Incremental prognostic value of acute serum biomarkers for functional outcome after traumatic brain injury (CENTER-TBI) : an observational cohort study

Incremental Predictive Ability of Acute Serum Biomarkers for Functional Outcome Following Traumatic Brain Injury (CENTER-TBI): an Observational Cohort Study

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Abstract

Background: Several studies have reported an association between serum biomarker values and functional outcome following traumatic brain injury (TBI). We aimed to examine the incremental (added) prognostic value of serum biomarkers over demographic, clinical and radiological characteristics and over established prognostic models, such as IMPACT and CRASH, for prediction of functional outcome.

Methods: We used data from the Collaborative European NeuroTrauma Effectiveness Research in Traumatic Brain Injury (CENTER-TBI) Core study. Incremental prognostic value of six serum biomarkers (S100B, NSE, GFAP, UCH-L1, NFL and T-Tau), collected <24h of injury, was determined separately, and in combination. The primary outcome was the Glasgow Outcome Scale Extended (GOSE) six-months post-injury. Incremental prognostic value, using proportional odds and a dichotomized analysis, was assessed by delta concordance (C) statistic and delta R^2 between models with and without serum biomarkers, corrected for optimism with a bootstrapping procedure.

Findings: Serum biomarker values and 6-month GOSE were available in 2283/4509 patients. Higher biomarker levels were associated with worse outcome. Adding biomarkers improved the C-statistic and R^2 compared to demographic, clinical and radiological characteristics by 0·014 (95% CI 0·009-0·020) and R^2 by 4·9% (95% CI 3·6%-6·5%) for predicting GOSE. UCH-L1 had the greatest incremental prognostic value. Adding biomarkers to established prognostic models resulted in a relative increase in R^2 of 48%-65% for IMPACT and 30%-34% for CRASH prognostic models, respectively.

Interpretation: Serum biomarkers have incremental prognostic value for functional outcome following TBI. Our findings support integration of biomarkers, in particular UCH-L1, in established prognostic models.

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Key words: Prognosis; Prognostic model; Serum biomarkers; Traumatic Brain Injury; Glasgow Outcome Scale Extended.

Introduction

Traumatic brain injury (TBI) poses a major and increasing health burden with global socio-economic implications,[1] and represents a leading cause of death. In those who survive, long-term disability or residual complaints are common, even if they experienced 'mild' TBI as indicated by a Glasgow Coma Score of 13-15.[2]

Functional outcome following TBI depends on many different aspects, including patient and injury characteristics, mechanisms of trauma, patient response and the quality of care provided.[1] Establishing a reliable prognosis early after injury is challenging, but can be facilitated by the use of a prognostic model. Prognostic models combine information from multiple predictors to support clinicians in providing reliable information to patients and their relatives, help guide clinical decision making, inform benchmarking quality of care, and guide the design and analysis of clinical trials. Validated models are available to predict functional outcome following moderate and severe TBI,[3] including the IMPACT and CRASH models.[4, 5] However, these models only explain 35% of variance in outcome. Prognostic models for mild TBI (mTBI) are less well established.[6] Improving prognostication has been recognized as a high priority by clinicians and researchers.[7]

Prognostic value may increase by adding biomarkers. Over the past decade, blood-based protein biomarkers, and in particular S100 calcium-binding protein B (S100B), glial fibrillary acidic protein (GFAP), and ubiquitin C-terminal hydrolase L1 (UCH-L1) have received much attention for their role in diagnosing mTBI and triaging patients for computed tomography (CT) scanning of the head.[8] S100B has been implemented in the Scandinavian TBI Guidelines, and the combination of GFAP and UCH-L1 was approved by the FDA as a diagnostic test in patients suspected of mTBI based on the results of the ALERT-TBI study.[9]

In addition to the diagnostic role of biomarkers in TBI, an increasing body of evidence indicates the potential for a prognostic role. A substantial number of studies have shown an association between serum biomarkers and functional outcome following TBI.[10-16]

However, most prior studies have mainly focused on the unadjusted prognostic effect of biomarkers rather than estimating their value over and above established prognostic factors, which is considered essential.[17] As a consequence, the independent prognostic value of biomarkers remains uncertain and their incremental value unknown.

The aim of our study was to determine the incremental prognostic value of six serum biomarkers (S100B, GFAP, UCH-L1, NSE, NFL, T-Tau) over patient's demographic, clinical and radiological characteristics for the prediction of six-month functional outcome after TBI. Furthermore, we aimed to examine the incremental prognostic value of biomarkers when added to the IMPACT core and CRASH basic models for predicting mortality and unfavorable outcome after TBI.

Methods

Study population and design

Participants were drawn from the Collaborative European NeuroTrauma Effectiveness Research in Traumatic Brain Injury (CENTER-TBI) Core study (version 3.0). CENTER-TBI was a prospective, multicenter, longitudinal, observational cohort study,[18, 19] that recruited patients between December 2014 and December 2017 from 18 countries across Europe and Israel. Inclusion criteria for the Core study were 1) a clinical diagnosis of TBI; 2) a clinical indication for computed tomography (CT) scanning; and 3) presentation within 24h of injury. Patients with a severe pre-existing neurological disorder were excluded. For the current analysis, selection of patients was limited to those with 1) blood sampling within 24h of injury, 2) availability of results from CT scan, 3) and for whom outcome assessment according to the Glasgow Outcome Scale-Extended (GOSE) was available at six-months.

Patients were stratified at enrollment by care path into the Emergency Department (ER) (assessed in the ER and discharged out of hospital), Admission (admitted to hospital ward), and Intensive Care Unit (ICU) strata (primary admission to the ICU). Informed consent was obtained from all participants or their legal representative according to local and national requirements. The use of biological samples was in accordance with the terms of the informed consent. The study was registered with ClinicalTrials.gov (NCT02210221), and is reported in accordance with the STROBE recommendations (see Supplementary material).

Clinical data were collected using a web-based electronic case report form (eCRF), with variables coded in accordance with the Common Data Elements (CDE) scheme (https://commondataelements.ninds.nih.gov/). Data were entered on the Quesgen e-CRF (Quesgen Systems Inc, USA), hosted on the International Neuroinformatics Coordinating Facility (INCF) platform and extracted via the INCF Neurobot tool (INCF, Sweden). We extracted data on demographic, clinical and radiological predictors of outcome, results of biomarker assays and outcome. The selection of predictors was based on established

prognostic models for functional outcome after mild and moderate to severe TBI (Suppl Table 1).[3, 6] Radiological parameters were obtained from central readings of the first CT scan. Missing predictor values were imputed with five iterations with multiple imputation using the mice package.[20] All demographic, clinical, and radiological characteristics, serum biomarkers, stratum, injury severity score (ISS) and six-month GOSE were included in the imputation model. Most observations showed low missingness (2 - 5%); the only exception being level of education, where missingness was higher (18%).

