). Using the

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pAUC in the range of 90-100% sensitivity or the best specificity at 90-100% sensitivity as measures, GFAP and tau showed the best results (Table 2B).

In patients with isolated mTBIs, the levels of H-FABP, GFAP, S100B, tau and NF-L ($p<0.05$) were significantly different between the CT+ and CT- groups. The biomarkers with the highest AUC values were slightly different in isolated mTBIs compared to all mTBIs. The AUCs varied from 0.515 to 0.749, with GFAP and H-FABP having the best values (0.749 and 0.699, respectively). The Js and pAUCs were generally higher in the isolated mTBIs, with H-FABP and GFAP showing the best values (H-FABP: 0.43 and 3.0 and GFAP: 0.39 and 3.13, respectively). When sensitivity was set to 100%, tau, GFAP, and H-FABP showed the best specificities (22.2%, 19.4% and 19.4%, respectively) (Table 2D).

Combinations of biomarkers in CT- and CT+ patients with TBIs of all severities

We studied if various combinations of biomarkers could improve the ability to discriminate patients with intracranial CT abnormalities from those without. In patients with TBIs of all severities, the optimal combinations varied slightly depending on if the sensitivity was set to 100% or to 90-100%. With 100% sensitivity, the best specificity (38.5%) was reached with a combination of GFAP+H-FABP+IL-10. The best combination of two biomarkers was IL-10+GFAP, which reached 35.4% specificity. Using the sensitivity range from 90-100%, the best specificity was shown by a combination Aβ40+IL-10+NF-L (69.2% specificity with 90.5% sensitivity), whereas the best combination of two biomarkers was Aβ40+NF-L (61.5% specificity with 91.6% sensitivity) (Table 3A).

A corresponding analysis was conducted for patients with isolated TBIs of all severities. When sensitivity was set to 100%, the best combination of three biomarkers was GFAP+S100B+IL-10 with a specificity of 66.7%. A similar result was obtained for two-biomarker combination, where IL-10+GFAP was still the best, but with improved specificity of 48.7%. With a sensitivity of 90-100%, the best panel of three biomarkers was totally different from the whole TBI group, namely GFAP+H-FABP+S100B with 79.5% specificity and 90.9% sensitivity. The best combination of two biomarkers was Aβ42+NF-L with 69.2% specificity and 90.9% sensitivity. The results are demonstrated in Table 3C.

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Combinations of biomarkers in CT- and CT+ patients with mTBI

In patients with mTBI, with 100% sensitivity, the best combination of three biomarkers was H-FABP+S100B+tau (with 46.4% specificity), and of two biomarkers either H-FABP+tau or IL-10+GFAP (both with 37.5% specificity). If the sensitivity range of 90-100% was used, the best specificity was reached by a combination of H-FABP+S100B+tau (60.7% specificity with 91.9% sensitivity), and the best combination of two biomarkers was H-FABP+tau (50.0% specificity with 91.9% sensitivity) (Table 3B).

In isolated mTBIs with sensitivity set to 100%, the best panel of three biomarkers was a combination H-FABP+S100B+tau (specificity of 66.7%), and the best two-biomarker combination was H-FABP+tau (specificity of 58.3 %). With a sensitivity of 90-100%, the best panel of three biomarkers was GFAP+H-FABP+IL-10 (specificity of 69.4% with sensitivity of 94.7%), and the aforementioned combination H-FABP+tau remained as the best option for two biomarkers combined. The results are shown in Table 3D.

Discussion

This prospective, observational study of patients with acute TBIs investigated the ability of eight protein biomarkers in discriminating CT+ and CT- patients, utilizing modern highly sensitive immunoassays in a well-characterized cohort. NF-L, GFAP, and tau exhibited the best abilities in discriminating CT+ and CT- patients, both in patients with mTBI and TBIs of all severities. In patients with isolated TBIs of all severities, NF-L, GFAP, and tau again performed again the best, but in patients with isolated mTBI H-FABP, GFAP and S100B showed the best results. Overall, single biomarkers had very low specificities (0-22.2%) when sensitivity was set to 100%.

Hence, we studied if panels of biomarkers would give better specificity/sensitivity. In the whole group, a combination of GFAP+H-FABP+IL-10 yielded the best specificity in separating CT+ and CT- patients when sensitivity was set to 100%. In patients with mTBI, a panel of H-FABP+S100B+tau showed the best specificity when sensitivity set to 100%.

Next, we hypothesized that in case of isolated TBIs, the optimal biomarker combinations may be different, because none of the proteins are apparently 100% brain-specific. In

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isolated TBIs of all severities, when sensitivity was set to 100%, the best specificity was reached with a panel of GFAP+S100B+IL-10. For patients with isolated mTBI, a panel of H-FABP+S100B+tau showed the best specificity when sensitivity set to 100%. The panel is the same as in all mTBI patients group with relatively similar thresholds for the panel to be classified as positive.

These results suggest that the best diagnostic value in discriminating CT+ and CT- patients can be achieved by utilizing biomarkers that do not necessarily perform best when applied alone. In the current study, NF-L, GFAP and tau exhibited the best AUCs and Js when studied individually, but H-FABP, IL-10 and S100B appeared in several of the best panel options, along with GFAP. Intriguingly, S100B alone showed 0% specificity and statistically non-significant difference between the CT+ and CT- groups in TBIs of all severities, isolated TBIs of all severities, and patients with mTBI, yet it appeared in some of the best combinations of biomarkers. The levels of S100B in CT+ patients were significantly higher in the non-isolated vs. isolated mTBIs, suggesting extracerebral release of the protein at the time of injury, which is in line with the previous literature[12].

