Prognostic value of serum biomarkers in patients with moderate-severe traumatic brain injury, differentiated by Marshall computer tomography classification

Prognostic value of serum biomarkers in patients with moderate-severe traumatic brain injury, differentiated by Marshall CT classification

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Abstract

Prognostication is challenging in traumatic brain injury (TBI) patients in whom the CT fails to fully explain a low level of consciousness. Serum biomarkers reflect the extent of structural damage in a different way than CT does, but it is unclear if biomarkers provide additional prognostic value across the range of CT abnormalities. This study aimed to determine the added predictive value of biomarkers, differentiated by imaging severity.

This prognostic study used data from the Collaborative European NeuroTrauma Effectiveness Research in Traumatic Brain Injury (CENTER-TBI) study (2014-2017). The analysis included patients aged ≥16 years with a moderate-severe TBI (Glasgow Coma Scale, GCS < 13) who had an acute CT and serum biomarkers obtained ≤24h of injury. Out of six protein biomarkers (GFAP, NFL, NSE, S100B, Tau, UCH-L1) the most prognostic panel was selected using lasso regression. The performance of established prognostic models (CRASH and IMPACT) was assessed before and after the addition of the biomarker panel, and compared between patients with different CT Marshall scores (Marshall score <3 versus Marshall score ≥3). Outcome was assessed at 6 months post-injury using the extended Glasgow Outcome Scale (GOSE), and dichotomized into favorable and unfavourable (GOSE <5).

We included 872 patients with moderate-severe TBI. The mean age was 47 years (range 16 - 95), 647 (74%) were male and 438 (50%) had a Marshall CT score <3. The serum biomarkers GFAP, NFL, S100B and UCH-L1 provided complementary prognostic information, NSE and Tau showed no added value. The addition of the biomarker panel to established prognostic models increased the area under the curve (AUC) by 0.08 and 0.03, and the explained variation in outcome by 13-14% and 7-

8%, for patients with a Marshall score of <3 and ≥3, respectively. The incremental AUC of biomarkers for individual models was significantly greater when the Marshall score was <3 compared to ≥3 ($p < 0.001$).

Serum biomarkers improve outcome prediction after moderate-severe TBI across the range of imaging severities and especially in patients with a Marshall score <3.

traumatic brain injury, prospective study, CT scanning, biomarkers, adult brain injury

Introduction

Traumatic brain injury (TBI) is estimated to affect 1 in 2 people, account for 1 in 3 injury-related deaths and consume approximately 0.5% of the annual economic output worldwide.[1] The Glasgow Coma Scale (GCS) is used to grade the initial injury severity, with a GCS <13 classified as moderate-severe TBI.[2] The detection of a low GCS usually prompts a computed tomography (CT) scan to look for a mass lesion or signs of raised intracranial pressure which would explain the low conscious level. When the CT does not show any of these (CT Marshall score <3)[3] the low GCS may pose a diagnostic challenge.[4] In some of these patients, the low GCS may be caused by alcohol, drugs, or early seizures and improves when these effects resolve. In others, prognosis-defining traumatic axonal injury may be present but missed by CT imaging, so that the CT is falsely reassuring.[5] A more sensitive measure than CT for the degree of brain damage is thus needed.

Being able to quantify the degree of brain damage more sensitively, is critical to predict clinical course and outcome, to guide communication with families and inform treatment decisions. For example, in patients with substantial brain injury the clinician could delay sedation holds or non-emergent surgical procedures to prioritise neuroprotection.

Serum protein biomarkers, especially glial fibrillary acidic protein, have been shown to be more sensitive than CT for the detection of traumatic (axonal) injury defined on magnetic resonance

imaging, at least in mild TBI.[6,7] However, it is unclear if the additional injury detected by serum biomarkers is prognostically relevant. For example, the impact of axonal injury on outcome is driven by its location, rather than its volume.[8] Serum biomarkers however correlate with total lesion burden, not with lesion type or location.[9] So it is possible that biomarkers become elevated equally for prognostically relevant and irrelevant injury, in which case they would not enhance outcome prediction and may even confound it.

Two independent multi-center studies have shown a significant benefit when serum biomarkers were added to the established prognostic models, IMPACT (International Mission for Prognosis and Analysis of Clinical Trials in TBI) and CRASH (Corticosteroid Randomisation after Significant Head Injury) in the general TBI population.[10,11] However, it is unclear whether these findings generalize across the range of imaging severity, quantified by the Marshall CT classification.

We therefore aimed to determine the added predictive value of biomarkers, differentiated by imaging severity as quantified by the Marshall CT classification.

Methods

Data collection

This study was conducted and reported in accordance with the TRIPOD statement.[12] Patients were selected from the prospective multi-centre Collaborative European NeuroTrauma Effectiveness Research in Traumatic Brain Injury (CENTER-TBI) study, which recruited from December 19, 2014, to December 17, 2017.[13] Ethical approval was obtained by each site in accordance with local regulations and details may be found at https://www.center-tbi.eu/project/ethical-approval. Whilst the patient lacked capacity assent was given by their next of kin and consent sought when the patient regained capacity. Clinical data was accessed via the Neurobot platform (RRID/SCR_017004,

core data, version 3.0; International Neuroinformatics Coordinating Facility; released November 24, 2020).

The present analysis included patients aged ≥16 years with a moderate-severe TBI (GCS < 13), in whom serum biomarkers and CT images were obtained within 24h of injury. The GCS refers to the best score recorded after resuscitation in the emergency department if available, otherwise the best recorded pre-hospital score was used. CT images were acquired using local site protocols and reported by central reviewers blinded to outcome, using the common data elements.[14] Patients were split into two groups, based on whether the CT showed evidence of mass lesion/ raised intracranial pressure (Marshall score ≥3) or not (Marshall <3).[3]

Outcome was assessed using the extended Glasgow Outcome Scale[15] (GOSE) at six months post-injury, by investigators blinded to biomarker levels. GOSE was dichotomized into favorable versus unfavourable outcome (GOSE <5) in line with established prognostic models.[10,11]

Serum biomarkers included glial fibrillary acidic protein (GFAP), neurofilament light (NFL), neuron-specific enolase (NSE), S100 calcium-binding protein B (S100B), total tau (Tau) and ubiquitin carboxy-terminal hydrolase L1 (UCH-L1). Samples were stored at -80°C, transported and quality controlled as previously described.[16] GFAP, NFL, Tau and UCH-L1 were analysed with a Single Molecule Arrays (SiMoA) based assay on the SR-X benchtop assay platform (Quanterix Corp., Lexington, MA) at University of Florida, USA.[16] NSE and S100B were quantified using an electrochemiluminescence immunoassay kit (ECLIA) on the Cobas 8000 modular analyser (Roche Diagnostics, Mannheim, Germany) at the University of Pecs.[16] The detectable ranges in pg/ml were as follows: GFAP 1.32–40,000; NFL 0.0971–2000, Tau 0.0231–400 and UCH-L1 1.34–40,000. Values exceeding the upper level of detection were handled by stepwise dilution until in range. No samples in our cohort had values below the lower level of detection.