The primary outcome was the Glasgow Outcome Scale Extended (GOSE), the most widely used measure of global functional outcome following TBI, six-months post-injury. The GOSE was assessed by structured interview, conducted either by face to face or telephone interview, or by postal questionnaire (Suppl Table 2). Data collection for the GOSE interview was standardized using a manual for CENTER-TBI.[21] GOSE interviews and questionnaires were scored centrally using an algorithm to derive the GOSE rating. In subjects for whom both interview and questionnaire assessments were available, we used the interview-based rating. Categories 2 (vegetative state) and 3 (lower severe disability) were combined. Using a multi-state model, missing GOSE values for six months were imputed based on GOSE measurements obtained at other time points up to 18 months post-injury.[22] Biomarker values were not available at the time of outcome assessment, so all ratings were blinded to biomarker values.

We analysed the association of biomarkers with six-month GOSE adjusted for demographic, clinical and radiological parameters, and determined their incremental prognostic value. GOSE was analysed across all severities, and dichotomized into clinically relevant endpoints, namely mortality (GOSE=1), unfavorable outcome (GOSE≤4) and incomplete recovery (GOSE<8). Subgroup analyses were performed by stratum, and by injury severity. Finally, we determined the incremental prognostic value of biomarkers when added to the IMPACT core and CRASH basic models.

Ethical approval

The CENTER-TBI study (EC grant 602150) has been conducted in accordance with all relevant laws of the EU if directly applicable or of direct effect and all relevant laws of the country where the Recruiting sites were located, including but not limited to, the relevant privacy and data protection laws and regulations (the "Privacy Law"), the relevant laws and regulations on the use of human materials, and all relevant guidance relating to clinical studies from time to time in force including, but not limited to, the ICH Harmonised Tripartite Guideline for Good Clinical Practice (CPMP/ICH/135/95) ("ICH GCP") and the World Medical Association Declaration of Helsinki entitled "Ethical Principles for Medical Research Involving Human Subjects". Informed Consent by the patients and/or the legal representative/next of kin was obtained, accordingly to the local legislations, for all patients recruited in the Core Dataset of CENTER-TBI and documented in the e-CRF. Ethical approval was obtained for each recruiting site. The list of sites, Ethical Committees, approval numbers and approval dates can be found on the website: https://www.center-tbi.eu/project/ethical-approval

Sample Collection and Biomarker Measurements

Blood samples were collected using gel-separator tubes for serum and centrifuged within 60 (45±15) minutes. The serum was processed, aliquoted (8x0·5ml), and stored at −80°C locally until shipment on dry ice to the CENTER-TBI serum biobank (Pécs, Hungary).

We assayed S100B, NSE, GFAP, UCH-L1, NFL, and T-Tau. Details of the analyses procedures have been previously described.[23] In brief: S100B and NSE were measured with a clinical-use automated system, using an electrochemiluminecesence immunoassay kit (Elecsys S100 and NSE assays on the Cobas 8000 modular analyzer, Roche Diagnostics, Mannheim, Germany). GFAP, UCH-L1, t-tau, and NFL were analysed using Single Molecule Arrays (SiMoA) based assay on the SR-X benchtop assay platform (Quanterix Corp., Lexington, MA). Unique aliquots were used for analyses on two platforms to avoid repeated freeze-thaw cycles and analyzed in one round of experiments using the same batch of reagents by qualified laboratory technicians blinded to clinical information. All biosamples have reportable values above the LLOD value for the respective markers, with the exception of n=19 samples that have UCH-L1 values below its LLOD (1·34 pg/mL). For those samples, we assigned their UCH-L1 levels as 1·34

pg/mL. A technical summary of the biomarker sample collection and measurements can be found in the Supplementary materials (Suppl Table 3).

Statistical analysis

Descriptive statistics are presented as means, medians or frequencies. Differences in biomarker values by stratum (ER, Admission, ICU) and injury severity (mild and moderate/severe TBI) were compared using independent sample t tests.

We used proportional odds analysis to quantify the relationship between serum biomarkers and six-month GOSE across all severities, adjusted for demographic, clinical and radiological parameters, and binary logistic regression for the GOSE dichotomized for mortality (GOSE=1), unfavorable outcome (GOSE≤4) and incomplete recovery (GOSE<8). For the serum biomarkers we assessed nonlinearity with spline functions. The six biomarkers were considered separately, and in combination, with a particular focus on the combination of GFAP and UCH-L1, as this combination has been approved by the FDA as a diagnostic test for patients after mTBI in the US.[9]

Model performance was expressed in terms of discrimination (C-statistic), which indicates how well the model can differentiate between patients with a low and high risk of the outcome, and the R^2 (quantified as a percentage from 0-100 (%)), which indicates the goodness of fit of a logistic regression model.[24]

The incremental value of biomarkers in prognosticating outcome was assessed by calculating the difference in C-statistic (delta C; ΔC) and R^2 (delta R^2; ΔR^2) between the models with and without the serum biomarkers ('reference model'). A bootstrapping procedure was used to reduce optimistic model performance estimates.[24] Bootstrapping entails drawing random samples (n = 200) with replacement from the derivation cohort, with sample size equal to that of the derivation cohort. We also used bootstrapping to obtain confidence intervals for C, ΔC, R^2, and ΔR^2. Finally, we assessed the incremental prognostic value of biomarkers relative to the IMPACT core (Age, GCS motor, pupillary

reactivity),[4] and CRASH basic (Age, GCS, pupillary reactivity, major extracranial injury (MEI)) models.[5]

Sensitivity analyses

We accounted for differences in predictor effects following mild, and moderate/severe TBI by fitting the models with interaction terms for GCS and the demographic, clinical and radiological parameters.

Subgroup analyses

The following subgroup analyses were performed:

- by care path as defined by stratum (ER; Admission; ICU)
- by injury severity, differentiated as moderate to severe (GCS 3-12) and mild (GCS 13-15)
- uncomplicated very mTBI (GCS=15, no traumatic abnormalities on first CT))
- mTBI with and without traumatic abnormalities on first CT

Statistical analysis was performed using R statistical software (http://www.r-project.org, version 3.6.0) in RStudio (http://www.rstudio.com, version 1.1.456). We used the 'rms' package to fit the logistic regression models.[25]

Role of funding source

The funders had no role in the collection, analysis and interpretation of data, nor in the writing of the report or in publication decisions. The authors had full access to study data and the senior authors had final responsibility for the decision to publish.

