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Early levels of GFAP and NF-L in predicting the outcome of mild TBI

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ABSTRACT

The purpose of this study was to correlate the early levels of glial fibrillary acidic protein (GFAP) and neurofilament light protein (NF-L) with outcome in patients with mild traumatic brain injury (mTBI). 107 patients with mTBI [Glasgow Coma Scale (GCS) ≥13] having the blood samples for GFAP and NF-L available within 24 hrs from arrival were included. Patients with mTBI were divided into computed tomography (CT)-positive and CT-negative groups. Glasgow Outcome Scale extended (GOSE) was used to assess the outcome. Outcomes were defined as complete (GOSE 8) vs. incomplete (GOSE <8), and favorable (GOSE 5-8) vs. unfavorable (GOSE 1-4). GFAP and NF-L concentrations in blood were measured using ultrasensitive single molecule array technology. Patients with incomplete recovery had significantly higher levels of NF-L compared to those with complete recovery (p=0.005). The levels of GFAP and NF-L were significantly higher in patients with unfavorable outcome than in patients with favorable outcome (p=0.002 for GFAP and p <0.001 for NF-L). For predicting favorable outcome, the area under the ROC curve for GFAP and NF-L was 0.755 and 0.826, respectively. In a multivariate logistic regression model, the level of NF-L was still a significant predictor for complete recovery (OR=1.008, 95%CI, 1.000-1.016). Moreover, the level of NF-L was a significant predictor for complete recovery in CT-positive patients (OR=1.009, 95%CI, 1.001-1.016). The early levels of GFAP and NF-L are significantly correlated with the outcome in patients with mTBI. The level of NF-L within 24 hrs from arrival has a significant predictive value in mTBI also in a multivariate model.

Introduction

Of all subjects with traumatic brain injury (TBI), mild TBIs (mTBI) comprise 80-90%, a number that is believed to be an underestimate as it only includes those presenting to the emergency departments.[1,2] Currently, there is no unanimous definition of mTBI,[2] and current prediction models for mTBI have shown fairly poor performance.[3] **Computed tomography (CT), a standard tool to assess acute TBI, is insensitive at detecting microbleeds and is unable to detect either diffuse axonal injury (DAI)[4] or metabolic disturbances[5], which are the most common mechanisms of TBI.** Many patients with mTBI suffer from neurological, cognitive and behavioural symptoms for days – weeks, and even one in four patients still have residual symptoms after one year.[6]

Brain injury biomarkers provide objective measures of pathophysiological events following a TBI and could reflect TBI severity, thus aiding the clinician in predicting outcome.[7] In case of mTBI, blood biomarkers could be useful in evaluating the risk for prolonged problems.[8,9,10] S100 calcium binding protein (S100-B), which is principally expressed in glial cells, has been the most studied biomarker in mTBI.[11,12,13,14] Regrettably, it neither seems to be a useful prognosticator of TBI outcome, partly due to extracerebral expression;[15,16,17] nor has it been able to discriminate patients who will develop prolonged or persistent symptoms.[18]

Glial fibrillary acidic protein (GFAP) is also an indicator of glial damage and almost exclusively found in the CNS, is a monomeric intermediate filament protein located in glial skeleton.[19,20,21] Several papers have reported the potential utility of this biomarker in predicting clinical outcome in TBI.[17,22,23,24,25]

Neurofilament light (NF-L) protein is the most abundant of the three neurofilament proteins[26] and is mainly expressed in the long myelinated white-matter axons.[27,28] One study showed a very marked increase in the serum levels of NF-L in patients with severe TBI, with gradually increasing levels up to 10-12 days post trauma, and the levels predicted clinical outcome at several time-points, also at admission.[29] Another study showed that the levels of NF-L 7-10 days after bout in amateur boxers correlated with the number of head impacts during the match, and the levels were elevated also in professional hockey players suffering from symptoms following repetitive mTBI.[30] The levels of NF-L in

cerebrospinal fluid (CSF) were able to discriminate two groups of contact sports athletes, those with rapidly resolving from prolonged concussion symptoms.[31]

The aim of the current study was to correlate the levels of GFAP and NF-L during the first 24 hours after admission with outcome in patients with mTBI, in order to find out their potential for clinical use in assessing mTBI.

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. We recruited 107 patients with mTBI [Glasgow Coma Scale (GCS) ≥13] having blood samples available within 24 hours from the arrival to the emergency department (ED) of Turku University Hospital, Finland.

Inclusion criteria were: GCS ≥13, age ≥18 years, clinical diagnosis of TBI and indications for acute head CT according to NICE criteria (http://www.nice.org.uk/guidance/cg176).

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 native language, or no consent received.

The ethical review board of Hospital District of South-West Finland approved the study protocol. All patients or their next of kin were informed about the study in both oral and written forms and a written informed consent was obtained.