Statistical analysis

Statistical analysis was conducted in R 4.2.0 (R Project for Statistical Computing).. Unless otherwise indicated, data is presented as mean (95% confidence interval). Statistical tests were two-tailed and p-values were considered significant if <0.05 after adjustment for multiple comparisons using the Benjamini-Hochberg method (applied within each results table or figure).[17]

Adjusting biomarkers for time of sampling

The median time to sampling was 14h in both patient groups (Marshall score <3 and ≥3). However, the authors were concerned about a theoretical risk of unmeasured confounders which might relate to both sampling time and outcome. For example, prolonged pre-hospital extrication might lead to later biomarker sampling, past the peak timing of a biomarker and may result in a falsely low biomarker concentration. For the main analysis biomarker concentrations were therefore adjusted as if they had all been sampled at 14h post-injury exactly (**Supplementary methods**). In brief, for each biomarker the average time trajectory was estimated. Imagine a patient's S100B was taken at 8h instead of 14h and measured 0.2ng/ml. Assume further that S100B is estimated to rise by x ng/ml between 8h and 14h in a patient with similar clinical features. The patient's adjusted S100B concentration would then be 0.2 + x ng/ml. As a sensitivity analysis we also repeated the main analysis with raw instead of adjusted biomarker concentrations.

Missing data handling

Missing data ranged between 0-10% per variable, with most variables missing <5% of data (**Table 1**). Missing data were handled under the missing at random assumption using multiple imputation by chained equations as implemented in the mice package.[18] Ten imputed datasets were generated and results pooled using Rubin's rules.[19] **Supplemental Figure 1** illustrates how multiple imputation was integrated with model derivation and testing.

Selecting the most prognostic protein biomarkers

Using a smaller panel of proteins, provided they contain the same amount of prognostic information, will be cheaper when applied in clinical practice. We therefore used lasso regression to identify and include only the most predictive proteins in the final prognostic model.[20] Lasso regression uses a tuning parameter or penalty factor lambda to reduce the model coefficients for all biomarkers in the panel; the higher the chosen lambda, the smaller the model coefficients. For some biomarkers the model coefficient will so be reduced to zero, which effectively excludes them from the panel. Within each imputed dataset 10x10 cross-validation was used to identify the magnitude of the tuning parameter lambda that would minimize the mean prediction error. Imputation sets were then stacked and a lasso model applied using the mean lambda from 10 imputed datasets. To avoid inflating the sample size by a factor of 10 after having stacked the imputation sets each observation was weighted as 0.1.[21] All data were scaled and centered to produce standardized coefficients (without bootstrapping) so that they could be compared across biomarkers measured on different scales and between patient groups (Marshall score <3 versus Marshall score ≥3). These coefficients were used to calculate a summary biomarker score for each patient (i.e., a summary measure of all biomarkers weighted by their relative prognostic value within the panel), which was used in subsequent prognostic models.

Note that the proteins selected for the final panel are not necessarily those with the highest prognostic ability when used in isolation. For example, it might be that S100B is the best biomarker, so the lasso model will select that one for its panel. It might further be that GFAP is the second-best marker, individually. However, if GFAP provides some information already obtained from S100B (e.g., the degree of astroglial injury), then the lasso model will prioritise a different biomarker, such as NFL, as the next most important panel member, even if individually NFL was not as prognostic as GFAP. We therefore also undertook a secondary analysis using individual biomarkers only.

Deriving prognostic models

We chose two established prognostic models as a reference benchmark: the corticosteroid randomisation after significant head injury (CRASH-CT) model and International Mission for Prognosis and Analysis of Clinical Trials in TBI (IMPACT-CT) model.[10,11] Four logistic regression models were defined: CRASH-CT with and without proteins and IMPACT-CT with and without proteins. The CRASH-CT model contained the variables age, GCS, pupil reactivity and major extra cranial injury plus the following CT features: presence of petechial haemorrhages, obliteration of the third ventricle or basal cistern, subarachnoid hemorrhage (SAH), midline shift and a non-evacuated haematoma.[10] The IMPACT-CT model contained the variables age, motor score, pupil reactivity, hypoxia, hypotension, Marshall CT score, SAH and epidural hematoma.[11] We fitted the models to the study population with and without the biomarker score, i.e. the variables are the same as in the original models (± biomarker score) but the coefficients are not. This is to provide a fair assessment of the value of adding biomarkers and is in line with previous publications on this subject.[22,23]

Testing model performance

Model performance was assessed in the domains of discrimination (the ability to distinguish between patients with and without the outcome of interest), calibration (the agreement between predicted risk and observed outcome prevalence) and the overall model fit.[24] Discrimination was assessed using the area under the receiver operating characteristic curve (AUC), where an AUC = 1 indicates a perfect model and an AUC = 0.5 a model that is no better than chance.[25] To assess calibration we fitted a Cox calibration regression plot with an intercept and a slope.[26] An intercept = 0 and a slope = 1 indicate perfect calibration.[26] Overall model fit was assessed using the Nagelkerke R^2 which captures the percentage of the variation in outcome explained by the model.[27] Two nested models (the same model with versus without protein biomarkers) were compared using a likelihood ratio test.[28]

The performance of prediction models is overestimated when models are tested on the same patient population that they were derived on.[29] To provide realistic estimates of model performance we corrected the aforementioned metrics of discrimination, calibration and overall fit using the bootstrapping technique recommended by Steyerberg et al.,[29] using 1000 bootstrap samples.

