

An outlier analysis for acute blood biomarkers of moderate and severe traumatic brain injury

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Abstract

Blood biomarkers have been studied to improve the clinical assessment and prognostication of patients with moderate–severe traumatic brain injury (mo/sTBI). To assess their clinical usability, one needs to know potential factors that might cause outlier values and affect clinical decision-making. In a prospective study we recruited patients with mo/sTBI (n = 85) and measured the blood levels of eight protein brain pathophysiology biomarkers, including glial fibrillary acidic protein (GFAP), S100 calcium-binding protein B (S100B), neurofilament light (NF-L), heart-type fatty acid-binding protein (H-FABP), interleukin-10 (IL-10), total tau (T-tau), amyloid β 40 (A β 40) and amyloid β 42 (A β 42), within 24h of admission. Similar analyses were conducted for controls (n = 40) with an acute orthopedic injury without any head trauma. The patients with TBI were divided into subgroups of normal vs. abnormal (n = 9/76) head computed tomography (CT) and favorable (Glasgow Outcome Scale Extended = GOSE 5-8) vs. unfavorable (GOSE < 5) (n = 38/42, 5 missing) outcome. Outliers were sought individually from all subgroups and the whole TBI patient population. Biomarker levels outside Q1 –1.5 IQR or Q3 +1.5 IQR were considered as outliers. The medical records of each outlier patient were reviewed in a team meeting to determine possible reasons for outlier values. A total of 29 patients (34%) combined from all subgroups and 12 patients (30%) among the controls showed outlier values for one or more of the eight biomarkers. Several patients had outlier values in more than one biomarker (up to 4). All outlier values were higher than Q3 +1.5 IQR. A logical explanation was found for almost all cases, except the amyloid proteins. Explanations for outlier values included extremely severe injury, especially for GFAP and S100B. In case of H-FABP and IL-10 the explanation was extracranial injuries (thoracic injuries for H-FABP and multi-trauma for IL-10), in some cases these also associated with abnormally high S100B. Timing of sampling and demographic factors such as age and pre-existing neurological conditions (esp. for T-tau), explained some of the abnormally high values especially for NF-L. Similar explanations also emerged in controls, where the outlier values were caused especially by pre-existing neurological diseases. To utilize blood-based biomarkers in clinical assessment of mo/sTBI, very severe or fatal TBIs, various extracranial injuries, timing of sampling and demographic factors such as age and pre-existing systemic or neurological conditions must be taken into consideration. Very high levels seem to be often associated with poor prognosis and mortality (GFAP and S100B).

Introduction

Traumatic brain injury (TBI) is considered one of the most complex and heterogeneous human diseases, which makes the clinical assessment a major challenge. TBIs are also recognized as a major global health issue with more than 50 million cases annually causing deterioration of quality of life and significant costs to society.¹ Moderate and severe traumatic brain injuries (mo/sTBI) represent only 10-20% of TBIs, but they have high mortality and disability rates, and also in many cases, they require neurosurgical intervention especially in case of sTBI.^{2,3} It has been estimated that the acute care of sTBI costs more than 16 000€, but the direct medical costs represent only a minor portion of the total economic burden as indirect productivity losses account for the majority.⁴ In the clinical management of TBI, one of the first steps is the assessment of severity, which is currently performed using imaging and clinical features such as level of consciousness (assessed with the Glasgow Coma Scale [GCS]) and duration of post-traumatic amnesia (PTA). However, these clinical features do not sufficiently reflect occurring complex pathophysiological processes, making the severity assessment and outcome prediction of TBI exceedingly challenging.

Biomarkers are being studied to improve prognostication, treatment monitoring, and severity assessment.⁵ Astroglial biomarkers S100 calcium-binding protein B (S100B) and glial fibrillary acidic protein (GFAP) have been widely studied in the acute setting. The levels of both S100B and GFAP seem to correlate strongly with the severity of the initial injury, and GFAP also with functional outcome.⁶⁻¹⁰ Also, serum levels of heart-type fatty acid-binding protein (H-FABP) and interleukin-10 (IL-10) have been able to distinguish patients with more severe TBIs from those with a mild TBI (mTBI), and they are shown to be promising candidates to predict the outcome.^{7,8,11} Neurofilament light (NF-L) is abundantly expressed in axons thus reflecting axonal damage. Recent studies indicate that NF-L has shown promise in predicting outcome and severity in the subacute phase.^{7,12} Along with NF-L, also less studied biomarkers β -amyloid isoforms 40 (A β 40) and 42 (A β 42) might have predictive value in the subacute phase for moderate and severe TBI (mo/sTBI).^{13,14} Tau is a neurodegenerative biomarker, that was initially used for neurodegenerative diseases and later found useful also in TBI diagnostics, as it has been associated with severity based on clinical and radiological variables, and the levels have also correlated with outcome.^{8,12,13}