Analysis of GFAP and NF-L

Plasma GFAP and NF-L levels 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). The measurements were performed in one round of experiments using one batch of reagents by board-certified laboratory technicians who were blinded to clinical data. Quality control (QC) samples were analyzed

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in each run, with coefficients of variations (CV) of 3.1% at 113 pg/mL and 3.8% at 86 pg/mL for GFAP, and 4.4% at 13.9 pg/mL and 6.1% at 7.1 pg/mL for NF-L.

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TBI severity and outcome grading

GCS scores were assessed by paramedics at the scene of accident or during transport, and/or by an emergency physician at the time of admission. In assessing the severity, the lowest recorded post-resuscitation GCS was used.[24,32] The overall injury severity of the patients was assessed using the Injury Severity Score (ISS).[33] The duration of posttraumatic amnesia (PTA) was assessed at the outcome visit using the Rivermead method.[34] Analysis of CT scans was conducted according to descriptive system proposed by Marshall et al.,[35] where class 1 corresponds with normal CT, classes 2-4 diffuse injuries, and classes 5-6 CTs with mass lesions.

Outcome

The outcome was assessed 6-12 months after the injury using the Extended Glasgow Outcome Scale (GOSE).[36] Outcomes were defined as complete recovery (GOSE 8), incomplete recovery (GOSE <8), favorable outcome (GOSE 5-8), and unfavorable outcome (GOSE 1-4). Rivermead Post Concussion Symptoms Questionnaire (RPCSQ)[37] was used to assess the presence and severity of mTBI-related symptoms.

Statistical analyses

Demographics of the subjects are presented as mean ±SD. Normality of distribution of biomarkers levels was assessed with the Kolmogorov-Smirnov test and by visually inspecting data histograms. As the levels of GFAP and NF-L were not normally distributed, nonparametric tests were used in the statistical analyses. Data are presented as medians and interquartile range [IQR]. Correlations between the levels of biomarkers and the outcomes were analyzed with Spearman rank correlation coefficient. Pearson's correlation coefficient was used to evaluate the correlation between biomarker levels with age and gender. Mann-Whitney U test was used to compare the levels of biomarkers between the outcome groups. Multivariate logistic regression analysis was done to study the prognostic ability of the biomarkers to predict the dichotomized outcomes. The regression analysis was done including the following variables: age, ISS, worst GCS, Marshall score, PTA, time

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from injury to blood sampling, pupillary reactivity, and the levels of GFAP and NF-L. Marshall score and pupil reactivity were used as categorical variables. Marshall class I denoting CT-negative finding and reactive pupils were used as reference categories in multivariate logistic regression. GFAP and NF-L were used in the multivariate logistic regression models independently with the other variables and together in the same models. Area under the receiver operating characteristic (ROC) curve (AUC) was also used to evaluate the prognostic ability of the biomarkers. AUC of 0.8 to 1.0 was considered very good; AUC of 0.7 to 0.8 was considered adequate; and AUC of 0.5 to 0.7 was considered poor.[21] A p value <0.05 was considered statistically significant. Cut-off values for the prediction of dichotomized outcomes were defined using the ROC curve at the sensitivity >90%. **Pearson's correlation coefficient was used to assess the correlation between the levels of GFAP and NF-L in the whole study population. Besides this, the correlation in the different outcome groups: complete, incomplete, favorable, and unfavorable outcome were also measured.** For the data analyses, IBM SPSS Statistics 22 (IBM Corp, Armonk, New York) and MATLAB R2015b (Math Works, Natick, Massachusetts) were used. Additionally, panels of biomarkers were generated using PanelomiX software[38] based on their best cut-off values. Panels of biomarkers were used to assess the performance of combination of biomarkers to discriminate complete and incomplete recovery, as well as favorable and unfavorable outcome. Cut-off values were selected for a sensitivity of >90%.

Results

Demographics, Injury Severities, CT imaging and Outcomes

One-hundred-and-seven patients with mTBI were recruited, with a mean age of 47.6 ±20.2 years and most of them being male (n=73, 68.2%). 55 patients (51.4%) were CT-positive, and 52 (48.6%) were CT-negative. Patient characteristics are shown in Table 1. For 105 (98.1%) patients the GOSE scores were available. Regarding the outcome, 37 patients (34.6%) had complete recovery, 68 patients (63.5%) had incomplete recovery, 90 patients (84.1%) had favorable outcome, 15 patients (14%) had unfavorable outcome, and the mortality was 3.7% (n=4).

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GFAP and outcome

The levels of GFAP were compared between patients with complete recovery vs. incomplete recovery, and favorable outcome vs. unfavorable outcome (Figures 1A and 1B). The levels were not significantly different between patients with complete (median, 612pg/mL; interquartile range [IQR], 1996pg/mL) and incomplete recovery (median, 1467pg/mL; IQR, 6453pg/mL). The levels of GFAP (median, 4867pg/mL; IQR, 24667pg/mL) were significantly higher in patients with unfavorable outcome than in patients with favorable outcome (median, 875pg/mL; IQR, 2280pg/mL; p=0.002).