The incremental value that biomarkers add to established models was compared between patient groups (Marshall score <3 versus Marshall score ≥3) using a t-test. The incremental value was measured both as the incremental R^2 and, as recommended by Snell et. al, the incremental logit(AUC).[30]

To understand the drivers of the incremental value we also checked for an interaction between patient group and predictions made by the established models, and between patient group and the biomarker score.

Sensitivity analysis

As a sensitivity analysis we tested for any differences between subgroups. Patients with a Marshall score <3 where split into those with CT-occult injury (Marshall score = 1) versus those with a Marshall score of 2. Patients with a Marshall score ≥3 where split into those with only diffuse injury (Marshall score 3 and 4) versus those with mass lesions (Marshall score 5 and 6). We accepted that the smaller sample sizes (e.g., N = 73 for Marshall score = 1) rendered this analysis purely exploratory.

Previous studies have suggested that the performance of biomarkers may be reduced in the elderly,[31,32] and so a subgroup analysis was performed in patients aged 65 years and above. Given biomarker levels can also be elevated by extra-cranial injuries[33] we conducted a sub-group analysis of patients with isolated TBI.

Data and code availability

The CENTER-TBI investigators are committed to data sharing to advance TBI research. Researchers can request access to de-identified patient data by submitting a proposal at https://www.center-tbi.eu/data to the CENTER-TBI Management Committee. Researcher will need to sign a data sharing agreement and adhere to the regulatory restrictions of the original CENTER-TBI study.

The statistical code is freely available at https://github.com/DrSophieRichter/BioPred

Results

Participants

Inclusion criteria were met by 872 patients with moderate-severe TBI (**Supplemental Figure 2**). Their mean age was 47 years (range 16 - 95), 647 (74%) were male and 771 (88%) were intubated. In 438 patients (50%) the Marshall score was <3. Compared to patients with a Marshall score ≥3, these patients tended to be younger, with more severe extra-cranial injuries and better pupil reactivity; their CT more often showed evidence of axonal injury and less often showed brain swelling or extra-axial injuries (**Table 1**). Only 143 (33%) patients with a Marshall score <3 had an unfavourable outcome compared to 262 (60%) with a Marshall score ≥3. Patients with an unfavourable outcome had higher median biomarker concentrations than patients with favourable outcomes (**Table 2**).

Selecting the biomarker panel for outcome prediction

The four biomarkers GFAP, NFL, S100B and UCH-L1 all added complementary prognostic value to each other (**Table 3**). In contrast, NSE and Tau added no prognostic value if these four markers were already present. The biomarker score used in subsequent prognostic models was a summary measure of all biomarkers weighted by their relative prognostic value within the panel (i.e., their lasso coefficient).

Looking at the biomarker score in isolation (not as an addition to established models), there was no significant difference between patients with Marshall scores <3 versus ≥3 (**Supplemental Tables S1 and S2**).

Comparing the incremental value of biomarkers between patient groups

Established models without the biomarker score had lower AUCs and lower R^2 in patients with a Marshall score <3 compared to those with a Marshall score ≥3 (**Table 4**). When tested using an interaction term however, this difference did not reach statistical significance (**Supplemental Table S2**).

The addition of the biomarker score resulted in a statistically significant improvement in measures of discrimination and model fit in both patient groups (**Table 4**, **Supplemental Figures S3-6**).

The incremental value of biomarkers was significantly greater for patients with a Marshall score <3 compared to those with a Marshall score ≥3 for both models (**Figures 1-2**). The panel outperformed individual biomarkers, at least for Marshall scores <3. The best performing individual marker was S100B, followed by UCH-L1 (**Figures 1-2**).

Model coefficients for all models are provided in **Supplemental Tables S3-S4**.

Sensitivity analysis

There was no difference in the incremental value of biomarkers between Marshall score 1 versus Marshall score 2 patients, nor between Marshall score 3-4 vs Marshall score 5-6 patients (**Supplemental Figure S7)**. However, the incremental value was higher in Marshall score 2 patients compared to Marshall score 3-4 patients, when quantified using the AUC (CRASH-CT and IMPACT-CT models) and the variation explained (only IMPACT-CT model).

Biomarkers also provided incremental benefits in patients aged ≥ 65 years (**Supplemental table S5**) and to patients with isolated TBI (**Supplemental table S6**).

Repeating the analysis with raw biomarker concentrations, unadjusted for sampling time, yielded almost identical results to the main analysis (**Supplemental Tables S7-S8, Supplemental Figures S8-S9**).

Discussion

This study assessed the prognostic value of serum biomarkers in traumatic brain injury patients with a GCS <13 with or without signs of mass lesion or raised intracranial pressure on CT. Prior to this study biomarkers were known to be a more sensitive marker of brain injury than CT, but it was unclear whether the additional detected brain damage would be prognostically relevant across the range of imaging severities. Our findings show that serum biomarkers not only improve established models in those patients with a Marshall score <3, but do so to a greater degree than in patients with higher Marshall scores.

The first step in serum biomarker-based outcome prediction is to choose the most relevant biomarker(s). Using a panel rather than individual biomarkers may be preferable given the varied cellular origin and pathological processes leading to the release of specific proteins.[34] This however needs to be balanced against the risk of redundancy, which reduces model performance and increases cost. To the authors' knowledge this is the first study that employed a data-driven approach to select the optimal panel. From our data we learned that most of the prognostic information could be captured in just two markers: S100B, an astroglial marker also elevated after skeletal injury, and NFL, derived from myelinated sub-cortical axons.[35] Together they may provide a summary measure of the burden of extra- and intra-cranial injury. Since some complementary information was added by GFAP and UCH-L1 we included those markers in our prognostic model. Whether the added information of these two markers is worth the extra cost would need to be assessed in a formal health economics analysis. Interestingly, NSE and Tau provided no additional value. If single biomarkers rather than a panel are being used, then S100B is the most prognostic marker. UCH-L1, rather than NFL, takes the second place as it is not being penalized for providing

information that is already obtained via S100B. This conclusion will be relevant to both researchers and developers of assay platforms.