H-FABP[23] and IL-10[24] have earlier been studied in screening patients with mTBI for a need for head CT. In this study, they perform well also in TBIs of all severities, whether isolated or not, although they both appear to be released also from extracerebral sources, because their levels were higher in non-isolated CT-positive patients compared to isolated ones, without a difference in the severity of TBI between the groups. The AUCs of H-FABP and IL-10 were relatively similar as reported earlier, but the specificities were lower both in mTBIs and TBIs of all severities in the current study[23,24].

GFAP is the second most studied biomarker of TBI, after S100B. In the current study, AUCs were slightly lower compared to earlier studies in patients with mTBI[17,37–39]. We have previously analyzed the levels of GFAP using a less sensitive assay from a cohort of patients with TBIs with all severities, including also the patients of the current study.[15] An ultrasensitive Simoa method[29] was used in the current study to analyze GFAP levels, and slightly higher AUCs were observed compared to our earlier study, but the patient cohorts were not identical.

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Aβ40, Aβ42, tau and NF-L have been less studied in acute diagnostics of TBI. In this study, NF-L and tau exhibited very good AUCs and Js in the group of all patients with TBI. However, in patients with isolated mTBIs, H-FABP, GFAP and S100B all outperformed NF-L and tau, suggesting that the less brain-specific biomarkers H-FABP and S100B are useful in cases of isolated mTBI. Levels of NF-L and tau were higher in patients with non-isolated CT+ mTBI than isolated CT+ mTBI, suggesting possible extracerebral release. The levels of Aβ42 did not differ between CT+ and CT- patients in any of the analyzed groups, while the levels of Aβ40 levels were significantly higher in CT+ patients, both in TBIs of all severities and isolated TBIs of all severities.

Both S100B and the combination of GFAP and UCH-L1 have been used as biomarkers to screen for CT-positivity/negativity in patients with acute TBI[19–21]. We analyzed also the levels of UCH-L1 from our samples, but the coefficients of variation were at a level where we could not consider the results sufficiently reliable, and therefore they have not been included in the analyses. For S100B, the publications have yielded an AUC of 0.69–0.78, and with 98-100% sensitivity and specificity from 5% to 22.9%[19,20]. FDA recently approved the combination of GFAP+UCH-L1 to be used to screen the need for a CT-scan in acute mTBI and supported by a study reporting 36.5% specificity with 97.5% sensitivity for patients with GCS of 9-15 and 36.7% specificity with 97.3% sensitivity for a subset of patients with GCS of 14-15 (AUC values not given)[21]. Compared to the above-mentioned results, the best panels in this study suggest that clearly improved specificities might be reached with 100% sensitivity using optimal biomarker combinations for targeted patient groups.

In this study, the NICE criteria[8] for head CT scanning were used. In a validation study including several international guidelines for indications of head CT, the NICE criteria yielded a sensitivity of 82.1% and a specificity of 46.1% for detecting traumatic intracranial findings in patients with GCS of 13-15.[40] The biomarker panels for both mTBI groups in the current study outperform the NICE criteria, but a proper comparison for diagnostic accuracy is not possible because of the study design and because most blood samples were drawn after the head CT scans.

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There are both considerable strengths and limitations of this study. Strengths are the use of several different biomarkers in the same cohort, use of sensitive advanced analytics, and a prospectively collected well-characterized study population. Our results are comparable with recent studies utilizing the same methodologies, but it is uncertain how studies utilizing different assays give comparable results especially at low biomarker levels. The main shortcomings include variable delays between the injury and the sampling, and the timing of the sampling after the CT scan. Due to the latter, we are not able to present our results as predictors for CT findings. This may affect the results especially in case of those biomarkers, which have a fairly short half-life, such as H-FABP, IL-10 and S100B. Indeed, most of our blood samples have not been collected within the 6-hour time window recommended for the use of S100B. For other biomarkers, *e.g.*, NF-L, the sampling time point may have been too close to the injury; NF-L is a slow marker that reaches its maximum more than 10 days after the injury.[21] It may thus not be the optimal biomarker for acute injury detection. Earlier sampling might had either improved or attenuated the diagnostic capabilities of the biomarker panels. When interpreting our results, it has to be noticed that especially what comes to patients with mTBI, our series cannot be considered to represent cases with mTBI at large, since the mildest cases were often discharged before possibility for recruitment, and a fairly large percentage showed traumatic intracranial abnormalities in CT and required hospital admission. In addition, as the inclusion criteria was based on using the NICE criteria for head CT, the results are not necessarily applicable for other head CT-rules. Pre-selection using any head CT-rule also gives different sensitivities and specificities than using biomarkers for screening the whole population of patients with head trauma attending emergency care.

This study analyzed only the associations of different biomarkers with visible intracranial abnormalities in CT. Biomarkers or biomarker panels that are needed to separate patients with TBI from patients with acute injuries without a TBI, or to predict the outcome of TBI may well be different from those found in this study. In addition, the optimal biomarkers do not depend only on the indication and patient population, but also on timing, why these results should be replicated and widened in further studies.