In mo/sTBI, the diagnosis does not require biomarkers, because the decision to perform a head CT is usually clear and the findings are sufficient to make clinical decisions. The main need for biomarkers in mo/sTBIs lies in monitoring treatment and predicting outcome.¹⁵ In patients with mo/sTBI it would be clinically important to identify those with an apparent unfavorable outcome and progressive brain injury to target treatment resources. Currently, the only clinical use of biomarkers is in assessing the need for head CT as this has attracted the most research interest in the field. However, several studies have reported outlier (exceptionally high or low) values in serum biomarker levels.^{6,7,13,16-18} Many factors may affect these levels such as patient's age^{16,19}, integrity of the blood-brain barrier²⁰, glymphatic system functioning²¹, extracellular proteolysis²², and hepatic and renal functions^{23,24}. Before biomarkers can be fully implemented into clinical practice, factors that may cause outlier biomarker levels that do not reflect actual injury have to be investigated to avoid misinterpretations.

It has been shown that several biomarker levels are consistently very high in patients with mo/sTBI⁷, therefore we considered it clinically relevant to investigate the background of outlier levels in these patients. The aim of this study was to identify factors that might cause outlier serum levels of S100B, GFAP, H-FABP, NF-L, IL-10, total tau (T-tau), A β 40 and A β 42 in a well-characterized cohort of patients with mo/sTBI.

Methods

Study population

In this post-hoc analysis of a prospective study, altogether 85 patients with moTBI [GCS 9-12, n=48] or sTBI [GCS 3-8, n=37] were recruited at Turku University Hospital between November 2011 and October 2013 as a part of the EU funded TBicare (Evidence-based Diagnostic and Treatment Planning Solution for Traumatic Brain Injuries, EU FP7 Grant Agreement 270259) project. Injury Severity Score (ISS) was assessed for all patients to classify the presence and magnitude of extracranial injuries. Also, 40 control patients with acute orthopedic injuries in absence of head trauma were recruited.

Inclusion criteria for the study were age \geq 18 years, clinical diagnosis of moTBI or sTBI (GCS $<$ 13), and indication for acute head CT according to National Institute for Health and Care Excellence (NICE) criteria²⁵ assessed by the physician on call. Exclusion criteria were age $<$ 18 years, blast-induced or penetrating injury, chronic subdural hematoma, inability to live independently due to pre-existing brain disease, TBI or suspected TBI not needing head CT, more than 2 weeks

from the injury, not living in the district thereby preventing follow-up visits, not speaking the native language, or no consent received.¹⁷ Inclusion criteria for control patients were age \geq 18 years and orthopedic injury without any head trauma. Control patients with inability to live independently due to pre-existing brain diseases or not speaking the native language were excluded.

The study protocol was approved by the ethical review board of the Hospital District of South-West Finland. Written informed consent was obtained from all patients or their legal guardians.

Biomarker analysis

Blood levels for A β 40, A β 42, GFAP, H-FABP, IL-10, NF-L, S100B, and T-tau were analyzed in this study. Blood samples were obtained within 24h of admission, however, they were not necessarily obtained within 24h of injury. Time elapse from injury to sampling was included as a dichotomized (within 24h or over 24h) variable. All samples were kept in cold ice and processed within 1h and stored at -80 °C until the analyzes.

H-FABP and IL-10 plasma levels were analyzed using the K151HTD and K151QUD kits, respectively (Meso Scale Diagnostics, Rockville, MD). For H-FABP and IL-10, the lower limits of detection (LLoD) were 0.103 ng/mL and 0.04 pg/mL with the calibration ranges of 0.137-100 ng/mL and 0.0774-317.0 pg/mL, respectively. The lower limit of quantification (LLoQ) for IL-10 was 0.298 pg/mL. The H-FABP test has not yet been fully validated by Meso Scale, therefore there is no established lower limit of quantification (LLoQ).