Previous studies in moderate-severe TBI reported AUCs ranging from 0.66-0.92 and 0.66-1.00 for IMPACT and CRASH models, respectively.[36] The AUCs of the IMPACT-CT model (before the addition of biomarkers) in our study were 0.72 and 0.78 in patients with Marshall scores <3 and ≥3, respectively. The AUCs of the CRASH-CT model without biomarkers in our study were 0.73 and 0.79 in patients with Marshall scores <3 and ≥3, respectively. Testing for the difference in model performance using an interaction term only showed an insignificant trend towards poorer performance in patients with a Marshall score <3. Overall, this suggests that model performance may be slightly worse in patients with a Marshall score <3, which may reflect the potentially conflicting information that CT and GCS provide to the models in such patients.

Serum protein biomarkers were able to significantly improve the performance of established prognostic models in our study. A previous study also conducted on the CENTER-TBI cohort but including a large proportion of mild TBI patients, reported improvements in the AUC by 0.05-0.08 (all 6 biomarkers) or 0.04-0.07 (GFAP + UCH-L1) and improvements in the explained variance by 12-15% (all 6 biomarkers) or 10-13% (GFAP + UCH-L1) when biomarkers were added to the IMPACT-Core and CRASH-Core models.[23] A similar study conducted in the US-based TRACK-TBI cohort recorded improvements of 0.05 for the AUC and 12% for the explained variance when adding UCH-L1 and GFAP to the IMPACT-CT model.[22] These values agree with those observed in our study, where the AUC increased by 0.08 and 0.03, and the variation explained by 13-14% and 7-8%, for patients with a Marshall score of <3 and ≥3, respectively. Importantly, we showed that an incremental value of serum biomarkers for AUC and R^2 was present across all imaging severities and was greater for those with Marshall scores <3.

Our sensitivity analysis showed no differences in the incremental value of biomarkers between Marshall scores 1 versus 2, or between Marshall scores 3-4 versus 5-6, but did show differences

between the Marshall score 2 and the Marshall score 3-4 group. This supports our a priori dichotomization at Marshall ≥3.

Limitations

First, our sample size was limited to 872 patients compared to the original derivation cohorts for CRASH-CT (n = 10,008) and IMPACT-CT (n = 8,509).[10,11] However, we believe that the multi-center multi-national design of our study and the use of bootstrapping for optimism correction, mean that our results will still generalize to future patients. Second, the Quanterix assay kits used in our study have not yet been licenced for use in clinical practice. Another platform is already approved for GFAP and UCHL-1 in the context of CT triage, and others are likely to be developed.[37] Further studies are needed to understand how to cross-calibrate and translate results between assay platforms. Finally, we only tested six protein biomarkers. It is possible that the prognostic value of the protein panel could be enhanced by yet another class of proteins (e.g. the oligodendrocyte marker myelin basic protein), by proteins extracted from extra-cellular vesicles (which facilitates blood brain barrier transit and prevents their degradation) or by non-protein markers such as microRNAs or metabolomics.[35,38,39]

Conclusion

Serum biomarkers improve outcome prediction after moderate-severe TBI across the range of imaging severities and especially in patients with a Marshall score <3.

Transparency, Rigor and Reproducibility Summary

The study was pre-registered at clinicaltrials.gov (NCT02210221). The analysis plan was not formally pre-registered, but the team members with primary responsibility for the analysis (lead author and senior author) certify that the analysis plan was pre-specified. The sample size of 434 and 438 patients per group was not pre-planned but based on the number of patients meeting inclusion criteria. 4509 CENTER-TBI participants were screened and 872 fulfilled inclusion criteria (Supplemental figure S2). Participants were blinded to biomarker results throughout the study, even after primary clinical observations were complete. Handling of biofluid samples and analysis was performed by team members blinded to relevant characteristics of the participants. Samples were analyzed in a single round of experiments with the same batch of reagents. Quantitative test-retest reproducibility using the same participants assessed repeatedly showed a coefficient of variation of 7% for S100B and NSE, and 22-30% for GFAP, UCH-L1, NFL and Tau.[16] All equipment and analytical reagents used to perform measurements on the fluid biomarkers are widely available from Roche Diagnostics, Mannheim, Germany and Quanterix Corp., Lexington, MA, respectively. The key inclusion criteria and outcome evaluations are established standards. Missing data has been handled using multiple imputation, as reported in the text. Correction for multiple comparisons was performed using the Benjamini-Hochberg method. This report includes documentation of internal validation using bootstrapping. Deidentified CENTER-TBI data, including the subset used for this study, will be available to researchers who provide a methodologically sound study proposal for review and approval by the Management Committee (submitted online at: https://www.center-tbi.eu/data).

Researchers will need to sign a data sharing agreement and adhere to the regulatory restrictions of the original CENTER-TBI study. Analytic code used to conduct the analyses presented in this study are available in a public code repository https://github.com/DrSophieRichter/BioPred. This paper

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Competing interests

DM received personal fees from Lantmannen AB, GlaxoSmithKline plc, Calico Life Sciences LLC, PresSura Neuro, Integra Neurosciences, and NeuroTrauma Sciences, LLC; grants from GlaxoSmithKline plc; and a shared National Institutes of Health grant from Gryphon Collaborators on a grant application outside the presented work. VFJN holds grants from Roche Pharmaceuticals for an analysis outside the presented work. AIRM declares personal fees from NeuroTrauma Sciences and Novartis and participated on the DSMB of PresSura Neuro during the conduct of the study

Author contributions

Virginia Newcombe had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

- **Concept and design:** Menon, Newcombe, Richter
- **Acquisition, analysis, or interpretation of data:** all authors
- **Drafting of the manuscript:** Richter
- **Critical revision of the manuscript for important intellectual content:** all authors
- **Statistical analysis:** Richter, Steyerberg
- **Obtained funding:** Menon, Maas
- **Administrative, technical, or material support:** Verheyden
- **Supervision:** Menon, Newcombe