Conclusions

The main finding was that panels of protein biomarkers perform better in discriminating CT+ and CT- patients than individual biomarkers. A panel including GFAP+H-FABP+IL-10 yielded the best specificity (38.5%) in separating CT+ and CT- patients with 100% sensitivity within 24 h from admission in TBIs of all severities. In patients with mTBI, a panel of H-FABP+S100B+tau gave the best specificity (46.4%) with 100% sensitivity not depending on whether TBI was isolated or not. The true diagnostic value of these biomarker panels compared to existing head CT rules should be addressed in further studies. Our results also suggest that different biomarkers may be needed when searching for optimal diagnostic tools for different types of patients.

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Table 1. Demographics of the different TBI subgroups

	All severities	Isolated all	mTBI (n=93)	Isolated mTBI
Age (mean ±	47.22 ± 19.59	48.59 ± 19.15	42.78 ± 18.60	42.76 ± 18.39
Sex	117 / 43	62 / 32	60 / 33	30 / 25
GCS (mean ±	12.45 ± 3.91	12.70 ± 3.41	14.65 ± 0.58	14.60 ± 0.63
CT-negative	65 (40.6%)	39 (41.5%)	56 (60.2%)	36 (65.5%)
CT-positive	95 (59.4%)	55 (58.5%)	37 (39.8%)	19 (34.5%)
Marshall II	27 (16.9%)	11 (11.7%)	21 (22.6%)	9 (16.4%)
Marshall III	3 (1.9%)	-	1 (1.1%)	-
Marshall IV	1 (0.6%)	1 (1.1%)	1 (1.1%)	1 (1.8%)
Marshall V	38 (23.8%)	23 (24.5%)	5 (5.4%)	2 (3.6%)
Marshall VI	26 (16.3%)	20 (21.3%)	9 (9.7%)	7 (12.7%)

mTBI, mild traumatic brain injury

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Table 2A. Ability of the individual biomarkers in discriminating CT-negative and CT-positive patients in TBIs of all severities (all n=160, CT-negative n=65, CT-positive n=95)

	Mann U CT+ vs. CT-	AUC (95%CI)	J (%SP/%SE; cut off)	%SP @ 100%SE (cut off)	pAUC (95%CI)	%SP/ 90-100%SE (cut off)
GFAP	**<0.001**	0.822	0.52	13.8	3.26	41.5/93.7
NF-L	**<0.001**	0.817	0.55	6.2 (4.43)	2.20	40/90.5
tau	**<0.001**	0.781	0.51	4.6 (0.29)	1.93 (1.0-	23.1/94.7
Aβ40	**<0.001**	0.680	0.36	0 (-)	1.17	12.3/98.9
IL-10	**<0.001**	0.676	0.30	4.6 (0.14)	1.82	20.0/96.8
H-FABP	**<0.001**	0.666	0.34	1.5	1.33	30.8/90.5
Aβ42	**0.018**	0.610	0.29	1.5 (2.92)	0.54	6.2/96.8
S100B	0.072	0.584	0.20	0 (-)	0.57	4.6/97.9

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Table 2B. Ability of the individual biomarkers in discriminating CT-negative and CT-positive patients with mild TBI (all n=93, CT-negative n=56, CT-positive n=37)

	Mann U CT+ vs. CT-	AUC (95%CI)	J (%SP/%SE; cut off)	%SP @ 100%SE (cut off)	pAUC (95%CI)	%SP/ 90-100%SE (cut off)
GFAP	**<0.001**	0.720	0.33	16.1	2.86 (1.47-	32.1/97.3
tau	**0.002**	0.689	0.37	14.3	2.23 (1.09-	26.8/94.6
NF-L	**0.004**	0.676	0.33	7.1 (4.43)	1.62 (0.52-	26.8/91.9
H-FABP	**0.021**	0.642	0.31	1.8	1.1 (0.20-	14.3/97.3
Aβ40	0.066	0.613	0.23	14.3	1.51 (0.71-	14.3/100
S100B	0.265	0.569	0.18	0 (-)	0.33 (0-	12.5/91.9
Aβ42	0.350	0.557	0.19	1.8 (2.92)	0.51 (0-	8.9/94.6
IL-10	0.177	0.583	0.17	5.4 (0.14)	1.42 (0.42-	19.6/94.6

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Table 2C. Ability of the individual biomarkers in discriminating CT-negative and CT-positive patients in isolated TBIs of all severities (all n=94, CT-negative n=39, CT-positive n=55)

	Mann U CT+ vs. CT-	AUC (95%CI)	J (%SP/%SE; cut off)	%SP @ 100%SE (cut off)	pAUC (95%CI)	%SP/ 90-100%SE (cut off)
GFAP	**<0.001**	0.859	0.57	17.9	4.03	59.0/90.9
NF-L	**<0.001**	0.848	0.59	5.1 (4.18)	3.29	53.8/90.9
tau	**<0.001**	0.789	0.52	7.7 (0.29)	2.52	35.9/92.7
H-FABP	**<0.001**	0.721	0.45	2.6	2.49	43.6/90.9
IL-10	**<0.001**	0.721	0.39	2.6 (0.14)	1.38	17.9/96.4
Aβ40	**0.001**	0.695	0.39	12.8	1.31	12.8/100
Aβ42	0.253	0.570	0.27	2.6 (2.92)	0.49	2.6/100
S100B	0.573	0.466	0.09	0 (-)	0.02	0/100 (-)