Plasma levels of S100B were measured using EZHS100B-33K kit (Millipore, Billerica, MA) with a LLoD of 2.7 pg/mL and the calibration range of 2.7-2000.0 pg/mL. One patient was below the detection range of S100B, and we decided to attribute a concentration of 1 pg/mL to this patient. This did not affect the statistics obtained. Plasma A β 40 and A β 42 levels were measured with a duplex Simoa immunoassay (Quanterix). For A β 40, the LLoD was 0.045 pg/mL and the LLoQ was 0.142 pg/mL, with a calibration range between 0 pg/mL and 90.0 pg/mL. Corresponding concentrations for A β 42 were LLoD of 0.142 pg/mL, LLoQ of 0.69 pg/mL, and with a calibration range of 0 -11.0 pg/mL.

The GFAP, NF-L, and T-tau plasma 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, Billerica, MA). For GFAP, the LLoD was 0.221 pg/mL, while the LLoQ was 0.467 pg/mL and the calibration range was 0.987 pg/mL to 725.0 pg/mL. Respective values for NF-L were 0.104 pg/mL (LLoD), 0.241 pg/mL (LLoQ), and a calibration range between 0.533 pg/mL and 453.0 pg/mL. For T-tau the respective figures were 0.024 pg/mL (LLoD), 0.053 pg/mL (LLoQ) and with a calibration range between 0.136 pg/mL to 112.0 pg/mL.

All patients, excluding the one patient with S100B level below detection range, were over the lower detection range (LLoD). The measurements were performed according to instructions from manufacturers by board-certified laboratory technicians who were blinded to clinical data.

TBI severity and CT scan grading

The severity grading of TBI was initially based on the lowest GCS score before the intubation, evaluated at the scene of the accident or in transport by paramedics, or at the Emergency Department (ED) by the treating physician.^{6,17} Patients with GCS of 9-12 were considered having moderate TBI and those with GCS of 3-8 severe TBI. The duration of PTA²⁶ was assessed at the follow-up visit using the Rivermead scores²⁷. Analysis of CT scans was conducted according to the

descriptive system proposed by Marshall et al.²⁸. Class I or no visual pathology was considered CT- and the classes II-VI were considered CT+, as they included diffuse injuries (class II-IV) and/or mass lesions (V-VI). CT scans were double read by a neuroradiologist and a neurosurgeon.

Outcome

The outcome was assessed at 6-12 months after the injury at a follow-up visit using the Glasgow Outcome Scale-Extended (GOSE).²⁹ Outcomes were classified as favorable (GOSE 5-8) or unfavorable (GOSE < 5) outcome. All patients were evaluated by the same experienced neurologist at the Turku Brain Injury Center.

Cohort characteristics

Normality of the numeric variables, including age, GCS, ISS and biomarkers, was assessed by visually examining histograms and with the Kolmogorov-Smirnov test. Age was normally distributed and is presented as mean \pm standard deviation. Differences between groups are analyzed with independent samples t-test. Biomarker levels, GCS and ISS were not normally distributed and are presented as medians and inter-quartile ranges (IQRs). Differences between groups are analyzed with Mann-Whitney U test. Chi-square test is used for assessing the differences of categorical variables, including sex, pupil reactivity, isolated-TBI, hypoxia, hypotension, hypoglycemia, anemia, outcome (dichotomized), TBI-related deaths and Marshall grading, between groups. There was missing data on pupil reactivity, hypoxia, hypotension, hypoglycemia, anemia and outcome. Patients with missing data were excluded from the comparison analyzes, which were conducted with IBM SPSS Statistics version 28 (IBM Corp, New York).

Outlier analysis

For the analysis all patients with mo/sTBI were divided into subgroups of normal (CT -, n=10) vs. abnormal (CT+, n=75) head CT, and favorable (GOSE 5-8, n=42) vs. unfavorable (GOSE < 5, n=38) outcome. Patients with missing outcome data were excluded from the outcome subgroups (n=5). Statistical outliers were sought individually for each biomarker from these four subgroups and solely from the whole population (n=85) based on biomarker levels. In this study we used the common definition of statistical outliers, Tukey's fences³⁰, to identify the outliers. Therefore biomarker levels outside $Q1 - 1.5 \text{ IQR}$ or $Q3 + 1.5 \text{ IQR}$ were considered outliers. To identify possible clinical reasons for outlier values, the medical records of each patient were systematically reviewed in a team meeting and the clinical reasons were determined. The clinical reasons were classified as obvious or probable, as well as the following categories: TBI severity, timing of sampling, extracranial injuries, demographic, or unknown. Categories were created based on the obvious clinical reasons. The classification of each patient into these categories was independently cross-checked by a senior neurosurgeon (JPP) and a senior neurologist (OT), and potential conflicts solved by discussion. For the tables, we have listed all clinical reasons for each biomarker, so that an outlier value could be explained by several concurrent clinical reasons. A corresponding analysis was also performed for control patients. Outlier analysis was performed with Rstudio software version 1.4 (RStudio, PBC, Boston, USA).