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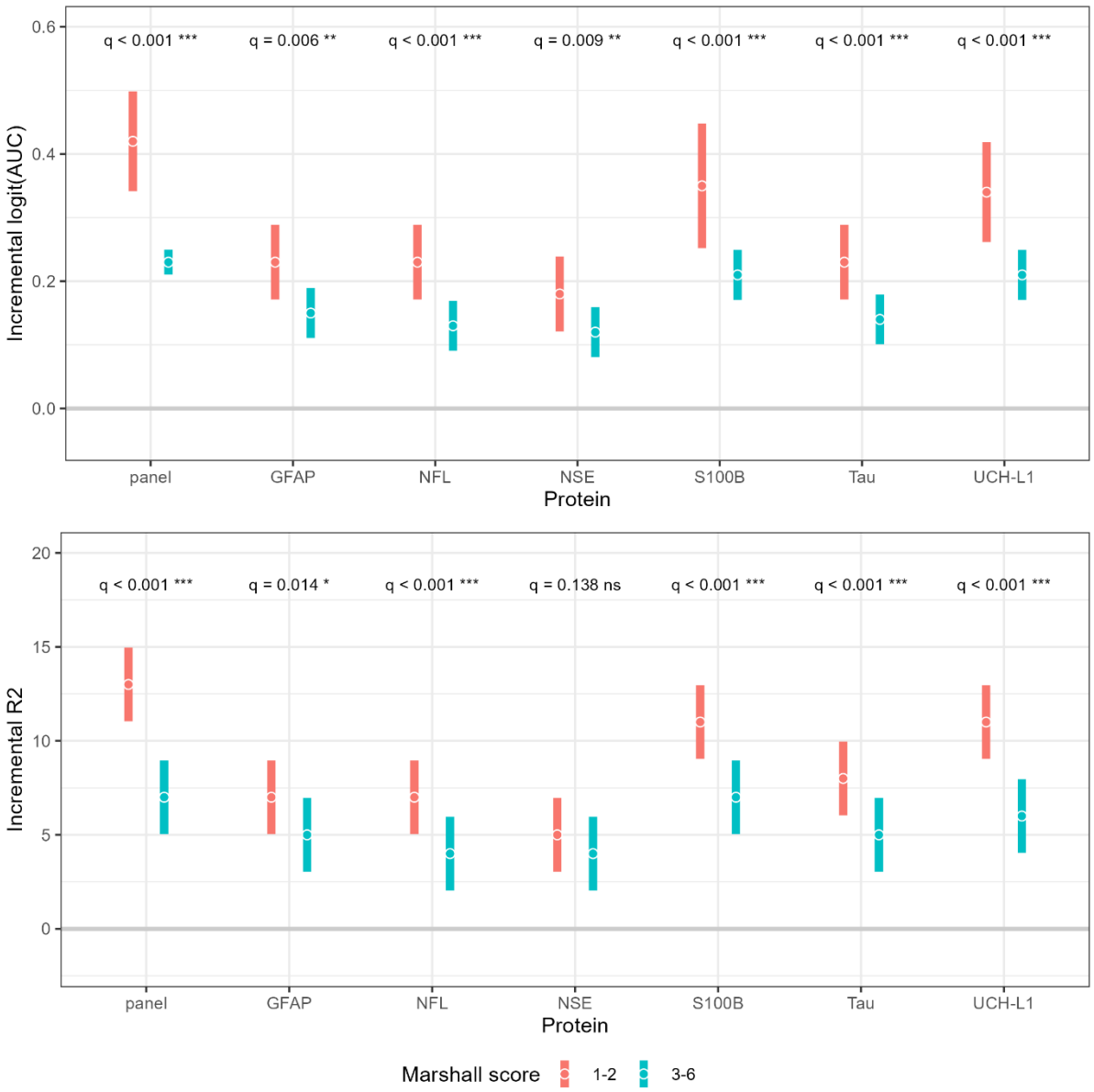
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0.6
0.4
0.2
0.0
q < 0.001 ***
q = 0.006 **
q < 0.001 ***
q = 0.009 **
q < 0.001 ***
q < 0.001 ***
q < 0.001 ***
panel
GFAP
NFL
NSE
S100B
Tau
UCH-L1
Protein
Incremental R2
20
15
10
5
0
q < 0.001 ***
q = 0.014 *
q < 0.001 ***
q = 0.138 ns
q < 0.001 ***
q < 0.001 ***
q < 0.001 ***
panel
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NSE
S100B
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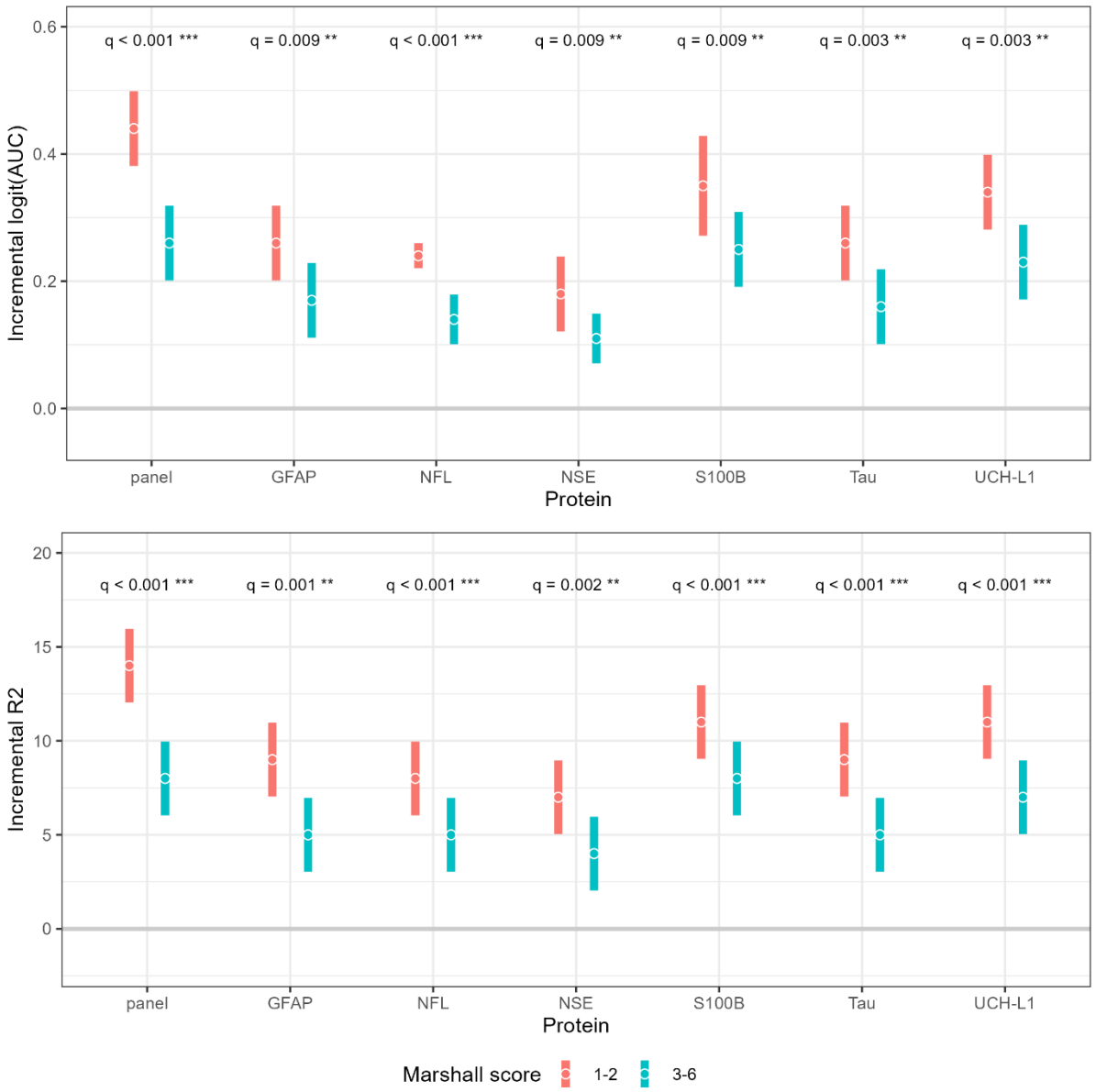
UCH-L1
Protein
Marshall score
1-2
3-6