There was a significant negative correlation between the levels of GFAP and GOSE score (Spearman ρ=-0.25, p=0.010) (Table 2). GFAP could predict complete recovery, with an AUC of 0.598 (95%CI 0.489-0.706, p=0.099) and favorable outcome, with an AUC of 0.755 (95%CI 0.628-0.882, p=0.002) (Figures 2A and 2B).

GFAP and outcome in CT-positive/negative mTBI

When patients were divided into CT-positive and CT-negative subgroups, the levels of GFAP did not differ between the outcome groups nor did the levels correlate significantly with the outcome within these subgroups.

In a multivariate logistic regression model, GFAP was not able to predict outcome independently or together with NF-L.

NF-L and outcome

Patients with incomplete recovery had significantly higher levels of NF-L (median, 17pg/mL; IQR, 47pg/mL) compared to those with complete recovery (median, 11pg/mL; IQR, 10pg/mL; p=0.005). The levels of NF-L (median, 66pg/mL; IQR, 35pg/mL) were also significantly higher in patients with unfavorable outcome than in patients with favorable outcome (median, 13pg/mL; IQR, 13pg/mL; p<0.001) (Figures 1A and 1B). There was a significant negative correlation between the levels of NF-L and GOSE score (Spearman ρ=-0.382, p<0.001) (Table 2). NF-L could predict complete recovery, with an AUC of 0.665 (95%CI 0.561-0.768, p=0.005) and favorable outcome, with an AUC of 0.826 (95%CI 0.694-0.958, p<0.001) (Figures 2A and 2B). In a multivariate logistic regression model, the level of NF-L was a predictor for complete recovery (OR=1.008, 95%CI, 1.000-1.016) having GFAP

in the model (Table 3). Furthermore, the level of NF-L was a statistically significant predictor for complete recovery in a multivariate logistic regression model (OR=1.006, 95%CI, 1.001-1.011).

NF-L and outcome in CT-positive/negative mTBI

Within the CT-negative mTBI subgroup, the levels of NF-L did not differ between the outcome groups, nor did the levels correlate significantly with the outcome. Within the CT-positive mTBI subgroup the patients with incomplete recovery had significantly higher levels of NF-L (median, 52pg/mL; IQR, 54pg/mL) compared to patients with complete recovery (median, 15pg/mL; IQR, 15pg/mL; p=0.007). Within the CT-positive mTBI subgroup, the levels of NF-L (median, 66pg/mL; IQR, 35pg/mL) were also significantly higher in patients with unfavorable outcome than in patients with favorable outcome (median, 20pg/mL; IQR, 41pg/mL; p=0.013).

Within the patients with CT-positive mTBI, there was a significant negative correlation between the levels of NF-L and GOSE score (Spearman ρ=-0.450, p=0.001). In this subgroup, NF-L could predict complete recovery, with an AUC of 0.750 (95%CI 0.593-0.908, p=0.007) and favorable outcome, with an AUC of 0.720 (95%CI 0.559-0.880, p=0.013). Furthermore, in CT-positive mTBI the level of NF-L was a significant predictor for complete recovery in a multivariate logistic regression model (OR=1.009, 95%CI 1.001-1.016).

Cut-off values

Cut-off values for GFAP and NF-L were derived from the ROC curves of full cohort for predicting complete recovery and favorable outcome. The minimum level of sensitivity was set to 90%.

For the prediction of complete recovery, the cut-off level of GFAP was 6438.05pg/mL with a sensitivity of 97% (95%CI, 86-100) and a specificity of 26% (95%CI, 68-99). For predicting favorable outcome, the cut-off value of GFAP was 12189.85pg/mL with a sensitivity of 92% (95%CI, 85-99) and a specificity of 47% (95%CI, 16-68).

For predicting complete recovery, the cut-off level of NF-L was 28.15pg/mL with a sensitivity of 94% (95%CI, 82-99) and a specificity of 44% (95%CI, 32-57). For predicting

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favorable outcome, the cut-off value of NF-L was 53.6pg/mL with a sensitivity of 90% (95%CI, 82-95) and a specificity of 67% (95%CI, 38-88) (eTable 4)

Combination of GFAP + NF-L and outcome

Using a combination of the two biomarkers with sensitivity set to >90% for complete recovery, the optimal sensitivity and specificity was 94.6% (95%CI, 86.5-100.0) and 47.1% (95%CI, 35.3-58.8), respectively (eFigure 3A), with GFAP and NF-L levels below 6438.05pg/mL and 28.15pg/mL, respectively.

When sensitivity was set to >90% for favorable outcome, the optimal sensitivity and specificity was 90.0% (95%CI, 83.3-95.6) and 86.7% (95%CI, 66.7-100.0), respectively (eFigure 3B), with GFAP and NF-L levels below 980.75pg/mL and 41.85pg/mL, respectively.

Correlation between the levels of GFAP and NF-L

Except for unfavorable outcome, the levels of GFAP and NF-L were significantly correlated. For the whole study population, Pearson's r=0.635, p<0.0001. For favorable outcome, complete, and incomplete recovery, Pearson's r=0.739, p<0.0001, Pearson's r=0.995, p<0.0001, Pearson's r=0.496, p<0.0001, respectively.