Results

A total of 85 mo/sTBI patients and 40 control patients were enrolled. The characteristics of whole TBI patient population and control patient population are presented in Table 1. Also, the characteristics of the CT and outcome subgroups are presented in Table 2.

Of the 85 patients with mo/sTBI 29 (34%) showed outlier values when outliers from all subgroups were combined. From the whole TBI group we found 17 patients (20%) with outlier values in one or more and up to four biomarkers. About a third of the outlier patients had outlier values in several biomarkers. The outliers in different subgroups were strongly overlapping. In the CT subgroups, 21 patients (25%), and in outcome subgroups, 23 patients (27%) showed outlier values. All outlier values were on the higher spectrum, meaning there were no low outliers. Cut-off values are presented in supplementary materials. Across all subgroups, IL-10 showed most often outlier values, but also for GFAP, NF-L, and S100B a considerable number of outlier values were found. Fewest outliers were seen for A β 40 and A β 42.

Clinical explanations for TBI outliers

Clinical explanations for TBI outliers are presented in Table 3 and the boxplots for the biomarker values in the outcome and CT subgroups are presented in Figures 1 and 2, respectively. Tables for the outcome and CT subgroups are presented in supplementary materials.

Across all subgroups, extremely severe TBIs (= large and widespread intracranial lesions with a respective clinical state) explained most of the outlier plasma GFAP levels. Outlier S100B levels were mainly explained either with extracranial injuries or extremely severe TBIs. In the case of IL-10 extracranial injuries, especially multi-trauma, explained most outlier values. Extracranial injuries, especially thoracic injuries and multi-trauma, accounted for all of the outlier H-FABP levels.

Timing of sampling, especially delay in sampling, and demographic factors, including old age and pre-existing neurological conditions, explained almost all the outlier NF-L levels. Outliers for T-tau were explained mainly with demographic factors, including age and pre-existing neurological conditions. No plausible explanation could be found for the outlier A β 40 and A β 42 levels, except for one patient whose outlier values were considered to be caused by multi-trauma.

Clinical explanations for control patients

Of the 40 control patients, 12 (30%) showed outlier values in at least one and up to four biomarkers. Also, here all outlier values were abnormally high, and almost half of the patients showed outlier values in more than one biomarker. Most outlier values were observed for T-tau and NF-L, and the smallest number of patients with outlier values were found for S100B. Two control patients had overlapping outlier levels with TBI outliers. One had higher IL-10 levels than any of the TBI outliers (obviously because of severe multi-trauma and internal bleedings), and this same patient had also very high H-FABP levels overlapping with those of TBI outliers. Another control patient had high NF-L levels overlapping with the TBI CT subgroups, obviously due to a cerebrovascular disease.

The clinical explanations for controls are presented in Table 4 and boxplots in Figure 3. Outlier levels of GFAP were explained by demographic factors, especially pre-existing neurological conditions, and old age. In case of S100B and IL-10, the explanation was often multi-trauma, but for some there were no obvious reasons. As for the H-FABP, thoracic injuries explained most of the outlier values. Outlier values for NF-L were explained by demographic factors, including pre-existing neurological conditions and age. In most cases there was no obvious reason for outlier levels of T-tau, but some were explained with demographic factors or multi-trauma.

Discussion

The aim of this study was to examine the incidence of outlier biomarker levels in patients with mo/sTBI and if there was a reasonable explanation for these outlier values for S100B, GFAP, H-FABP, NF-L, IL-10, A β 40 and A β 42 in a well-characterized cohort. We found that a substantial part (25-34%) of patients showed outlier values in at least one and up to four biomarkers and that all the outlier values were abnormally high. Also, an obvious or probable clinical explanation was found for almost all outlier values, except the A β 40 and A β 42 outlier levels.