Figure 1.


0.6
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Incremental logit(AUC)
q < 0.001 ***
q = 0.009 **
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q = 0.009 **
q = 0.003 **
q = 0.003 **
panel
GFAP
NFL
NSE
S100B
Tau
UCH-L1
Protein
20
15
10
5
0
Incremental R2
q < 0.001 ***
q = 0.001 **
q < 0.001 ***
q = 0.002 **
q < 0.001 ***
q < 0.001 ***
q < 0.001 ***
panel
GFAP
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NSE
S100B
Tau
UCH-L1
Protein
Marshall score
1-2
3-6

Figure 2.

Figure legends

Figure 1. Comparison of the incremental value of serum biomarkers between patient groups – CRASH-CT model. The incremental value of adding serum biomarkers to established prognostic models (CRASH-CT and IMPACT-CT) was compared between patients with a Marshall score <3 and those with a Marshall score ≥3, using a t-test. Dot and bar plots show means and 95% confidence intervals. Estimates were down-corrected for optimism via bootstrapping. q denotes the p-value corrected for multiple comparisons. ns = not significant, */**/** indicate q <0.05/ <0.01/ <0.001. AUC = area under the curve. The logit transformation ensures a fair comparison across models which had different baseline AUCs prior to the addition of serum biomarkers. R2 = Nagelkerke R^2 expressed as the percentage of the variation in outcome explained by the model.

Figure 2. Comparison of the incremental value of serum biomarkers between patient groups – IMPACT-CT model. The incremental value of adding serum biomarkers to established prognostic models (CRASH-CT and IMPACT-CT) was compared between patients with a Marshall score <3 and those with a Marshall score ≥3, using a t-test. Dot and bar plots show means and 95% confidence intervals. Estimates were down-corrected for optimism via bootstrapping. q denotes the p-value corrected for multiple comparisons. ns = not significant, */**/** indicate q <0.05/ <0.01/ <0.001. AUC = area under the curve. The logit transformation ensures a fair comparison across models which had different baseline AUCs prior to the addition of serum biomarkers. R2 = Nagelkerke R^2 expressed as the percentage of the variation in outcome explained by the model.

	Marshall ≥3 (N=434)	Marshall <3 (N=438)	Overall (N=872)
Age (years)[C, I]			
Median (Q1-Q3)	53 (36 - 68)	42 (25 - 58)	47 (29 - 64)
Age, dichotomized (years)[S]			
<65	295 (68 %)	367 (84 %)	662 (76 %)
>=65	139 (32 %)	71 (16 %)	210 (24 %)
Sex[D]			
F	116 (27 %)	109 (25 %)	225 (26 %)
M	318 (73 %)	329 (75 %)	647 (74 %)
Care path[D]			
not admitted	0 (0 %)	2 (0 %)	2 (0 %)
ward	13 (3 %)	22 (5 %)	35 (4 %)
ICU (self-ventilating)	26 (6 %)	34 (8 %)	60 (7 %)
ICU (intubated)	394 (91 %)	377 (86 %)	771 (88 %)
ICU (airway unknown)	1 (0 %)	3 (1 %)	4 (0 %)
Major extra-cranial injury[C]			
absent	250 (58 %)	194 (44 %)	444 (51 %)
present	184 (42 %)	244 (56 %)	428 (49 %)
Isolated TBI[S]			
isolated	188 (43 %)	128 (29 %)	316 (36 %)
not isolated	246 (57 %)	310 (71 %)	556 (64 %)
Glasgow Coma Scale[C]			
12	31 (7 %)	25 (6 %)	56 (6 %)
11	22 (5 %)	39 (9 %)	61 (7 %)
10	27 (6 %)	40 (9 %)	67 (8 %)
9	24 (6 %)	20 (5 %)	44 (5 %)
8	36 (8 %)	42 (10 %)	78 (9 %)
7	40 (9 %)	47 (11 %)	87 (10 %)
6	30 (7 %)	40 (9 %)	70 (8 %)
5	17 (4 %)	18 (4 %)	35 (4 %)
4	33 (8 %)	30 (7 %)	63 (7 %)
3	174 (40 %)	137 (31 %)	311 (36 %)
Motor score[I]			
6	32 (7 %)	40 (9 %)	72 (8 %)
5	97 (22 %)	132 (30 %)	229 (26 %)
4	44 (10 %)	50 (11 %)	94 (11 %)
3	20 (5 %)	23 (5 %)	43 (5 %)
2	26 (6 %)	19 (4 %)	45 (5 %)
1	214 (49 %)	174 (40 %)	388 (44 %)
Missing	1 (0.2%)	0 (0%)	1 (0.1%)
Reactive pupils[C, I]			
2	273 (63 %)	359 (82 %)	632 (72 %)
1	43 (10 %)	29 (7 %)	72 (8 %)
0	97 (22 %)	38 (9 %)	135 (15 %)
Missing	21 (4.8%)	12 (2.7%)	33 (3.8%)
Hypoxia[I]			
absent	343 (79 %)	332 (76 %)	675 (77 %)
present or suspected	59 (14 %)	85 (19 %)	144 (17 %)