Discussion

We found that in this population and at this time-point of sampling, NF-L was useful in predicting favorable outcome, and to a lesser degree also complete recovery. Also, GFAP could predict favorable outcome adequately. Our most important finding is that for all patients with mTBI, in a multivariate logistic regression model including known outcome predictors and the level of GFAP, NF-L was a statistically significant predictor for complete recovery. Moreover, NF-L was an independent predictor for complete recovery in patients with CT-positive mTBI in a multivariate logistic regression model including the same clinical variables. A combination of these two biomarkers showed a higher sensitivity (94.6%) and specificity (47.1%) for predicting complete recovery compared to a single biomarker. This combination was also found to have a higher sensitivity (90.0%) and specificity (86.7%) for predicting favorable outcome compared to a single biomarker. Patients with incomplete recovery had significantly higher levels of NF-L than those with complete recovery. The levels of GFAP and NF-L were significantly higher in patients with unfavorable outcome

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than in patients with favorable outcome. There was also a strong negative correlation between the levels of GFAP and NF-L and outcome assessed with GOSE. **Since GFAP and NF-L are expressed by different cell types as well as structures and the cytoskeleton of astroglia and the axoskeleton of long axons likely respond the same physical forces differently, the fact that their correlation was weakest in those patients with unfavorable outcome supports the need to use a panel of biomarkers for reliable outcome prediction following mTBI.**

Recently, our research group reported that in a cohort of patients with TBIs of all severities, the levels of GFAP on day 1 after the injury were higher in patients with incomplete recovery. The levels of GFAP on arrival day, day 1 and 2 were higher in patients with unfavorable outcome and also correlated negatively with GOSE. In addition, GFAP upon arrival was able to distinguish between favorable and unfavorable recovery.[24] An earlier study reported that the levels of GFAP were lower in patients with favorable outcome than with unfavorable outcome within the first 12 hours of injury.[21] One study demonstrated that elevated levels of GFAP on day 2 were able to predict mortality in patients with TBI,[39] and another study found that the levels of GFAP were good predictors of outcome within 6 hours from TBI.[40] In one study, GFAP breakdown products could poorly predict complete recovery, but predicted adequately favorable outcome.[41] Interestingly, Metting et al. found that the levels of GFAP measured within 3 hours after injury could not predict complete recovery when outcome was assessed with GOSE at 6 months after mTBI,[42] which may be related to the finding that GFAP levels appear to rise up to 16–24 h after the injury.[43] In the TRACK-TBI study, where 83% of the study subjects were patients with mTBI, the levels of GFAP were not able to discriminate those with complete recovery from those with incomplete recovery.[23]

Two previous studies, including TBIs of all severities, have reported that GFAP is able to distinguish patients with favorable outcome from patients with unfavorable outcome.[23,24] The TRACK-TBI investigators reported that the levels of GFAP could adequately discriminate favorable outcome from unfavorable outcome, with an AUC of 0.74 (95%CI, 0.61-0.87).[23] Our earlier study reported that the levels of GFAP could adequately distinguish favorable outcome from unfavorable outcome, with an AUC of 0.723 (95%CI,

0.602-0.814) [24]. The results from the present study are thus consistent with the aforementioned studies, with an AUC of 0.755 (95%CI 0.628-0.882). **It has been suggested that combining several astroglia-derived markers may improve the predictive ability[44]**.

NF-L has been reported to be a highly sensitive blood-based protein biomarker for severe TBI.[29] The same group has also investigated the relationship between diffusion tensor imaging of DAI following a severe TBI and the levels of NF-L in the CSF, showing that the levels of NF-L could predict the degree of axonal injury as well as the outcome following TBI.[28] A novel finding in our study is that the levels of NF-L were able to distinguish patients with complete recovery from incomplete recovery and favorable outcome from unfavorable outcome, not only in the whole population but also in patients with CT-positive mTBI. Although in earlier studies, the levels of NF-L were able to differentiate athletes with prolonged symptoms from those with rapid recovery following concussion,[30,31] in our study neither the levels of NF-L nor GFAP correlated with the RPCSQ scores. However, the levels of NF-L correlated with the GOSE scores. By combining the levels of GFAP and NF-L we obtained better sensitivity and specificity compared to use of either biomarker alone.

Both biomarkers failed to predict recovery in CT-negative patients. This could potentially be due to inadequate statistical power – though there were approximately similar numbers of CT-positive and CT-negative patients in our cohort, the event rate for poor outcomes was lower in the latter group. However, it is also possible that the drivers of poorer outcome are different in CT-positive and negative groups, with outcome in the latter group driven more by host factors (such as education, premorbid psychological health, and coping strategies)[45] rather than by structural injury that is detectable by blood biomarkers or structural neuroimaging.

Ultrasensitive single molecule array (Simoa) technology was used to measure the levels of these blood-based protein biomarkers, which is strength in our study. Simoa has been reported as more sensitive than conventional enzyme-linked immunosorbent assay (ELISA) or electro-chemiluminescence (ECL)-based assay.[46] Another strength of our study is the considerable sample size in a well-characterized cohort.