We also identified and described the most obvious clinical explanations for the outlier values, which included extremely severe TBIs, especially for GFAP and S100B. In case of IL-10 and H-FABP, a common explanation was extracranial injuries. Outlier NF-L levels were mainly explained by demographic and timing of sampling. Also, high T-tau was explained by demographic factors. We did not find obvious reasons for high levels of A β 40 and A β 42. Similar explanations occurred among control patients for NF-L, IL-10, H-FABP, and S100B, but for GFAP the outlier values were associated with demographic factors, and for T-tau we did not find any probable explanation.

Patients with extremely severe TBIs showed often outlier values and especially high outliers levels of GFAP were seen, which is reasonable considering that several studies suggest that GFAP is strongly associated with the severity of the initial injury and intracranial findings.^{6,31-33} Consequently, in this study we also observed an association between high outlier levels of GFAP and poor outcome, as all outlier patients in the CT+ -subgroup or in the whole population died because of their injury. Interestingly, in those with favorable outcome many of the GFAP outliers had a disproportion between low admission GCS and high GOSE, which was explained with multiple small cortical contusions causing the high GFAP release. There are many studies indicating that GFAP has potential in outcome prediction.^{8,9,17} In some cases, outlier levels of S100B were also seen, and we observed a respective connection with high outlier levels of S100B and poor outcome. S100B has been associated with the severity of the injury in the acute phase^{7,8,10}, and some studies suggest that S100B has predictive value for unfavorable outcome and death³⁴⁻³⁷.

Timing of sampling, including delayed or very rapid sampling and sampling after surgery, were also identified as one of the potential contributors for unexpected levels. Especially majority of the outlier values for NF-L were associated with the timing of sampling, usually delay in sampling. NF-L is released slowly from the axons, peaking at 1-2 weeks after the injury, which is in line with our observations.³⁸ In some cases, outlier levels of S100B and GFAP were also associated with either delays in sampling (>24h), or in case of S100B also with rapid sampling (<12h). Previous studies have suggested, that peak values for serum S100B and GFAP are at approximately 2-6 h and 24-48 h, respectively^{38,39}, which would also explain our findings. In case of IL-10, delay in sampling and sampling after surgery explained some of the outlier values. However, there is no clear consensus on the kinetic profile of IL-10 - some studies suggest the plasma levels peaking at 3 h⁴⁰ and others at 5-6 days after injury⁴¹. We also found that in some cases outlier levels of IL-10 were associated with samples being drawn after surgery. Several studies have shown that IL-10 is not a brain-specific biomarker, therefore it might be released also from extracranial sources.^{40,42} It is however unclear if surgical measures affect the levels significantly.

Our study suggests that extracranial injuries are the most common confounder for TBI biomarkers. In this respect, IL-10, S100B, and H-FABP were particularly associated. As mentioned previously, elevated IL-10 levels are common also in trauma patients without head trauma^{31,40,42}, which supports our suggestions of multi-trauma causing the outlier values. Several studies have reported that S100B also has extracerebral sources, such as bone fractures, and that high levels have been seen in critically ill patients without head trauma, which is also consistent with our observations of high outlier

levels of S100B in patients with multi-trauma.^{43–45} Outlier patients with high H-FABP had thoracic injuries and/or multi-trauma. H-FABP is not TBI-specific; it has been also identified as a biomarker for myocardial infarction^{46,47} and elevated levels have also been present in patients with multi-trauma, especially affecting the thoracic area^{31,48}. Spinal cord injury was associated with outlier values for GFAP and NF-L, and also the literature suggests elevated levels in patients with SCI.^{49,50}

Demographic factors, including age as well as pre-existing neurological and systemic conditions, were also found as obvious sources for confounding values. Previous studies suggest that age affects the levels of GFAP, NF-L, Tau, and S100B^{16,19,51,52}, and accordingly we found that age was associated with some outlier values for these biomarkers. At least for some TBI-related biomarkers the normal range should be age dependent. Chronic neurological conditions, including earlier TBIs, epilepsy, and cerebral atrophy, have been associated with elevated NF-L and T-tau levels. After a TBI, NF-L may be elevated for up to one year^{38,53}, why it can be assumed that earlier TBIs might cause permanently abnormally high NF-L blood levels in some patients. Tau has been used as a biomarker for Alzheimer's disease⁵⁴, and elevated plasma levels have been associated with neurodegeneration of grey matter^{12,18}. Hence, we suspected that an observed T-tau elevation was caused by AD-type neurodegeneration. Some IL-10 outliers were possibly associated with pre-existing systemic conditions, which included hepatic cirrhosis, lymphoma, and cachexia. One study has reported that IL-10 could be produced by lymphoma cells leading to elevated serum levels⁵⁵, but it is not certain if these systemic conditions were the cause for outlier levels seen in these subjects.