Results

Study Population

We included 2283/4509 (51%) adult patients (≥14 years) with available serum biomarker values within 24h after injury and six-month GOSE (Suppl Fig 1). Patients had a median age of 51 years (IQR = 32-67), 68% were male, and most (67%) were diagnosed with mild TBI (mTBI; GCS 13-15) (Table 1). More than a third (37%) experienced major extracranial injury. Baseline characteristics were largely similar to those previously described in the overall cohort (Suppl Table 4).[23] Characteristics of patients not included (n=2226) were

similar to those analyzed (n=2283), although the percentage of patients with severe TBI was lower (20% versus 24%), and serum biomarker values were generally lower in patients not included.

The time from admission to sampling was shortest in the ER stratum (Median 5·0, IQR= [3·5-9·5]), compared to the admission (15·5 [(9·9-19·9]) and ICU strata (14·3 [7·7-19·6]) (Suppl Table 5; Suppl Fig 2).

At six months, 270 (12%) patients had died, 593 (26%) had unfavorable outcome, and 1443 (63%) patients had an incomplete recovery (Table 1).

Table 1: Patients' demographic, clinical and radiological characteristics at admission, serum biomarker values within 24h and six-months functional outcome for all patients and by stratum (ER, Admission and ICU).

Characteristics	**Overall[a] (n =2283)**	**ER (n =505, 22%)**	**Admission (n =624, 27%)**	**ICU (n =1154, 51%)**
Age (14-95) (Median [IQR])	51 [32-67]	50·00 [32-66]	54 [35-69]	49 [31,66]
% Male sex	68% (1559)	57% (287)	67% (420)	74% (852)
Level of education	(N=1881)	(N=479)	(N=538)	(N=864)
College/Uni degree	467 (25)	156 (33)	141 (26)	170 (20)
Currently in school/With diploma or degree-oriented program	395 (21)	84 (18)	129 (24)	182 (21)
None/primary school	347 (18)	94 (20)	100 (19)	153 (18)
Secondary/High school	672 (36)	145 (30)	168 (31)	359 (42)
Pre-injury mental health problems	272 (12)	60 (12)	68 (11)	144 (13)
GCS baseline	*(N=2209)*	(N=503)	(N=605)	(N=1101)
Mild (13-15)	1472 (67)	499 (99)	578 (96)	395 (36)
Moderate (9-12)	186 (8)	2 (0·4)	21 (4)	163 (15)
Severe (3-8)	551 (25)	2 (0·4)	6 (1)	543 (49)
GCS motor score	(N=2241)	(N=503)	(N=606)	(N=1132)
None	361 (16)	2 (0·4)	2 (0·3)	357 (32)
Extension	35 (2)	0 (0·0)	1 (0·2)	34 (3)
Abnormal flexion	40 (2)	0 (0·0)	1 (0·2)	39 (3)

Normal flexion	89 (4)	0 (0·0)	4 (1)	85 (8)
Localizes	235 (11)	4 (1)	14 (2)	217 (19)
Obeys	1481 (66)	497 (99)	584 (96)	400 (35)
Reaction of Pupils	(N=2178)	(N=483)	(N=591)	(N=1104)
Both	1944 (89)	474 (98)	577 (98)	893 (81)
One	90 (4)	1 (0·2)	8 (1)	81 (7)
None	144 (7)	8 (2)	6 (1)	130 (12)
Marshall CT	(N=2182)	(N=497)	(N=597)	(N=1088)
I	836 (38)	428 (86)	292 (49)	116 (11)
II	834 (38)	67 (14)	252 (42)	515 (47)
III	90 (4)	0 (0)	6 (1)	84 (8)
IV	19 (1)	0 (0)	0 (0)	19 (2)
V	6 (0·3)	0 (0)	1 (0·2)	5 (0·5)
VI	397 (18)	2 (0·4)	46 (8)	349 (32)
Traumatic Subarachnoid Hemorrhage	1015 (47)	44 (9)	195 (32)	776 (73)
Epidural Hematoma	233 (11)	1 (0·2)	42 (7)	190 (18)
Hypotension	172 (9)	3 (1)	9 (2)	160 (15)
Hypoxia	163 (8)	1 (0·2)	9 (2)	153 (14)
Glucose (Median [IQR])	7·1 [6·0-8·6]	6·0 [5·3-7·0]	6·6 [5·8-7·8]	7·7 [6·4-9·3]
Hemoglobin (Median [IQR])	13·5 [12·0-14·6]	14·1 [12·8-14·9]	13·9 [12·8-14·9]	13·2 [11·6-14·5]
ISS (0-75) (Median, [IQR])	16 [9-29]	4 [2-6]	10 [9-16]	29 [25-41]
MEI[b]	848 (37)	17 (3)	172 (28)	659 (57)
Serum biomarkers within 24 hours				
S100B mg/L	0·12 [0·07-0·26]	0·09 [0·05-0·15]	0·08 [0·06-0·2]	0·19 [0·10-0·43]
NSE ng/ml	15·5 [11·7-23·4]	13·7 [10·9-17·5]	13·6 [11·1-18·4]	19·3 [13·4-29·5]
GFAP ng/ml	3·0 [0·48-15·7]	0·30 [0·11-0·91]	1·3 [0·32-4·8]	12·3 [3·4-38·0]

UCH-L1 pg/ml	88·5 [35·1-281·3]	35·8 [15·8-62·6]	49·1 [22·2-108·2]	232·6 [93·4-563·1]
T-Tau pg/ml	2·6 [1·2-7·0]	1·1 [0·63-1·7]	1·7 [0·99-3·2]	5·9 [2·7-13·8]
NFL pg/ml	23·7 [9·4-74·6]	8·7 [5·3-15·1]	13·7 [7·3-25·9]	58·8 [27·7-139·9]
Sampling time (h) ((Median [IQR])	12·6 [6·0-18·9]	5·0 [3·5-9·5]	15·5 [9·9-19·9]	14·3 [7·7-19·6]
Functional outcome six months post-injury				
Death	290 (13)	3 (1)	31 (5)	236 (21)
Vegetative state/Lower Severe disability	175 (8)	9 (2)	18 (3)	194 (17)
Upper Severe disability	80 (4)	7 (1)	14 (2)	81 (7)
Lower Moderate disability	171 (8)	15 (3)	42 (7)	168 (15)
Upper Moderate disability	217 (10)	26 (5)	50 (8)	133 (12)
Lower good recovery	266 (12)	94 (19)	160 (26)	162 (14)
Upper good recovery	1029 (46)	351 (70)	309 (50)	180 (16)

[a] Patients <14 years of age (N=43) were excluded.