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Table 2D. Ability of the individual biomarkers in discriminating CT-negative and CT-positive patients with isolated mild TBI (all n=55, CT-negative n=36, CT-positive n=19)

	Mann U CT+ vs. CT-	AUC (95%CI)	J (%SP/%SE; cut off)	%SP @ 100%SE (cut off)	pAUC (95%CI)	%SP/ 90-100%SE (cut off)
GFAP	**0.003**	0.749	0.39	19.4	3.13	44.4/94.7
H-FABP	**0.016**	0.699	0.43	19.4	3.0 (1.11-	41.7/94.7
S100B	**0.022**	0.689	0.36	11.1	2.56	41.7/94.7
tau	**0.044**	0.667	0.31	22.2	2.6 (1.23-	30.6/94.7
NF-L	**0.049**	0.662	0.34	5.6 (4.18)	1.61	27.8/94.7
Aβ40	0.124	0.627	0.25	13.9	1.39	13.9/100
Aβ42	0.608	0.542	0.23	2.8 (2.92)	0.54	8.3/94.7
IL-10	0.860	0.515	0.17	2.8 (0.14)	0.67	11.1/94.7

Biomarkers are presented in order according to their AUC, area under the curve; Mann U, Mann-Whitney U-test; Youden's Index (J); %SP @ 100%SE, specificity at 100 % sensitivity; pAUC%, partial area under the curve in the range of 90-100% sensitivity; %SP/90-100%SE, specificity / sensitivity in the range of 90-100 %; Aβ40, β-Amyloid isoform 1-40; Aβ42 β-Amyloid isoform 1-42; GFAP, glial fibrillary acidic protein; H-FABP, heart fatty-acid binding protein; IL-10, interleukin 10; NF-L neurofilament light; S100B, S100 calcium-binding protein B; statistically significant p values are in bold

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Table 3A. Panels of the best biomarker combinations in discriminating CT-negative and CT-positive patients in TBIs of all severities

Sensitivity	Number of biomarkers	Biomarkers (threshold to be classified as positive, pg/mL)			No of biomarkers needed to be +	%Specificity (95%CI)	%Sensitivity (95%CI)
90-100%	2	Aβ40 (>19.9)	NF-L (>17.7)	-	1	61.5 (49.2-	91.6 (85.3-
	3	Aβ40 (>19.3)	IL-10 (>0.21)	NF-L (>17.7)	2	69.2 (58.5-	90.5 (84.2-
100%	2	IL-10 (>0.39)	GFAP (>467)	-	1	35.4 (24.6-	100 (100-100)
	3	GFAP (>467)	H-FABP (>2520)	IL-10 (>0.39)	2	38.5 (26.2-	100 (100-100)

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Table 3B. Panels of the best biomarker combinations in discriminating CT-negative and CT-positive patients with mild TBI

Sensitivity	Number of biomarkers	Biomarkers (threshold to be classified as positive, pg/mL)			No of biomarkers needed to be +	%Specificity (95%CI)	%Sensitivity (95%CI)
90-100%	2	H-FABP (>4620)	Tau (>2.56)	-	1	50.0 (37.5-	91.9 (83.8-
	3	H-FABP (>4490)	S100B (<105)	Tau (>2.42)	2	60.7 (48.2-	91.9 (83.8-
100%	2	IL-10 (>0.39)	GFAP (>468)	-	1	37.5 (25.0-	100 (100-100)
	2	H-FABP (>4170)	Tau (>2.53)	-	1	37.5 (25.0-	100 (100-100)
	3	H-FABP (>4170)	S100B (<136)	Tau (>2.42)	2	46.4 (33.9-	100 (100-100)

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Table 3C. Panels of the best biomarker combinations in discriminating CT-negative and CT-positive patients in isolated TBIs of all severities

Sensitivity	Number of biomarkers	Biomarkers (threshold to be classified as positive, pg/mL)			No of biomarkers needed to be +	%Specificity (95%CI)	%Sensitivity (95%CI)
90-100%	2	Aβ42 (>20.9)	NF-L (>17.7)	-	1	69.2 (53.8-82.1)	90.9 (81.8-98.2)
	3	GFAP (>4510)	H-FABP (>4480)	S100B (<110)	2	79.5 (66.7-92.3)	90.9 (83.6-98.2)
100%	2	GFAP (>468)	IL-10 (>0.39)	-	1	48.7 (33.3-64.1)	100 (100-100)
	3	GFAP (>468)	S100B (<110)	IL-10 (>0.39)	2	66.7 (51.3-82.1)	100 (100-100)

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Table 3D. Panels of the best biomarker combinations in discriminating CT-negative and CT-positive patients with isolated mild TBI

Sensitivity	Number of biomarkers	Biomarkers (threshold to be classified as positive, pg/mL)			No of biomarkers needed to be +	%Specificity (95%CI)	%Sensitivity (95%CI)
90-100%	2	H-FABP (>4490)	Tau (>2.46)	-	1	58.3 (41.7-75.0)	100 (100-100)
	3	GFAP (>540)	H-FABP (>3880)	IL-10 (>0.40)	2	69.4 (55.6-83.3)	94.7 (84.2-100)
100%	2	H-FABP (>4490)	Tau (>2.46)	-	1	58.3 (41.7-75.0)	100 (100-100)
	3	H-FABP (>4490)	S100B (<141)	Tau (>2.46)	2	66.7 (50.0-80.6)	100 (100-100)