Similar potential confounders for biomarkers were observed among our control patient outliers. GFAP was the only biomarker having a different explanation in controls vs. patients with TBI, as most of the GFAP control outliers had a cerebrovascular disease. An earlier study suggests an association with cerebrovascular diseases and blood levels of GFAP.⁵⁶ For control outliers as well as for TBI outliers we did not find any obvious reason for outlier levels of A β 40 and A β 42, except for one patient where a severe multi-trauma was a possible explanation. Both amyloid outliers with TBI were CT negative and had favorable outcome.

Our study has some strengths and limitations. The study population is small, and a larger number of similar associations would strengthen the observations. Due to the small number of observed associations, statistical analyses to establish the connections could not be done, why our findings remain merely on an observational level and should thus be interpreted with caution. A strength of this study is a prospectively collected well-characterized cohort where numerous biomarkers have been measured from these very same patients. Understanding the background of outlier values is very important for the use of biomarkers in the clinical setting, because unexpectedly high biomarker levels caused, for example, by advanced age or extracranial injuries may lead to an incorrect assessment of the patient's injury burden and outcome. In the worst case this could lead to limiting treatments or to treatment discontinuation. These facts also speak in favor of the simultaneous use of multiple TBI biomarkers.

Conclusions

Our study showed that in patients with a mo/sTBI, outlier levels of several biomarkers are found often, and that in most cases there seems to be a plausible clinical explanation for such a finding. These include very severe or fatal TBIs, various extracranial injuries, timing of sampling and demographic factors including age and pre-existing systemic or neurological conditions. Similar potential explanations could be seen also in orthopedic injury controls. Knowledge on potential

confounders when using TBI-related biomarkers for clinical decision-making is mandatory to avoid misleading diagnostics and false conclusions. The observations found in this study need to be confirmed in other or larger cohorts.

Conflict of interest:

KB has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, BioArctic, Biogen, JOMDD/Shimadzu, Julius Clinical, Lilly, MagQu, Novartis, Ono Pharma, Pharmatrophix, Prothena, 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, outside the work presented in this paper. VFJN holds a grant with Roche Pharmaceuticals. HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work).

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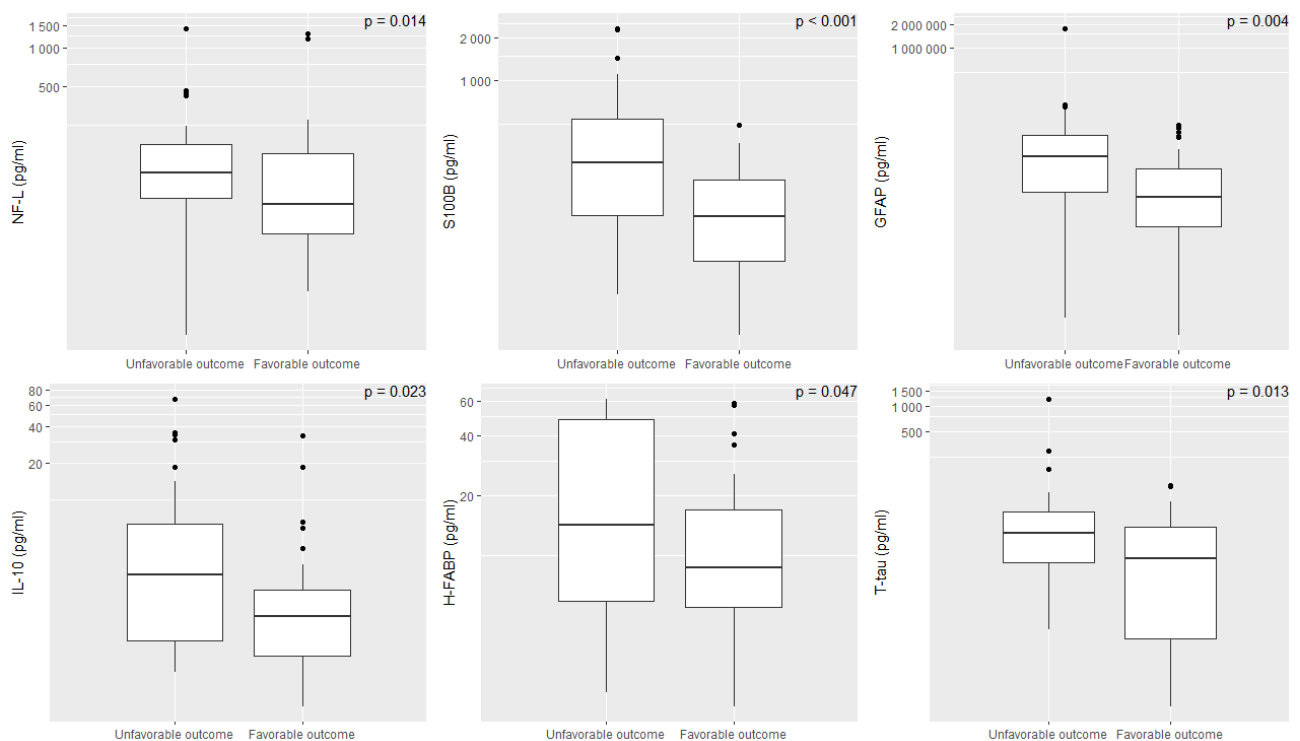