[b] Patients with an Abbreviated Injury Scale ≥ 3 regarding the following body regions; face, cervical spine, thorax/chest, abdomen/pelvic contents, extremities and pelvic girdle, or external (skin), thus excluding head and neck.

Abbreviations: GCS, Glasgow Coma Scale; ISS, Injury Severity Score; N, Number; MEI, Major Extracranial Injury; SD, Standard Deviation; IQR, Interquartile range

Serum biomarkers and functional outcome following TBI

Higher biomarker levels were associated with poorer outcome overall, and when differentiated by stratum and injury severity (Fig 1; Suppl Table 5; Suppl Fig 3). Associations were stronger for UCH-L1, NFL, S100B, T-tau, and GFAP compared to NSE. Biomarker levels scaled with the intensity of care (as defined by stratum), and with TBI severity (higher after moderate-severe TBI compared to those with mTBI). All serum biomarkers were negatively correlated with six-month GOSE (Spearman rank correlations: S100B -0·43; NSE -0·28; GFAP -0·50; UCHL1 -0·54; T-tau -0·52; NFL -0·56; Suppl Fig 4).

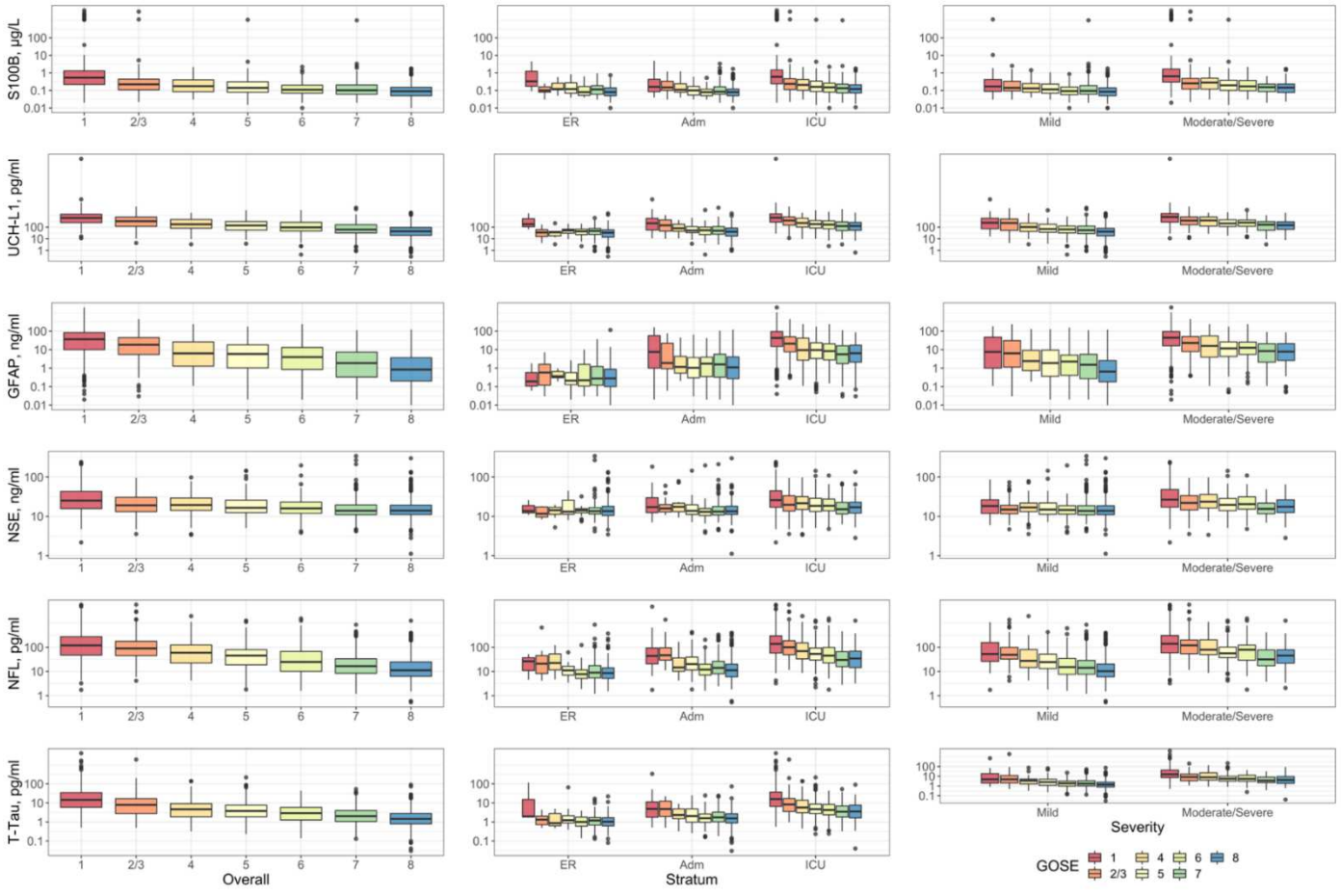

Figure 1: *Biomarker values by stratum and by clinical severity, differentiated for the Glasgow Outcome Scale Extended (GOSE). The serum biomarkers are shown on the log scale. The boxplots show the median serum biomarker value (thick black line). The first quartile is indicated by the line above and the third quartile by the line below the median. The whiskers are the minimum and maximum. The dots are outliers.*

Incremental prognostic value of serum biomarkers for prediction of GOSE

In proportional odds logistic regression analysis, biomarkers improved the prognostic value in addition to demographic, clinical and radiological characteristics for the prediction of six-month GOSE (Fig 2; Suppl Table 6). The C-statistic for the reference model was 0·781 (95% CI 0·768, 0·794), and increased with the addition of biomarkers. Improvements in C-statistic ranged from 0·002 (95% CI 0·000, 0·004) for NSE to 0·010 (95% CI 0·006, 0·015) for UCH-L1 (Suppl Table 6). Similarly, the addition of the biomarkers increased the R^2 of the reference model (44·8% (95% CI 41·4%, 47·8%), with improvements ranging from 0·8% R^2 (95% CI 0·3, 1·4) for NSE, to 3·8% R^2 (95% CI 2·8%, 5·1%) for UCH-L1 (Fig 2; Suppl Table 6). All six biomarkers taken together had substantial incremental value over single biomarkers (ΔC-statistic 0·014 (95% CI 0·009, 0·020; ΔR^2 4·9% (95% CI 3·6%, 6·5%)). Combinations of UCH-L1 with NFL, NFL with T-tau, and NFL with T-tau and S100B showed similar performance as all biomarkers together (Suppl Table 7). The combination of GFAP with UCH-L1 did not improve discrimination compared to UCH-L1 alone.