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The main limitation is that we did not have data for GFAP and NF-L available beyond 24 hrs after admission. Later levels could provide more information about the likelihood of recovery. **The time from injury to sampling was variable, which may well influence the levels of these biomarkers, although this was tried to take into account as a covariate. A more precise knowledge of the kinetics of these biomarkers after a TBI would be needed to assess the clinical significance of various levels measured.** Another limitation is that we did not have information about the duration of symptoms after the mTBI in those who had recovered before the outcome evaluation. It is also important to mention that our mTBI cohort does not represent typical general mTBI population, because only GCS was used to assess the severity and because there was a high percentage of patients with CT-positive findings, since they were more easily recruited due to hospital admission. **The variability in assessing the GOSE, between 6 to 12 months after the injury, is a limitation that needs to be recognized. However, every patient was evaluated by the same experienced clinician. In addition, studies suggest that most who recover fully from a mTBI recover fairly quickly, and that the majority of those who are symptomatic at 6 months have not recovered at one year, either[47]. Yet, it is possible that some who were still symptomatic at 6 months might have recovered by one year, which potentially causes some uncertainty in the outcome evaluation. While based on all available data our patients had a minimum GCS of 13 or more, it should be noted that using other severity measures used in the literature PTA some might have had a more severe injury. Because we found the duration of PTA impossible to assess reliably in many patients, and because there is no generally accepted severity classification of imaging findings in TBI, we chose to use only GCS which gives the least uncertainty and is the most often used. When interpreting the results of our study one should note the quite large confidence intervals for the prognostic ability of these biomarkers, which is obviously due to the fairly small sample size, especially taking into consideration the large variability within mTBI. Although the percentage of those subjects who showed an unfavorable outcome after mTBI (15/107, 14%) is in line with the general concept of mTBI outcome, our results should be confirmed in larger materials.**

Due to the complexity and individual variability of TBI, developing clinically useful biomarkers for this condition has proven to be a challenging task. This study shows that both GFAP and NF-L may be useful predictive biomarkers in mTBI, but because of the limitations discussed above, more studies are needed to clarify the useful time points as well as other potential limitations for their clinical use. These problems have been discussed in a recent systematic review[48].

Conclusions

The early levels of GFAP and NF-L are significantly correlated with the outcome in patients with mTBI. The level of NF-L within 24 hrs from arrival has a significant predictive value in mTBI also in a multivariate model.

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Table 1. Patient characteristic

Age (years)	47.64 ± 20.19
Sex	
Male	73 (68.2%)
Female	34 (31.8%)
Marshall Grade	
No visual pathology	52 (48.6%)
Diffuse injury	24 (22.4%)
Diffuse injury with swelling	1 (0.9%)
Diffuse injury with shift	1 (0.9%)
Mass lesions	29 (27.1%)
Pupil reactivity	
Unreactive	1 (0.9%)
Sluggish	2 (1.9%)
Reactive	99 (92.5%)
Missing data	5 (4.7%)
GOSE	
1	4 (3.7%)
2	0
3	6 (5.6%)
4	5 (4.7%)
5	7 (6.5%)
6	14 (13.1%)
7	32 (29.9%)
8	37 (34.6%)
Missing data	2 (1.9%)
Total	107 (100%)

Demographics are reported in mean ±SD or percentages (%)

Table 2. Correlation between biomarkers and GOSE, gender, RPCSQ and age

Biomarkers	GOSE			Gender			RPCSQ (PRQ, total)			Age			RPSQ (16 cutoff)		
	Spearman	p-value	N	Pearson's	p-	N	Pearson's	p-	N	Pearson's	p-	N	Pearson's	p-	N
GFAP	**-0.25**	**0.010**	105	0.145	0.137	107	0.030	0.769	96	0.157	0.104	107	0.025	0.809	96
NF-L	**-0.382**	**p<0.001**	105	0.096	0.327	107	-0.016	0.874	96	**0.223**	**0.021**	107	-0.019	0.851	96

GOSE: Glasgow Outcome Scale extended; RPCSQ: Rivermead Post Concussion Symptoms Questionnaire, statistically significant findings are in

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Table 3. Logistic regression analysis of GFAP and NF-L to distinguish mild TBI patients with complete recovery from patients with incomplete recovery

Number of patients = 98	**OR**	**95%CI**	
Age	0.987	0.961	1.013
PTA	0.483	0.158	1.473
Time elapse	**4.037**	**1.264**	**12.897**
Worst GCS	0.672	0.285	1.581
ISS	0.960	0.902	1.021
Marshall II-V	0.417	0.121	1.442
Marshall V	0.232	0.052	1.037
Pupillary reactivity	**29.760**	**1.543**	**574.069**
GFAP	1.000	1.000	1.000
NF-L	**1.008**	**1.000**	**1.016**

Time elapse of more than 24 hours, Marshall I, and pupil reactive are used as reference category