No of biomarkers needed to be +, number of biomarkers needed to be positive for the panel to be classified as positive; Aβ42, β-Amyloid isoform 1-42; GFAP, glial fibrillary acidic protein; H-FABP, heart fatty-acid binding protein; IL-10, interleukin 10; NF-L neurofilament light; S100B, S100 calcium-binding protein B

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Table 4A. Biomarker levels in patients with TBIs of all severities

	Non-isolated TBI CT+, median level (IQR) pg/mL (n=40, GCS 11.18 ± 4.94)	Non-isolated TBI CT-, median level (IQR) pg/mL (n=26, GCS 13.50 ± 3.46)	Isolated TBI CT+, median level (IQR) pg/mL (n=55, GCS 11.58 ± 3.91)	Isolated TBI CT-, median level (IQR) pg/mL (n=39, GCS 14.28 ± 1.52)
Aβ40	21.4 (14.2–29.8)	15.7 (10.5–21.3)	24.7 (15.3–32.2)	17.0 (11.9–21.4)
Aβ42	20.0 (14.9–26.1)	15.9 (11.6–19.0)	18.5 (11.5–29.0)	15.7 (11.7–20.1)
GFAP	5890 (1830–32700)	1140 (435–2210)	6840 (585–47600)	204 (82.0–530)
H-FABP	16800 (5570–42500)	8440 (4150–28000)	6720 (4980–12800)	4080 (2880–6770)
IL-10	1.10 (0.47–4.04)	0.79 (0.23–1.60)	0.86 (0.41–2.46)	0.37 (0.24–0.55)
NF-L	48.4 (19.1–85.7)	13.6 (10.4–22.7)	57.6 (15.9–116)	8.66 (6.26–16.8)
S100B	122 (63.2–259)	81.1 (45.3–143)	83.5 (49.9–209)	85.9 (50.4–120.3)
tau	11.0 (4.00–36.1)	2.35 (1.52–4.82)	6.83 (1.52–32.8)	1.53 (0.55–2.21)

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Table 4B. Biomarker levels in patients with mild TBI

	Non-isolated mTBI CT+, median level (IQR) pg/mL (n=18, GCS 14.61 ± 0.61)	Non-isolated mTBI CT-, median level (IQR) pg/mL (n=20, GCS 14.80 ± 0.41)	Isolated mTBI CT+, median level (IQR) pg/mL (n=19, GCS 14.53 ± 0.61)	Isolated mTBI CT-, median level (IQR) pg/mL (n=36, 14.64 ± 0.64)
Aβ40	17.1 (11.14–27.53)	14.2 (9.68–23.4)	20.1 (14.8–27.9)	16.9 (11.9–21.3)
Aβ42	17.4 (11.50–22.99)	15.9 (11.9–18.9)	17.6 (9.26–26.2)	15.7 (11.7–19.1)
GFAP	1830 (822.28–2600.97)	1140 (449–2040)	604 (214–2290)	186.64 (75.9–500)
H-FABP	15400 (4220–37900)	7250 (4150–24800)	5670 (4550–9640)	3880.90 (2860–6380)
IL-10	1.10 (0.33–5.97)	0.73 (0.22–1.45)	0.41 (0.23–0.56)	0.34 (0.23–0.53)
NF-L	19.1 (12.7–41.1)	13.0 (8.63–18.4)	14.0 (8.59–19.5)	8.23 (6.11–15.3)
S100B	92.3 (45.9–164)	81.1 (48.1–120)	51.4 (38.4–87.0)	83.46 (48.8–118)
tau	4.29 (3.08–7.61)	2.44 (1.52–4.40)	1.79 (1.19–2.92)	1.51 (0.55–2.08)

GCS is presented as group mean ± SD; IQR, interquartile range; Aβ40, β-Amyloid isoform 1-40; Aβ42 β-Amyloid isoform 1-42; GFAP, glial fibrillary acidic protein; H-FABP, heart fatty-acid binding protein; IL-10, interleukin 10; NF-L neurofilament light; S100B, S100 calcium-binding protein

Table 5A. Comparison GCS scores between different patient groups

	Non-isolated all severities TBI CT+ vs. isolated all severities TBI CT+	Non-isolated all severities TBI CT- vs. isolated all severities TBI CT-	Non-isolated mTBI CT+ vs. isolated mTBI CT+	Non-isolated mTBI CT- vs. isolated mTBI CT-
n	40 vs. 55	26 vs. 39	18 vs. 19	20 vs. 36
Mean GCS ± SD	11.18 ± 4.94 vs. 11.58 ±	13.50 ± 3.46 vs. 14.28 ±	14.61 ± 0.61 vs. 14.53 ±	14.80 ± 0.41 vs. 14.64 ±
p value	0.981	0.567	0.460	0.367

p values are from Mann-Whitney U-test

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Table 5B. Differences in the biomarker levels in different patient groups