	Marshall ≥3 (N=434)	Marshall <3 (N=438)	Overall (N=872)
Missing	32 (7.4%)	21 (4.8%)	53 (6.1%)
Hypotension[I]			
absent	348 (80 %)	335 (76 %)	683 (78 %)
present or suspected	55 (13 %)	80 (18 %)	135 (15 %)
Missing	31 (7.1%)	23 (5.3%)	54 (6.2%)
Time to blood protein sample (hours)[D]			
Median (Q1-Q3)	14 (8.4 - 19)	14 (7.7 - 19.4)	14 (8 - 19.1)
Marshall CT score[I, S]			
1	0 (0 %)	73 (17 %)	73 (8 %)
2	0 (0 %)	365 (83 %)	365 (42 %)
3	85 (20 %)	0 (0 %)	85 (10 %)
4	17 (4 %)	0 (0 %)	17 (2 %)
5 or 6	332 (76 %)	0 (0 %)	332 (38 %)
Petechial haemorrhage[C]			
absent	359 (83 %)	299 (68 %)	658 (75 %)
present	55 (13 %)	118 (27 %)	173 (20 %)
Missing	20 (4.6%)	21 (4.8%)	41 (4.7%)
Cisternal compression[C]			
absent	98 (23 %)	411 (94 %)	509 (58 %)
present	316 (73 %)	6 (1 %)	322 (37 %)
Missing	20 (4.6%)	21 (4.8%)	41 (4.7%)
Midline shift[C]			
absent	185 (43 %)	414 (95 %)	599 (69 %)
present	229 (53 %)	3 (1 %)	232 (27 %)
Missing	20 (4.6%)	21 (4.8%)	41 (4.7%)
SAH[C, I]			
absent	46 (11 %)	139 (32 %)	185 (21 %)
present	368 (85 %)	278 (63 %)	646 (74 %)
Missing	20 (4.6%)	21 (4.8%)	41 (4.7%)
EDH[I]			
absent	315 (73 %)	367 (84 %)	682 (78 %)
present	99 (23 %)	50 (11 %)	149 (17 %)
Missing	20 (4.6%)	21 (4.8%)	41 (4.7%)
Haematoma[C]			
absent	47 (11 %)	262 (60 %)	309 (35 %)
present	367 (85 %)	155 (35 %)	522 (60 %)
Missing	20 (4.6%)	21 (4.8%)	41 (4.7%)
WLST[D]			
not withdrawn	341 (79 %)	417 (95 %)	758 (87 %)
withdrawn	93 (21 %)	21 (5 %)	114 (13 %)
GOSE at 6 months[D]			
1	159 (37 %)	43 (10 %)	202 (23 %)
2 or 3	83 (19 %)	66 (15 %)	149 (17 %)
4	20 (5 %)	34 (8 %)	54 (6 %)
5	53 (12 %)	59 (13 %)	112 (13 %)
6	25 (6 %)	63 (14 %)	88 (10 %)
7	29 (7 %)	55 (13 %)	84 (10 %)
8	24 (6 %)	70 (16 %)	94 (11 %)
Missing	41 (9.4%)	48 (11.0%)	89 (10.2%)

Table 1. Patient characteristics. Values are given as mean (first quartile – third quartile) or frequency (percent). ICU = intensive care unit, CT = computed tomography, Cisternal compression = obliteration of the third ventricle or basal cistern, SAH = subarachnoid haemorrhage, EDH = epidural haematoma, Haematoma = Non-evacuated haematoma, WLST = Withdrawal of life-sustaining treatment, GOSE = extended Glasgow Outcome Score. Superscripts: C = variable included in CRASH_CT model, I = variable included in IMPACT_CT model, S = variable used for subgroup-analysis, D = descriptor of patient cohort not used in modelling

	Marshall ≥3		Marshall <3	
	fav (N=131)	unfav (N=262)	fav (N=247)	unfav (N=143)
GFAP (ng/ml)				
Median (Q1-Q3)	15 (5.4 - 30)	39 (13.9 - 79.5)	9.6 (4.7 - 21)	27 (9.2 - 50.5)
Missing	0 (0%)	3 (1.1%)	2 (0.8%)	1 (0.7%)
NFL (pg/ml)				
Median (Q1-Q3)	51 (24.2 - 97.9)	100 (46.7 - 224.8)	31 (16.2 - 55.3)	73 (32.6 - 137)
Missing	0 (0%)	3 (1.1%)	2 (0.8%)	1 (0.7%)
NSE (ng/ml)				
Median (Q1-Q3)	22 (16.3 - 32.1)	30 (21.1 - 47.3)	21 (15.5 - 29.4)	26 (18.6 - 40)
Missing	0 (0%)	0 (0%)	1 (0.4%)	0 (0%)
S100B (ng/ml)				
Median (Q1-Q3)	0.25 (0.1 - 0.4)	0.62 (0.3 - 1.4)	0.23 (0.1 - 0.4)	0.49 (0.3 - 0.7)
Missing	0 (0%)	1 (0.4%)	0 (0%)	0 (0%)
Tau (pg/ml)				
Median (Q1-Q3)	8.1 (3.3 - 15)	18 (7.7 - 41)	5.4 (3 - 10.4)	10 (5.4 - 22.6)
Missing	0 (0%)	3 (1.1%)	2 (0.8%)	1 (0.7%)
UCH-L1 (pg/ml)				
Median (Q1-Q3)	270 (145 - 572)	720 (295 - 1283)	220 (112 - 398)	540 (249 - 1011)
Missing	0 (0%)	4 (1.5%)	2 (0.8%)	1 (0.7%)

Table 2. Biomarker concentrations stratified by Marshall score and outcome. Outcome was measured at 6 months using the extended Glasgow Outcome Scale (GOSE) and deemed favourable ("fav") if GOSE ≥5 and unfavourable ("unfav") if GOSE <5. Figures are provided as medians (first quartile – third quartile).