N: number of subjects; OR: odds ratio; CI: confidence interval; PTA: posttraumatic amnesia; Time elapse: duration between the time of injury and the time of blood-biomarker sampling; Marshall I and pupil reactive as reference categories. Significant OR values in bold

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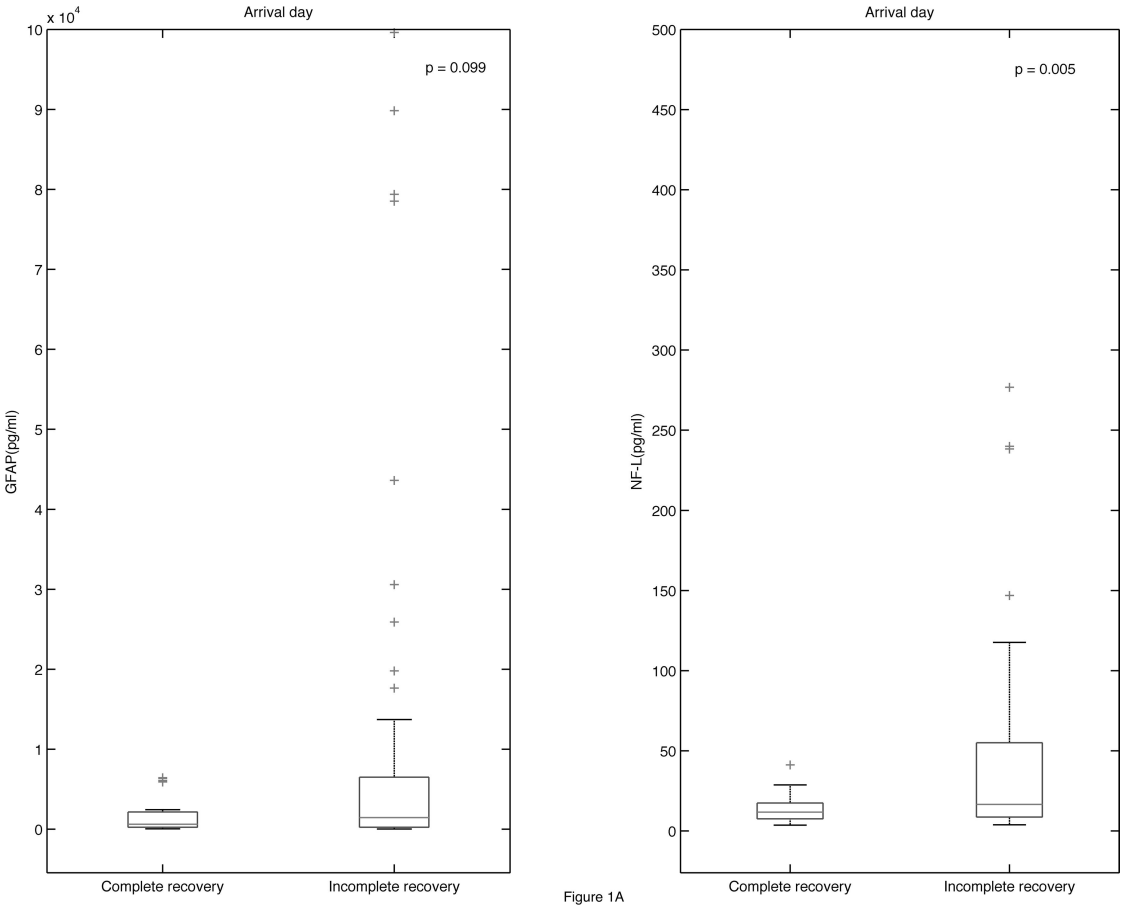

Figure 1A

Figure 1A. Levels of GFAP and NF-L in patients with complete (GOS 8) and incomplete (GOS <8) recovery (y axis is zoomed). (Title)

Box plots represent medians in picograms per milliliter and interquartile ranges. (Caption)