	Mann U, non-isolated all severities TBI CT+ vs. isolated all severities TBI CT+ (median levels, pg/mL)	Mann U, non-isolated all severities TBI CT- vs. isolated all severities TBI CT- (median levels, pg/mL)	Mann U, non-isolated mTBI CT+ vs. isolated mTBI CT+ (median levels, pg/mL)	Mann U, non-isolated mTBI CT- vs. isolated mTBI CT- (median levels, pg/mL)
Aβ40	0.449 (21.4 vs. 24.7)	0.533 (15.7 vs. 17.0)	0.775 (17.0 vs. 20.1)	0.521 (14.2 vs. 16.9)
Aβ42	0.438 (20.0 vs. 18.5)	0.888 (15.9 vs. 15.7)	0.753 (17.4 vs. 17.6)	0.898 (15.9 vs. 15.7)
GFAP	0.916 (5890 vs. 6840)	**0.001** (1140 vs. 204)	0.118 (1830 vs. 604)	**0.001** (1140 vs. 186)
H-FABP	**0.023 (**16800 vs. 6720)	**0.002** (8440 vs. 4080)	0.298 (15400 vs. 5670)	**0.004** (7250 vs. 3880)
IL-10	0.386 (1.10 vs. 0.86)	**0.024** (0.79 vs. 0.37)	**0.014** (1.10 vs. 0.41)	0.090 (0.73 vs. 0.34)
NF-L	0.871 (48.4 vs. 57.6)	**0.034** (13.6 vs. 8.66)	**0.036** (19.1 vs. 14.0)	0.070 (13.0 vs. 8.23)
S100B	0.107 (122 vs. 83.5)	0.851 (81.1 vs. 85.9)	**0.019** (92.3 vs. 51.4)	0.824 (81.1 vs. 83.5)
tau	0.216 (11.0 vs. 6.83)	**0.002** (2.35 vs. 1.53)	**0.005** (4.29 vs. 1.79)	**0.001 (2.44 vs. 1.51)**

Mann U, p value from Mann-Whitney U-test; Aβ40, β-Amyloid isoform 1-40; Aβ42 β-Amyloid isoform 1-42; GFAP, glial fibrillary acidic protein; H-FABP, heart fatty-acid binding protein; IL-10, interleukin 10; NF-L neurofilament light; S100B, S100 calcium-binding protein B; statistically significant p values are in bold

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Table 6. Correlations between the biomarker levels in all patients (n=160).

Biomarker		GFAP	NF-L	tau	Aβ40	IL-10	H-FABP	Aβ42	S100B
GFAP	Pearson's r	1	**0.638***	**0.948***	0.146	0.057	-0.017	0.154	**0.399***
	p value		**p<0.001**	**p<0.001**	0.065	0.470	0.831	0.052	**p<0.001**
NF-L	Pearson's r	**0.638***	1	**0.640***	**0.184***	0.114	0.135	**0.218***	**0.302***
	p value	**p<0.001**		**p<0.001**	**0.020**	0.151	0.089	**0.006**	**p<0.001**
tau	Pearson's r	**0.948***	**0.640***	1	0.148	0.097	0.080	**0.167***	**0.475***
	p value	**p<0.001**	**p<0.001**		0.062	0.223	0.313	**0.034**	**p<0.001**
Aβ40	Pearson's r	0.146	**0.184***	0.148	1	0.111	0.108	**0.386***	**0.296***
	p value	0.065	**0.020**	0.062		0.163	0.174	**p<0.001**	**p<0.001**
IL-10	Pearson's r	0.057	0.114	0.097	0.111	1	**0.384***	0.012	**0.383***
	p value	0.470	0.151	0.223	0.163		**p<0.001**	0.882	**p<0.001**
H-FABP	Pearson's r	-0.017	0.135	0.080	0.108	**0.384***	1	0.138	**0.413***
	p value	0.831	0.089	0.313	0.174	**p<0.001**		0.082	**p<0.001**
Aβ42	Pearson's r	0.154	**0.218***	**0.167***	**0.386***	0.012	0.138	1	0.117
	p value	0.052	**0.006**	**0.034**	**p<0.001**	0.882	0.082		0.141
S100B	Pearson's r	**0.399***	**0.302***	**0.475***	**0.296***	**0.383***	**0.413***	0.117	1
	p value	**p<0.001**	**p<0.001**	**p<0.001**	**p<0.001**	**p<0.001**	**p<0.001**	0.141	

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*p<0.05; Pearson's r, Pearson correlation coefficient; Aβ40, β-Amyloid isoform 1-40; Aβ42, β-Amyloid isoform 1-42; GFAP, glial fibrillary acidic protein; H-FABP, heart fatty-acid binding protein; IL-10, interleukin 10; NF-L neurofilament light; S100B, S100 calcium-binding protein B