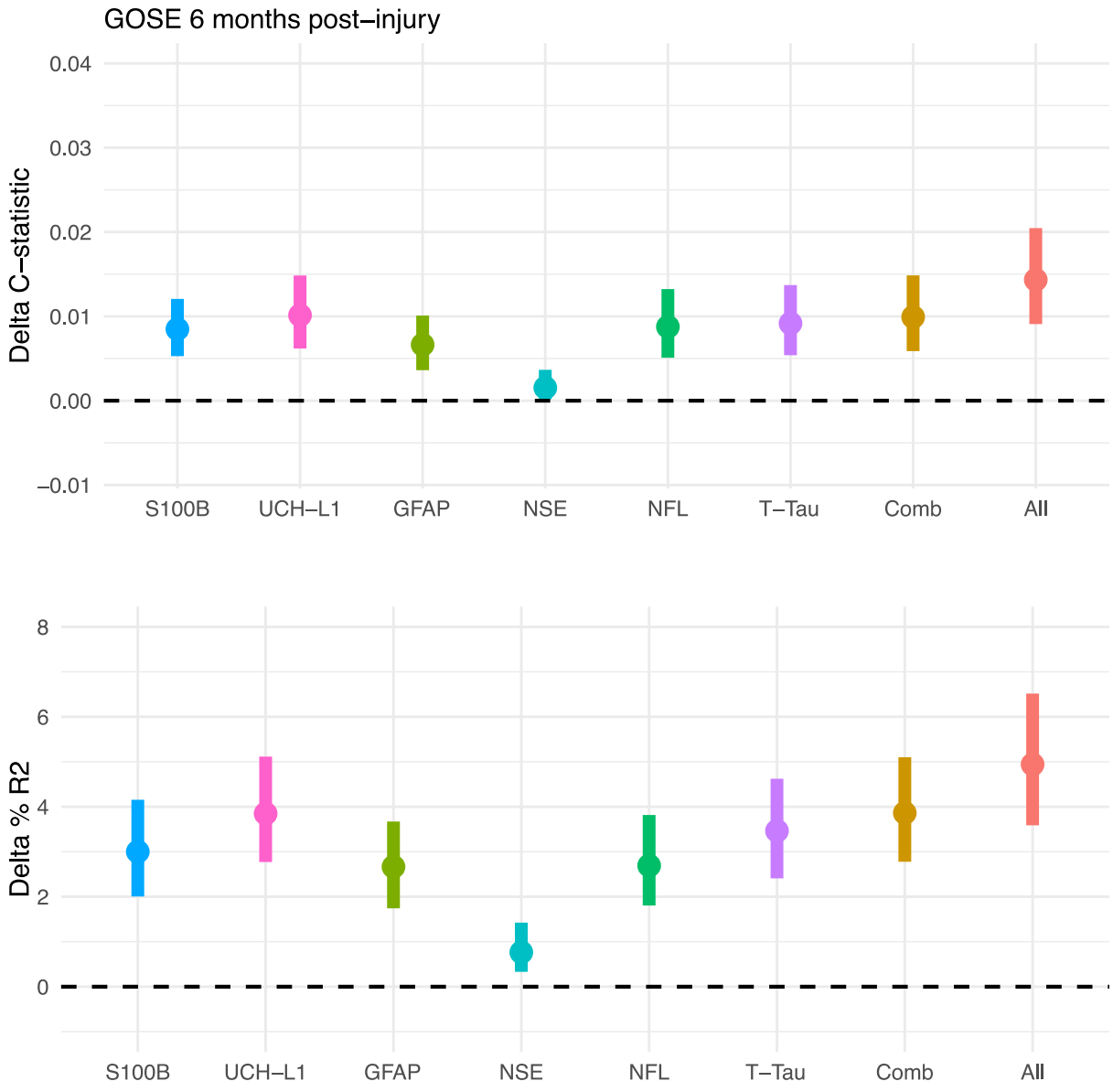

Figure 2: *The difference (delta) in C-statistic and % R^2 between the reference model and models including serum biomarkers of ordinal regression models adjusted for demographic, clinical and radiology parameters (see Supplementary Table 1 for model parameters) for the Glasgow Outcome Scale Extended 6 months post-injury. Six biomarkers are considered separately, in combination (Comb; GFAP + UCHL1) and taken together ("all"). The absolute values are presented in Supplementary Table 4. The points illustrate the delta C-statistic (above) and R^2 (below) and the vertical lines above and below the points illustrate the 95% CI around the estimate.*

In binary logistic regression analysis, the reference model discriminated very well: 0·922 (95% CI 0·906, 0·936) for mortality, 0·883 (95% CI 0·866, 0·898) for unfavorable outcome, and 0·802 (95% CI 0·783, 0·819) for incomplete recovery (Table 2). Nevertheless, biomarkers showed incremental prognostic value (Table 2, Suppl Fig 5). Incremental

value was highest for UCH-L1 and T-Tau in predicting mortality (ΔC-statistic for both biomarkers: 0·011 (95% CI 0·005, 0·017); ΔR2: 3·8% (95% CI 2·1%, 5·9%) for UCH-L1 and 3·8% (95% CI 2·0%, 6·2%) for T-Tau), and for NFL in predicting unfavorable outcome (ΔC-statistic: 0·015 (95% CI 0·009, 0·022) ; ΔR2: 4·2% (95% CI 2·9%, 5·9%)). Single biomarkers had lower incremental value for the prediction of incomplete recovery, and was highest for S100B, UCH-L1 and NFL, and lowest for NSE (Table 2). Results were similar for the prediction of incomplete recovery in patients with mTBI and uncomplicated very mTBI; the incremental prognostic value of biomarkers was highest for S100B, UCH-L1 and NFL (Suppl Table 8).

Results were consistent across strata and injury severity (Suppl Tables 9 and 10). Serum biomarkers had incremental prognostic value for the prediction of six-month functional outcome for patients in the ER, admission, and ICU strata (Suppl Table 9), and in patients following mild and moderate/severe TBI (Suppl Table 10). The incremental prognostic value of biomarkers was similar in mTBI and moderate/severe TBI (Suppl Table 10). In patients following mTBI with and without traumatic abnormalities on CT the incremental value remains, but the added value of biomarkers is more pronounced in mTBI patients with CT abnormalities (Suppl Table 11). The addition of interaction terms for GCS led to a decrease in incremental value of serum biomarkers for prediction of six-month GOSE (Suppl Table 12).