Figure 1. Levels of neurofilament light (NF-L), glial fibrillary acidic protein (GFAP), S100 calcium-binding protein B (S100B), T-tau, interleukin-10 (IL-10) and heart-type fatty acid-binding protein (H-FABP) in patients with favorable (GOSE 5-8) and unfavorable (GOSE <5) outcome. Boxplots describe medians and interquartile ranges measured in picograms per milliliter. The y-axis coordinate system is log10.

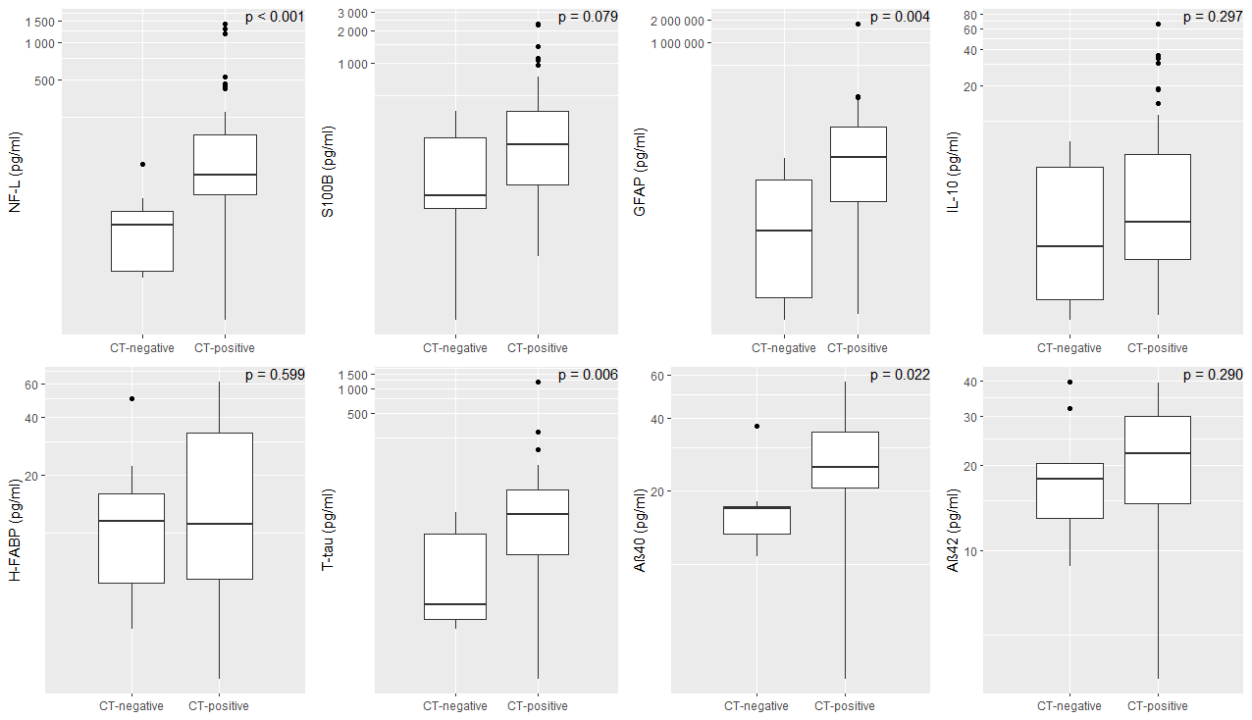


Figure 2. Levels of neurofilament light (NF-L), glial fibrillary acidic protein (GFAP), S100 calcium-binding protein B (S100B), T-tau, interleukin-10 (IL-10), heart-type fatty acid-binding protein (H-FABP), amyloid β 40 ($A\beta$ 40) and amyloid β 42 ($A\beta$ 42) in patients with traumatic intracranial findings (CT-positive) and without findings (CT-negative) in head CT. Boxplots describe medians and interquartile ranges measured in picograms per milliliter. The y-axis coordinate system is log10.

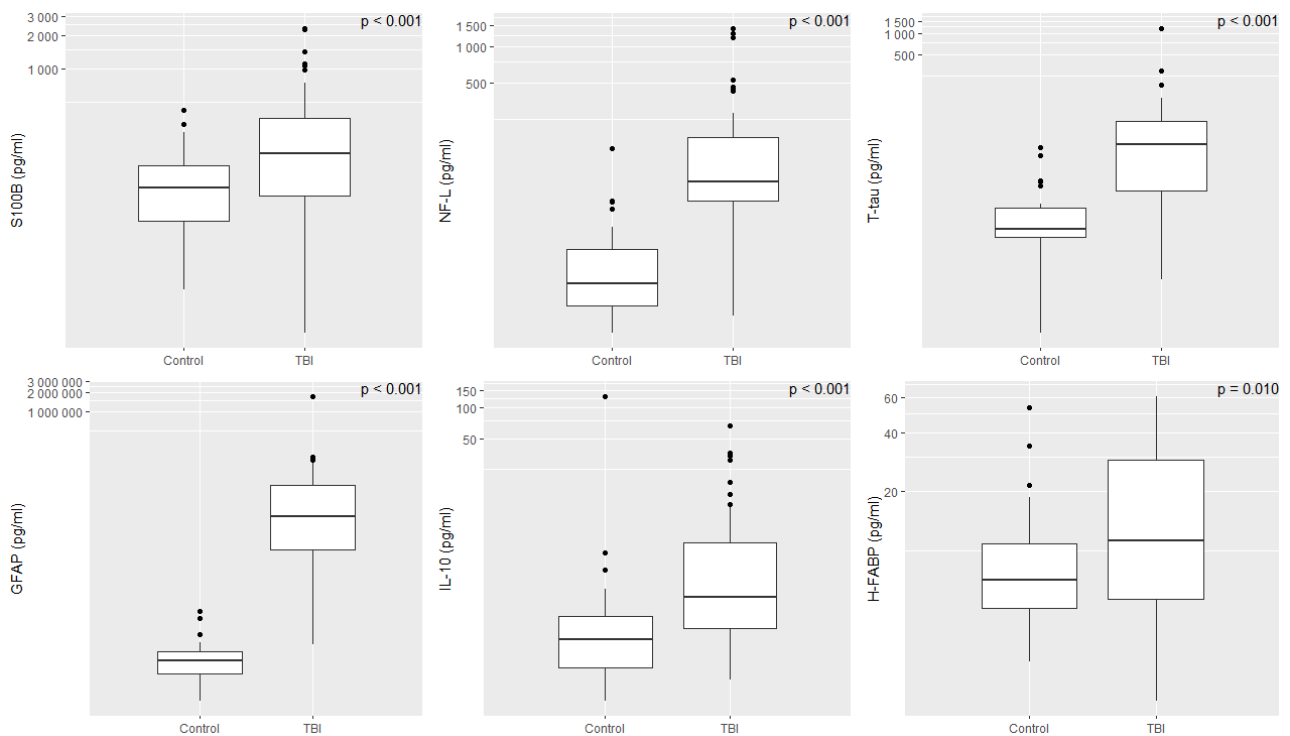


Figure 3. Levels of neurofilament light (NF-L), glial fibrillary acidic protein (GFAP), S100 calcium-binding protein B (S100B), T-tau, interleukin-10 (IL-10) and heart-type fatty acid-binding protein (H-FABP) in control patients and in patients with moderate/severe TBI. The y-axis coordinate system is log10.