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Figures

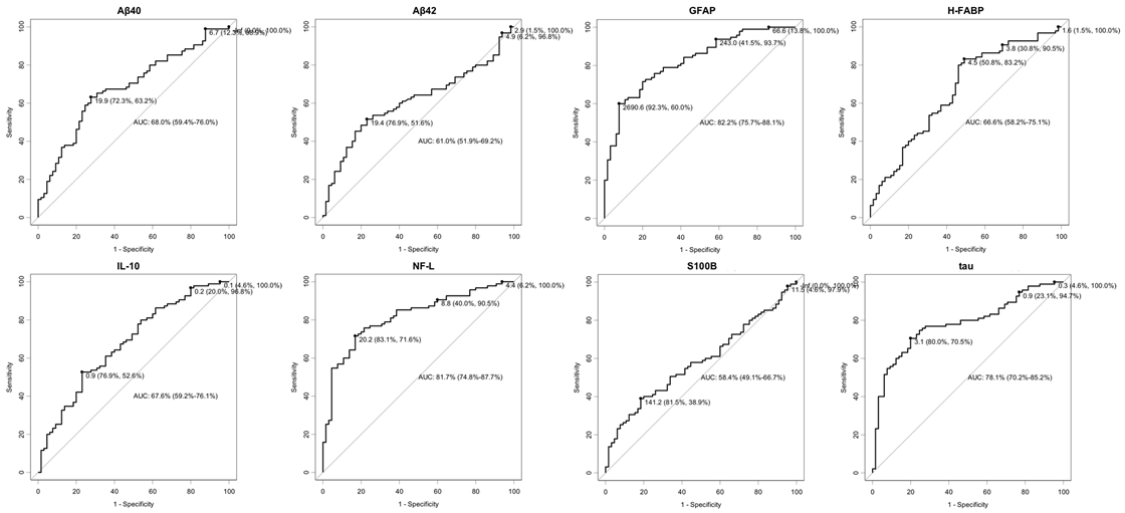

Figure 1. Ability of the individual biomarkers in discriminating CT-negative and CT-positive patients in TBIs of all severities. (Title)

Within each receiver operating characteristics curve, the uppermost value in parentheses are specificity at 100 % sensitivity, the centermost values are specificity at sensitivity set the range of 90-100 %, and the undermost values are specificity and sensitivity from Youden's index; a value before parentheses is a cut-off value of a protein at a particular specificity/sensitivity; AUC, area under receiver operating characteristic curve; Aβ40, β-Amyloid isoform 1-40; Aβ42 β-Amyloid isoform 1-42; GFAP, glial fibrillary acidic protein; H-FABP, heart fatty-acid binding protein; IL-10, interleukin 10; NF-L neurofilament light; S100B, S100 calcium-binding protein B (Caption)

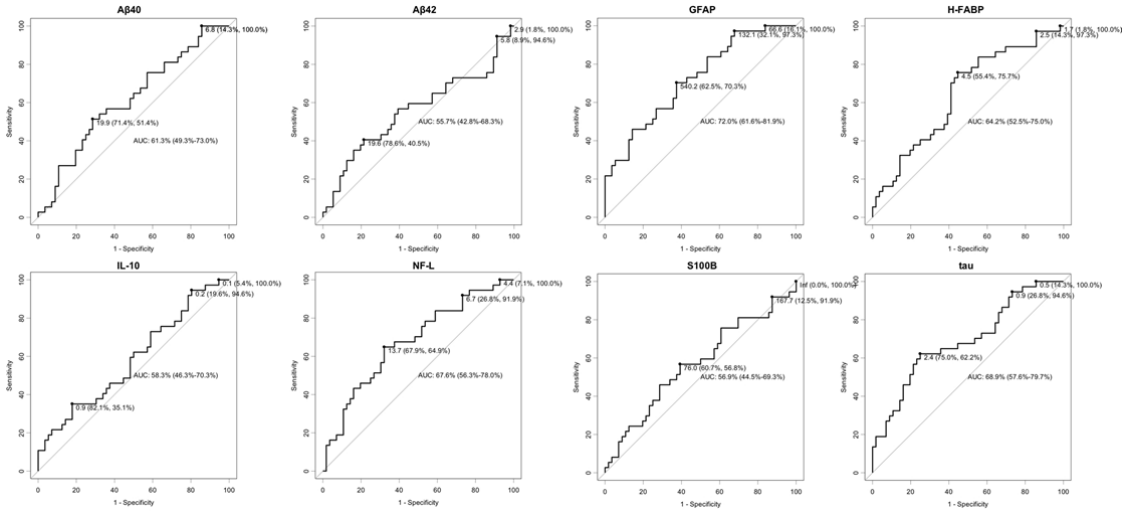

Figure 2. Ability of the individual biomarkers in discriminating CT-negative and CT-positive patients with mild TBI. (Title)

Within each receiver operating characteristics curve, the uppermost value in parentheses are specificity at 100 % sensitivity, the centermost values are specificity at sensitivity set the range of 90-100 %, and the undermost values are specificity and sensitivity from Youden's index (exception: Aβ40 same dot for 100% and 90-100%); a value before parentheses is a cut-off value of a protein at a particular specificity/sensitivity; AUC, area under receiver operating characteristic curve; Aβ40, β-Amyloid isoform 1-40; Aβ42 β-Amyloid isoform 1-42; GFAP, glial fibrillary acidic protein; H-FABP, heart fatty-acid binding protein; IL-10, interleukin 10; NF-L neurofilament light; S100B, S100 calcium-binding protein B (Caption)