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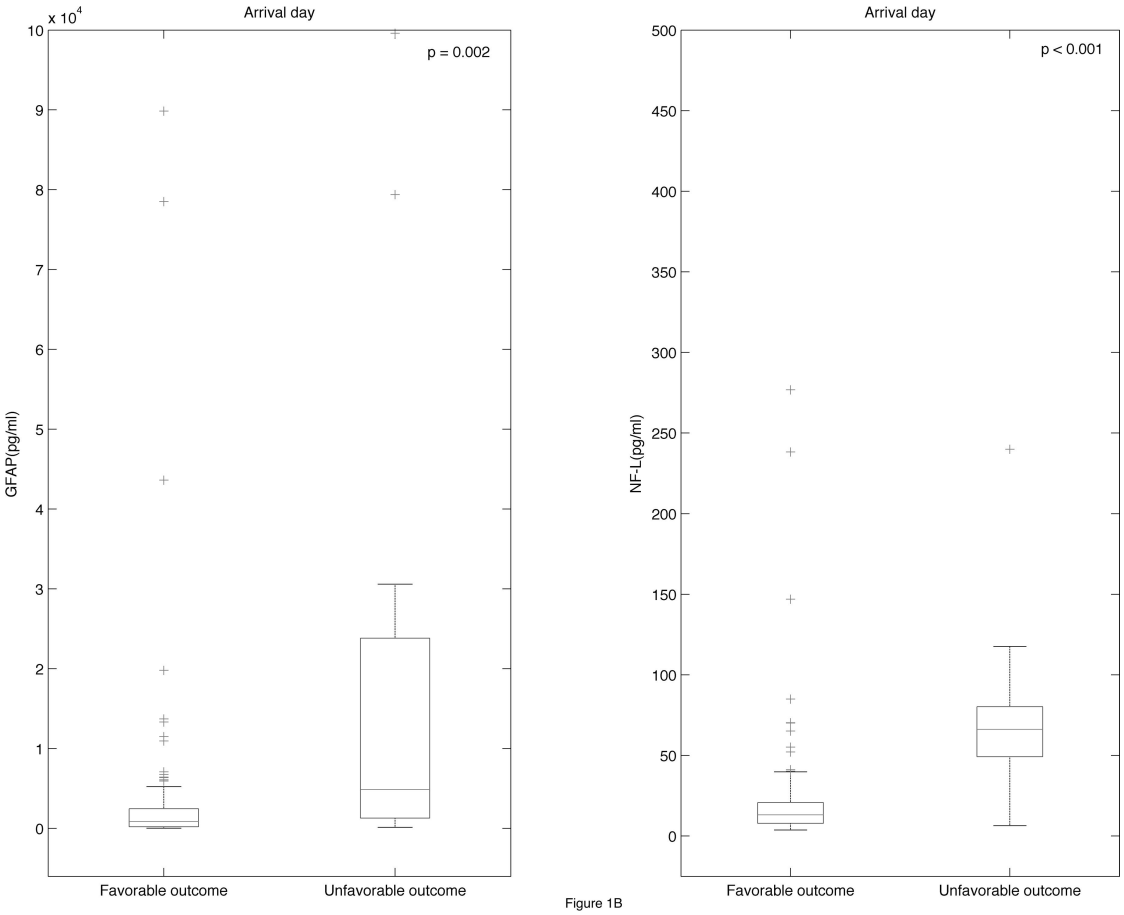

Figure 1B

Figure 1B. Levels of GFAP and NF-L in patients with favorable (GOS 5-8) and unfavorable (GOS 1-4) outcome (y axis is zoomed). (Title)

Box plots represent medians in picograms per milliliter and interquartile ranges. (Caption)

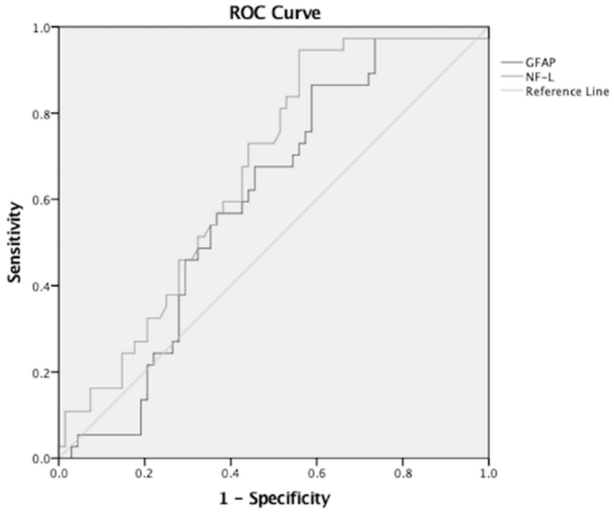

Figure 2A. ROC curves for predicting complete recovery (GOS 8). (Title)

AUC for GFAP, 0.598 (95%CI 0.489-0.706, p=0.099) and AUC for NF-L, 0.665 (95%CI 0.561-0.768, p=0.005). (Caption)

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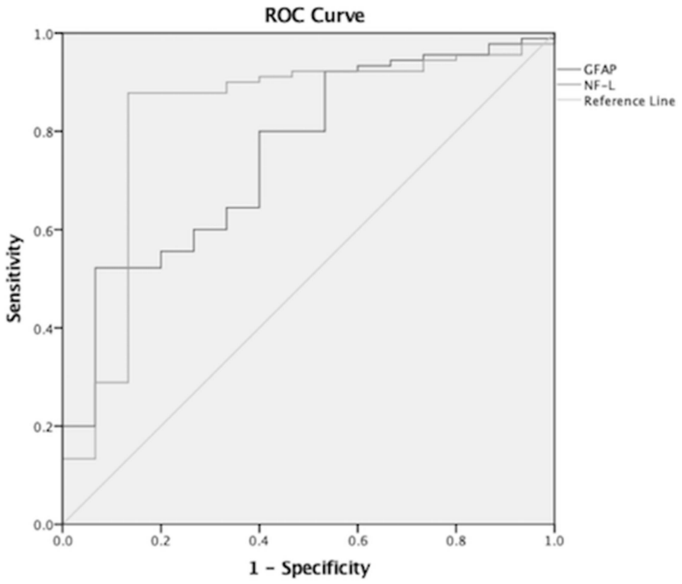