Table 2: Discriminative ability (C-statistic) and R^2 of serum biomarkers adjusted for demographic. clinical and radiology parameters to predict functional outcome six-months following traumatic brain injury for three clinically relevant thresholds; mortality, unfavorable outcome (GOSE ≤4) and incomplete recovery (GOSE <8).

	Mortality (N=290)				**Unfavorable outcome (N=545)**				**Incomplete recovery (N=1254)**			
	C-statistic (95% CI)		**R^2 (%) (95% CI)**		**C-statistic (95% CI)**		**R^2 (%) (95% CI)**		**C-statistic (95% CI)**		**R^2 (%) (95% CI)**	
Reference model	0·922	(0·906, 0·936)	51·0%	(45·7%, 56·1%)	0·883	(0·866, 0·898)	49·7%	(45·3%, 53·8%)	0·802	(0·783, 0·819)	33·8%	(29·8%, 37·7%)
Serum biomarkers	**Delta C-statistic (95% CI)**		**Delta R^2 (%) (95% CI)**		**Delta C-statistic (95% CI)**		**Delta R^2 (%) (95% CI)**		**Delta C-statistic (95% CI)**		**Delta R^2 (%) (95% CI)**	
S100B	0·009	(0·004, 0·015)	3·3%	(1·4%, 5·7%)	0·008	(0·004, 0·012)	2·4%	(1·3%, 3·8%)	0·007	(0·003, 0·012)	1·6%	(0·8%, 2·5%)

UCH-L1	0·011	(0·005, 0·017)	3·8%	(2·1%, 5·9%)	0·014	(0·009, 0·021)	4·6%	(3·0%, 6·5%)	0·007	(0·003, 0·012)	1·9%	(1·1%, 3·0%)
GFAP	0·006	(0·002, 0·012)	2·3%	(0·9%, 4·1%)	0·010	(0·005, 0·015)	3·0%	(1·8%, 4·5%)	0·003	(0·000, 0·006)	1·0%	(0·4%, 1·8%)
NSE	0·004	(0·001, 0·009)	1·6%	(0·4%, 3·3%)	0·003	(0·001, 0·007)	1·1%	(0·3%, 2·1%)	0·001	(0·000, 0·002)	0·2%	(0·1%, 0·5%)
NFL	0·005	(0·001, 0·009)	1·5%	(0·3%, 3·0%)	0·015	(0·009, 0·022)	4·2%	(2·9%, 5·9%)	0·007	(0·003, 0·013)	1·8%	(0·9%, 3·0%)
T-tau	0·011	(0·005, 0·017)	3·9%	(2·0%, 6·2%)	0·013	(0·008, 0·019)	4·2%	(2·6%, 5·9%)	0·006	(0·002, 0·011)	1·7%	(0·9%, 2·7%)
GFAP + UCH-L1	0·011	(0·006, 0·018)	4·1%	(2·2%, 6·2%)	0·014	(0·008, 0·020)	4·5%	(2·9%, 6·3%)	0·006	(0·002, 0·012)	1·8%	(0·9%, 2·9%)
All	0·012	(0·005, 0·019)	4·3%	(1·9%, 6·9%)	0·019	(0·012, 0·027)	5·6%	(3·7%, 7·7%)	0·010	(0·004, 0·018)	2·5%	(1·2%, 4·1%)

Incremental prognostic value of serum biomarkers relative to established prognostic models

The incremental value of biomarkers when added to the IMPACT core and CRASH basic models, for prediction of mortality and unfavorable outcome in patients with moderate to severe TBI, was substantial (Table 3). For mortality, improvements in C-statistic ranged from 0·016 (95% CI 0·000, 0·036) for NFL to 0·053 (95% CI 0·029, 0·080) for UCHL-L1 for the IMPACT models, and from 0·013 (95% CI 0·003, 0·026) for NFL to 0·035 (95% CI 0·019, 0·052) for UCHL-L1 for the CRASH models. For unfavorable outcome, improvements in C-statistic ranged from 0·030 (95% CI 0·015, 0·048) for NSE to 0·066 (95% CI 0·041, 0·093) for UCHL-L1 for IMPACT, and from 0·018 (95% CI 0·009, 0·029) for NSE to 0·041 (95% CI 0·026, 0·058) for UCHL-L1 for CRASH. The R^2 for the IMPACT and CRASH models was 30·7% (23·5%, 37·7%) and 35·2% (28·8%, 41·8%) for mortality, and 22·6% (95% CI 15·6%, 29·1%) and 33·8% (28·4%, 39·7%) for unfavorable outcome. For mortality, adding all biomarkers increased the prognostic value with 14·6% R^2 (95% CI 8·6%, 20·6%) for IMPACT and 10·7% R^2 (6·4%, 15·2%) for CRASH, corresponding to a relative increase of 48% (14·6/30·7) for IMPACT and of 30% (10·7/35·2) for CRASH. For unfavorable outcome, adding all biomarkers increased model performance with 14·6% R^2 (95% CI 9·5%, 20·2%) for IMPACT and 11·6% R^2 (95% CI 7·8%, 15·8%) for CRASH, corresponding to a relative

increase of 65% (14·6/22·6) for IMPACT and 34% (11·6/33·8) for CRASH. Of single biomarkers, UCH-L1 had the greatest incremental value in R^2: 12·5% (95% CI 7·3%, 17·8%) when added to IMPACT, and 9·2% (95% CI 5·6%, 13·2%) when added to CRASH for predicting mortality.

Table 3: Change in discriminative ability (C-statistic) and R^2 of serum biomarkers compared to IMPACT core and CRASH basic models to predict mortality and unfavorable outcome six-months following traumatic brain injury.