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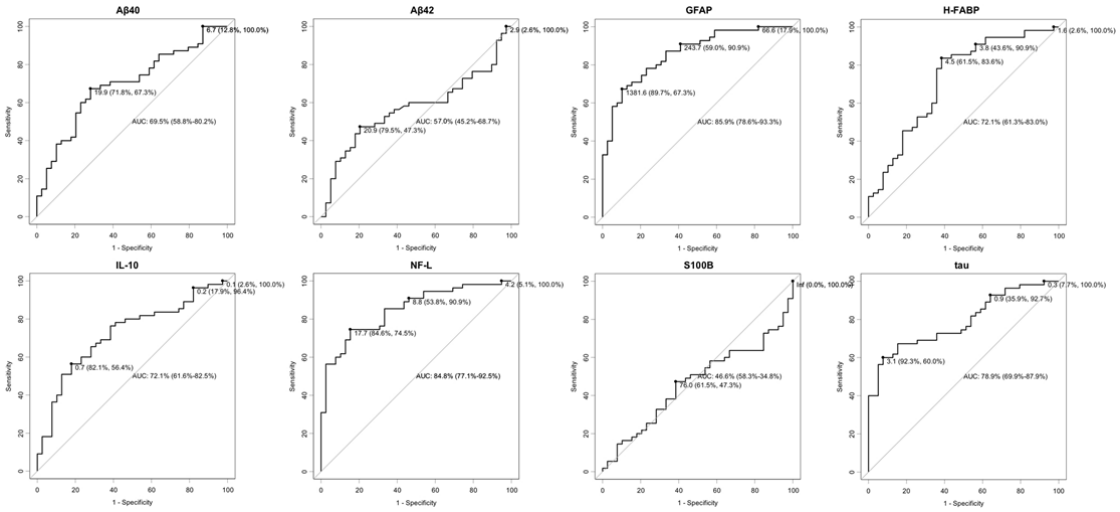

Figure 3. Ability of the individual biomarkers in discriminating CT-negative and CT-positive patients in isolated TBIs of all severities. (Title)

Within each receiver operating characteristics curve, the uppermost value in parentheses are specificity at 100 % sensitivity, the centermost values are specificity at sensitivity set the range of 90-100 %, and the undermost values are specificity and sensitivity from Youden's index (exceptions: same dot for S100B 100% and 90-100%, Aβ42 100% and 90-100%, Aβ40 100% and 90-100%); a value before parentheses is a cut-off value of a protein at a particular specificity/sensitivity; AUC, area under receiver operating characteristic curve; Aβ40, β-Amyloid isoform 1-40; Aβ42 β-Amyloid isoform 1-42; GFAP, glial fibrillary acidic protein; H-FABP, heart fatty-acid binding protein; IL-10, interleukin 10; NF-L neurofilament light; S100B, S100 calcium-binding protein B (Caption)

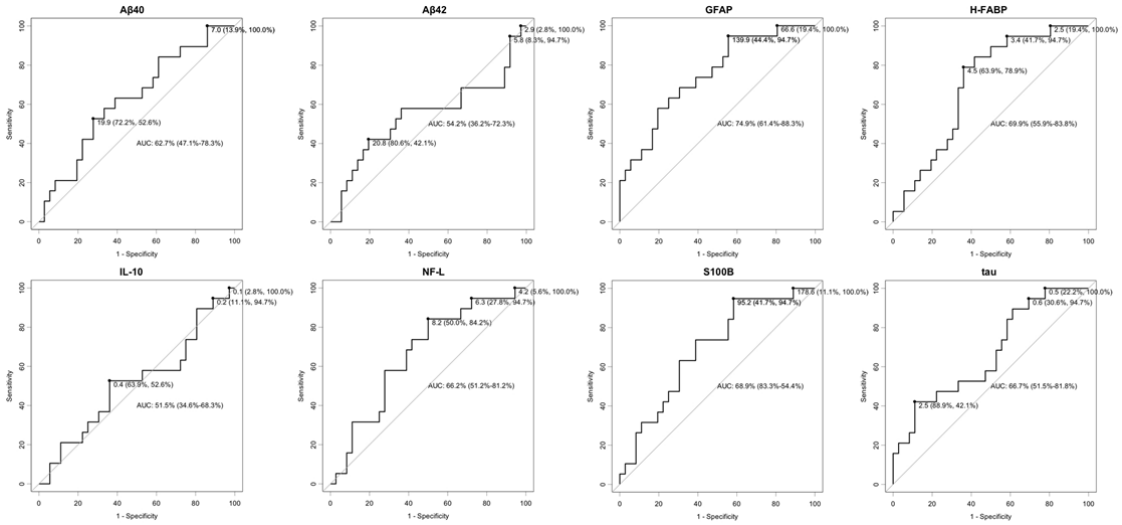

Figure 4. Ability of the individual biomarkers in discriminating CT-negative and CT-positive patients with isolated mild TBI. (Title)

Within each receiver operating characteristics curve, the uppermost value in parentheses are specificity at 100 % sensitivity, the centermost values are specificity at sensitivity set the range of 90-100 %, and the undermost values are specificity and sensitivity from Youden's index (exceptions: same dot for S100B 90-100% as Youden's Index, Aβ40 100% and 90-100%, GFAP 90-100% as Youden's Index); a value before parentheses is a cut-off value of a protein at a particular specificity/sensitivity; AUC, area under receiver operating characteristic curve; Aβ40, β-Amyloid isoform 1-40; Aβ42 β-Amyloid isoform 1-42; GFAP, glial fibrillary acidic protein; H-FABP, heart fatty-acid binding protein; IL-10, interleukin 10; NF-L neurofilament light; S100B, S100 calcium-binding protein B (Caption)

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