Figure 2B. ROC curves for predicting favorable outcome (GOSE 5-8). (Title)

AUC for GFAP, 0.755 (95%CI 0.628-0.882, p=0.002) and AUC for NF-L, 0.826 (95%CI 0.694-0.958, p<0.001). (Caption)

Supplementary data

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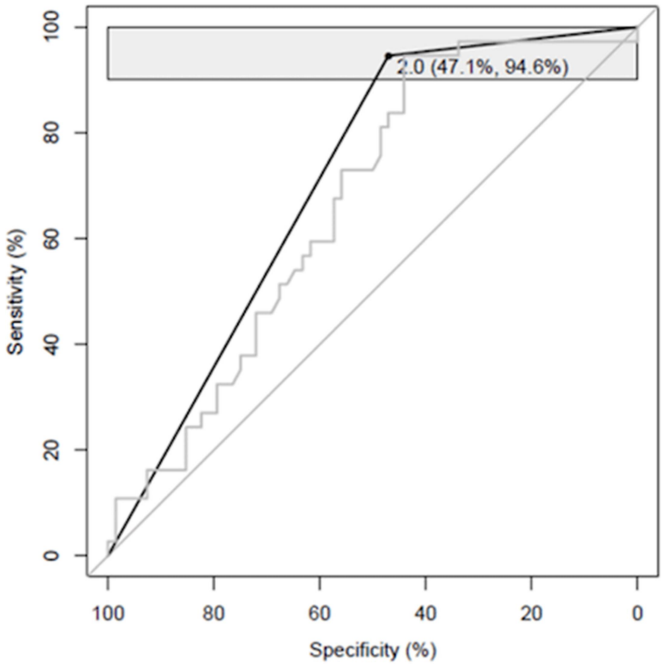
eTable 4. Cut-off value, sensitivity, specificity, and 95 % confidence interval of sensitivity and specificity of in receiver operating characteristic curve analysis for complete recovery or favorable outcome

Biomarker	**Dichotomized outcome**	**Cutoff value (pg/ml)**	**Sensitivity**	**95%CI Sensitivity**	**Specificity**	**95%CI Specificity**
GFAP	Complete recovery	6438.05	0.97	0.86 - 1	0.26	0.68 - 0.99
NF-L	Complete recovery	28.15	0.94	0.82 – 0.99	0.44	0.32 - 0.57
GFAP	Favorable outcome	12189.85	0.92	0.85 – 0.99	0.47	0.16 – 0.68
NF-L	Favorable outcome	53.6	0.90	0.82 – 0.95	0.67	0.38– 0.88



eFigure 3A. ROC curves for panels of biomarkers for predicting complete recovery (GOSE 8). (Title)

A combination of GFAP and NF-L showed a higher sensitivity (94.6%) and specificity (47.1%) for predicting complete recovery compared to a single biomarker. (Caption)

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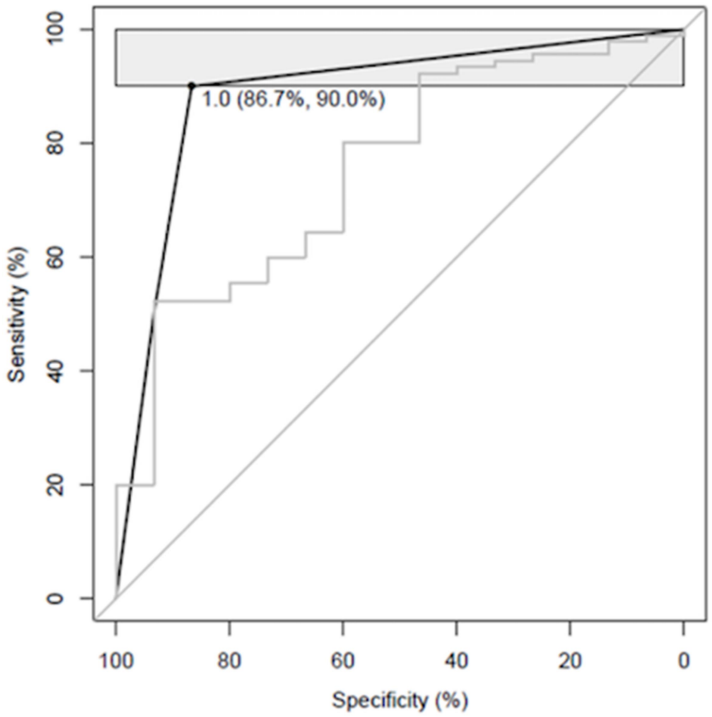

eFigure 3B. ROC curves for panels of biomarkers for predicting favorable outcome (GOSE 5-8). (Title)

A combination of GFAP and NF-L was found to have a higher sensitivity (90.0%) and specificity (86.7%) for predicting favorable outcome compared to a single biomarker. (Caption)