	IMPACT core (Age, GCS motor, GCS pupils)					
	GCS ≤ 12 (N=737)					
	Mortality			Unfavorable outcome		
	C-statistic (95% CI)		R^2 (%) (95% CI)	C-statistic (95% CI)		R^2 (95% CI)
Reference model	0·877	(0·827, 0·924)	38·3% (19·9%, 54·4%)	0·836	(0·798, 0·877)	37·8% (21·3%, 52·3%)
Serum biomarkers	Delta C-statistic (95% CI)		Delta R^2 (%) (95% CI)	Delta C-statistic (95% CI)		Delta R^2 (95% CI)
S100B	0·026	(0·005, 0·047)	8·3% (-7·7%, 21·1%)	0·022	(0·002, 0·041)	6·2% (-4·9%, 15·0%)
UCH-L1	0·034	(0·009, 0·061)	10·4% (-15·8%, 28·4%)	0·041	(0·018, 0·064)	11·5% (-5·1%, 24·9%)
GFAP	0·023	(0·004, 0·041)	7·5% (-4·8%, 16·6%)	0·030	(0·010, 0·051)	8·6% (-2·5%, 17·9%)
NSE	0·013	(-0·001, 0·027)	4·5% (-5·7%, 12·0%)	0·010	(-0·003, 0·022)	3·0% (-3·4%, 7·9%)
NFL	0·019	(0·001, 0·036)	5·2% (-12·5%, 16·8%)	0·041	(0·020, 0·064)	10·6% (-9·4%, 26·7%)
T-tau	0·033	(0·009, 0·059)	10·6% (-11·1%, 27·4%)	0·038	(0·014, 0·062)	10·9% (-6·9%, 25·4%)
GFAP + UCH-L1	0·033	(0·008, 0·061)	10·5% (-14·8%, 28·7%)	0·040	(0·018, 0·064)	11·5% (-5·6%, 24·6%)
All	0·035	(0·008, 0·064)	11·6% (-20·4%, 35·4%)	0·051	(0·025, 0·077)	13·8% (-14·5%, 34·2%)
	CRASH basic (Age, GCS, GCS pupils, MEI)					
	GCS < 15 (N= 1083)					
	Mortality			Unfavorable outcome		

	C-statistic (95% CI)		R^2 (%) (95% CI)		C-statistic (95% CI)		R^2 (%) (95% CI)	
Reference model	0·890	(0·846, 0·935)	41·8%	(0·846, 0·935)	0·859	(0·824, 0·890)	43·0%	(22·2%, 61·0%)
Serum biomarkers	**Delta C-statistic (95% CI)**		**Delta R^2 (%) (95% CI)**		**Delta C-statistic (95% CI)**		**Delta R^2 (%) (95% CI)**	
S100B	0·021	(0·005, 0·036)	7·0%	(0·005, 0·036)	0·014	(0·002, 0·026)	4·7%	(-8·9%, 15·0%)
UCH-L1	0·025	(0·007, 0·044)	8·3%	(0·007, 0·044)	0·027	(0·013, 0·041)	8·4%	(-9·0%, 21·2%)
GFAP	0·016	(0·004, 0·029)	5·7%	(0·004, 0·029)	0·018	(0·007, 0·030)	5·8%	(-5·9%, 14·7%)
NSE	0·010	(-0·001, 0·021)	4·0%	(-0·001, 0·021)	0·007	(-0·002, 0·016)	2·6%	(-4·8%, 8·1%)
NFL	0·012	(0·000, 0·023)	3·5%	(0·000, 0·023)	0·026	(0·011, 0·040)	7·4%	(-13·5%, 23·7%)
T-tau	0·025	(0·007, 0·045	8·4%	(0·007, 0·045	0·026	(0·010, 0·040)	7·9%	(-8·6%, 22·4%)
GFAP + UCH-L1	0·025	(0·005, 0·045)	8·4%	(0·005, 0·045)	0·027	(0·012, 0·041)	8·2%	(-9·4%, 21·2%)
All	0·026	(0·006, 0·047)	9·4%	(0·006, 0·047)	0·034	(0·017, 0·050)	10·0%	(-19·4%, 31·8%)

Discussion

We examined the incremental prognostic value of serum biomarkers, independent of patient's demographic, clinical and radiological characteristics, for prediction of six-month GOSE following TBI. All examined serum biomarkers – UCH-L1, S100B, GFAP, NFL, t-tau, and NSE - obtained within 24h after injury, improved the prognostic value for functional outcome. We found that UCH-L1 had the greatest incremental prognostic value. Combining all six biomarkers resulted in small further increments in C-statistic and R^2, compared to the best performing individual biomarkers separately. Adding biomarkers to the IMPACT and CRASH models resulted in an R^2 up to 45% and 46% for mortality and 37% and 45% for unfavorable outcome, respectively.

Previous studies have reported associations between serum biomarker levels and functional outcome following TBI.[11, 15, 26] These studies typically focused on the unadjusted effect of biomarkers rather than estimating their value over and above known predictors of outcome following TBI. We showed that the addition of biomarkers can improve prognostication over and above demographic, clinical, and radiological characteristics. We also provide greater detail on the context-specific performance and potential clinical application of our findings.

We showed that the prognostic performance of individual biomarkers may vary with injury severity. NFL provided the greatest incremental prognostic value in patients after mTBI for predicting incomplete recovery, followed by S100B, UCH-L1 and T-tau. However, in moderate to severe TBI, the greatest incremental value was provided by UCH-L1 for predicting unfavorable outcome,[27] closely followed by T-Tau, NFL, and S100B. Future studies should further examine differences in prognostic value of serum biomarkers between patients following mild versus moderate/severe TBI. As S100B can also be present outside the central nervous system,[28] questions have been raised about the specificity of S100B as a biomarker in TBI, particularly in patients with extracranial injuries. However, our results suggest that S100B has added value for the prediction of functional outcome after TBI, relative to known predictors, including major extracranial injury.

The prognostic performance of individual biomarkers may not be concordant with their diagnostic utility. In a prior CENTER-TBI study of the incremental value of these six serum biomarkers for the prediction of CT abnormalities, GFAP outperformed the other markers.[23] This is consistent with other studies of the diagnostic performance of GFAP.[9, 29, 30] The association between biomarkers and imaging phenotypes was described in greater detail in a prior CENTER-TBI publication.[31] Lesion volume showed stronger associations with biomarkers than pathoanatomical type of injury. Overall, GFAP showed the highest value in all pathology groups. In contrast, in the current study, GFAP showed relatively little added value for the prediction of functional outcome following TBI. Our findings indicate that GFAP is more relevant for diagnostic purposes, and less so for predicting functional outcome following TBI. Different pathobiological roles, marker-specific features (e.g., kinetics, abundance, localization), and their link with distinct injury types and pathophysiological mechanisms could underlie these differences in performance. Accordingly, previous studies have demonstrated different GFAP and UCH-L1 release patterns as a result of different patterns of structural damage, which in turn imply different clinical relevance and ensuing outcomes.[15, 31, 32] Previously, UCHL-1, assessed over the first 5 days after injury, displayed the best discrimination for predicting outcome in univariate analysis, outperforming other known predictors.[33] On multivariable analysis, however, GFAP and NFL added most independent information to predict unfavorable outcome. The differential diagnostic and prognostic effects of biomarkers may have various explanations. First, UCHL-1 and NFL are neuronal markers, whilst GFAP is an astroglial marker. Conceptually a marker that reflects neuronal damage could be expected to be better correlated with outcome than an astroglial marker. Second, temporal trajectories may be relevant. Future research should focus on the validation of our findings and explaining differences in the diagnostic and prognostic value of different biomarkers. It has been suggested that a panel of biomarkers, based on a multi-marker approach, might improve prognostic accuracy.[17] We found that a multi-marker approach of all six biomarkers together indeed has most incremental prognostic value. However, the combination of GFAP with UCH-L1, which has been proposed as a useful combination for diagnosis of TBI,[9] did not improve discrimination of outcome when compared to UCH-L1 alone. Based on our findings, combinations of UCH-L1 with S100B, NFL and T-tau may provide better opportunities in future research for the prediction of functional outcome following TBI. However, when compared to the

best performing individual biomarkers, the incremental discrimination provided by combining the entire biomarker panel was relatively small. Consequently, the use of a single marker or a combination of two markers, might be preferred in clinical practice, especially in low- and middle-income countries and austere environments.

The improvement in prognostic value by combining biomarker data with conventional predictors of outcome may translate into clinical application. First, integrating biomarker data with established prognostic models for the prediction of death or unfavorable outcome, resulted in a relative increase in R^2 of 48%-65% for IMPACT and 30%-34% for CRASH, respectively. These models are widely used to stratify patients in clinical trials, and for benchmarking quality-of-care assessments. These improvements in prognostic value were for IMPACT core and CRASH basic models, when only age, initial injury severity (based on GCS, motor score and pupillary reactivity) and MEI were considered. Second, even when all demographic, clinical and radiological parameters were used, biomarkers were still able to provide incremental value not just for the GOSE overall, but also for mortality, unfavorable outcome, and incomplete recovery, which are relevant to clinicians and patients; Biomarkers resulted in R^2 up to 55% for mortality, 55% for unfavorable outcome, and 36% for incomplete recovery. These results make a strong case for integrating serum biomarker data when developing or updating prognostic models for functional outcome following TBI.

Strengths and limitations

Strengths of our study include the use of a longitudinal prospective international cohort study (the CENTER-TBI study), resulting in an unprecedented large number of patients following TBI with available serum biomarkers obtained within 24h. Our sample included 2283/4509 (51%) patients from the overall CENTER-TBI cohort. Baseline characteristics were largely similar to those previously described in the overall CENTER-TBI cohort.[23] The analyses were performed across all severities of TBI, including mostly patients following mTBI, which reflects contemporary clinical practice. Furthermore, the CENTER-TBI study includes a relatively high percentage of patients with traumatic abnormalities on CT, reflecting the type of patients seen in large trauma referral centres. To study generalizability, our findings should be further validated in new patients and settings. In contrast to prior studies of serum biomarkers in TBI, we adjusted for known predictors of outcome following TBI. Furthermore, we examined

the incremental value of six serum biomarkers that have been studied most extensively in recent studies, both in isolation and in combination (including the specific combination of GFAP and UCH-L1, thought to have specific diagnostic utility). Prior CENTER-TBI studies have examined and explained differences between men and women in outcome after TBI.[34, 35] Future studies should explore the relationship between serum biomarkers and differences in outcomes between men and women following TBI.

Several limitations of our study must be considered. Most patients were categorized as mTBI based on the GCS. However, predictors of outcome following mTBI are less well established than those for moderate and severe TBI. Most demographic, clinical and radiological characteristics included in our study are relevant to predict outcome in patients following moderate and severe TBI, but less so in patients following mTBI. Therefore, we also included MEI, level of education and pre-injury mental health problems, which are known predictors outcome in patients following mTBI. Differences were noted in predictor effects for patients following mild versus moderate and severe TBI. Second, in the CENTER-TBI study the time of biomarker sampling is widely varying and typically late (Mean 12.6 hours after injury). Serial sampling of serum biomarkers, including S100B and NSE, has revealed different temporal trajectories.[36] Future research should consider mixed model approaches for the prediction of functional outcome following TBI including repeated measures of serum biomarkers. Third, the Quanterix platform on which we measured four of the six biomarkers is a research-use only device, and this platform currently cannot be used in clinical practice. Robust clinical assay platforms are required before biomarkers can be broadly implemented into clinical practice for either diagnostic or prognostic purposes. The high coefficients of variation (CVs) reported for the assays performed on the Quanterix platform are of some concern. However, we consider that these high CVs would be more likely to dilute prognostic effects than to inflate these. S100B and NSE tests are available as clinical lab tests, and have been cleared in the US and Europe as cancer marker tests. Procedures for regulatory approval of assays for other biomarkers are ongoing. Recently, a point-of-care assay for UCH-L1/GFAP obtained FDA clearance in the US and CE mark by EMN/European Medicines Agency as in vitro diagnostic test for mTBI patients with suspected brain lesions. Fourth, we recognize that levels of some biomarkers (e.g. NSE) could be artificially elevated in haemolytic

samples, and that this may have contributed to the relatively low prognostic strength of NSE. As the current study aims to assess the incremental value of biomarkers in clincal practice, and haemolysis may sometimes occur despite strict procedures for sampling, pre-processing and processing of samples, we opted not to exclude haemolytic samples.

Conclusion

Serum biomarkers obtained within 24h after injury have incremental prognostic value relative to demographic, clinical and radiological characteristics in predicting functional outcome following mild, moderate and severe TBI. Our findings support the integration of biomarkers in established models for predicting outcome after TBI.

Authors' contributions

All authors certify that they have participated in the concept, design, analysis, writing, or revision of the manuscript. AB and EC conceived the original idea. DvK and AIRM supervised the project. IRARH and DvK analysed the data. All authors participated in the interpretation of results relevant to their domain of interest. IRARH, AIRM, and DM prepared the draft manuscript and coordinated its finalisation. All authors approved the final manuscript. IRARH and DvK verified the underlying data, all main authors had full access to study data and IRARH, DvK and AIRM had final responsibility for the decision to publish

Conflicts of interest

The authors declare that there is no conflict of interest.

Data sharing

Individual participant data will be available immediately following publication, conditional to approved study proposal, with no end date. Data will be available to researchers who provide a methodologically sound study proposal that is approved by the management committee to achieve the aims in the approved proposal. Proposals can be submitted online at https://www.center-tbi.eu/data. A data access agreement is required and all access must comply with regulatory restrictions imposed on the original study.

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Supplementary material

Supplementary material is available at:
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