Variable	Overall	Marshall <3	Marshall ≥3
(Intercept)	0.50	0.34	0.65
GFAP	0.02	0.01	0.04
NFL	0.08	0.07	0.03
NSE	0.00	0.00	0.00
S100B	0.13	0.10	0.11
Tau	0.00	0.00	0.00
UCH-L1	0.03	0.03	0.03

Table 3. Relative prognostic value of individual proteins. The table shows the results of the lasso regression using acute serum biomarker concentrations to predict unfavourable six-month outcome. The higher the coefficient, the stronger the prognostic value of a biomarker. A coefficient of zero indicates that the protein does not add any further prognostic value if the other proteins are already available. The strength of the coefficients is color coded with darker shades indicating greater prognostic value. Biomarker concentrations are all log-transformed and adjusted for time of sampling so that individual proteins are directly comparable. All coefficients are standardized so that the two patient groups are directly comparable.

	CRASH-CT		IMPACT-CT	
Proteins added	none	panel	none	panel
Marshall score <3				
Area under the curve	0.73 (0.72-0.74)	0.81 (0.79-0.82)	0.72 (0.71-0.73)	0.80 (0.78-0.81)
Variation explained (%)	26 (24-27)	39 (37-41)	24 (22-25)	38 (35-41)
Calibration intercept	-0.16 (-0.21--0.11)	-0.10 (-0.14--0.07)	-0.08 (-0.11--0.06)	-0.05 (-0.07--0.03)
Calibration slope	0.73 (0.67-0.79)	0.80 (0.77-0.84)	0.84 (0.82-0.85)	0.87 (0.86-0.88)
Likelihood ratio test q-value		<0.001		<0.001
Marshall score ≥3				
Area under the curve	0.79 (0.77-0.80)	0.82 (0.81-0.84)	0.78 (0.76-0.79)	0.82 (0.81-0.83)
Variation explained (%)	36 (33-39)	43 (40-46)	35 (32-38)	43 (40-46)
Calibration intercept	0.04 (0.03-0.06)	0.04 (0.03-0.05)	0.07 (0.06-0.08)	0.06 (0.05-0.07)
Calibration slope	0.89 (0.88-0.90)	0.90 (0.89-0.91)	0.84 (0.83-0.86)	0.85 (0.84-0.87)
Likelihood ratio test q-value		<0.001		<0.001

Table 4. Performance of prognostic models with and without serum biomarkers. CRASH-CT and IMPACT-CT are the established prognostic models. Their performance was assessed before and after adding the biomarker score. Figures are Mean (95% confidence interval). Results were obtained through bootstrapping within multiply imputed datasets, which reduces mean estimates and widens confidence intervals to ensure results are generalizable. q-values are p-values corrected for multiple comparisons.

Supplement to
“Prognostic value of serum biomarkers in patients with moderate-severe traumatic brain injury, differentiated by Marshall CT classification”

Contents

15. **Supplemental table S7. Relative prognostic value of individual proteins – unadjusted for sample timing**
16. **Supplemental Table S8. Model performance in all patients – unadjusted biomarker concentrations**
17. **Supplemental Figure S8. Comparison of the incremental value of serum biomarkers between patient groups for CRASH-CT – unadjusted biomarker concentrations**
18. **Supplemental Figure S9. Comparison of the incremental value of serum biomarkers between patient groups for IMPACT-CT – unadjusted biomarker concentrations**

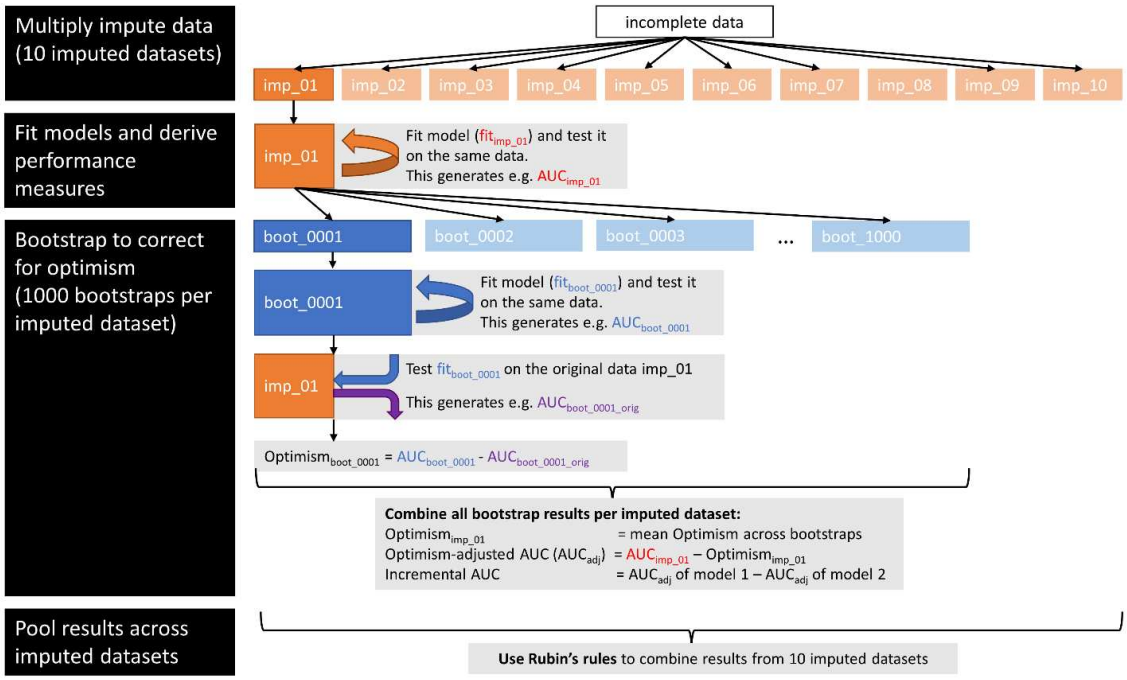
Supplemental methods: Adjusting biomarkers for time of sampling

Serum biomarkers were adjusted for time between injury of sampling (from now on just called "time") by estimating what each patient's biomarker concentration would have been, had the sample been taken two hours post-injury. This was done as follows:

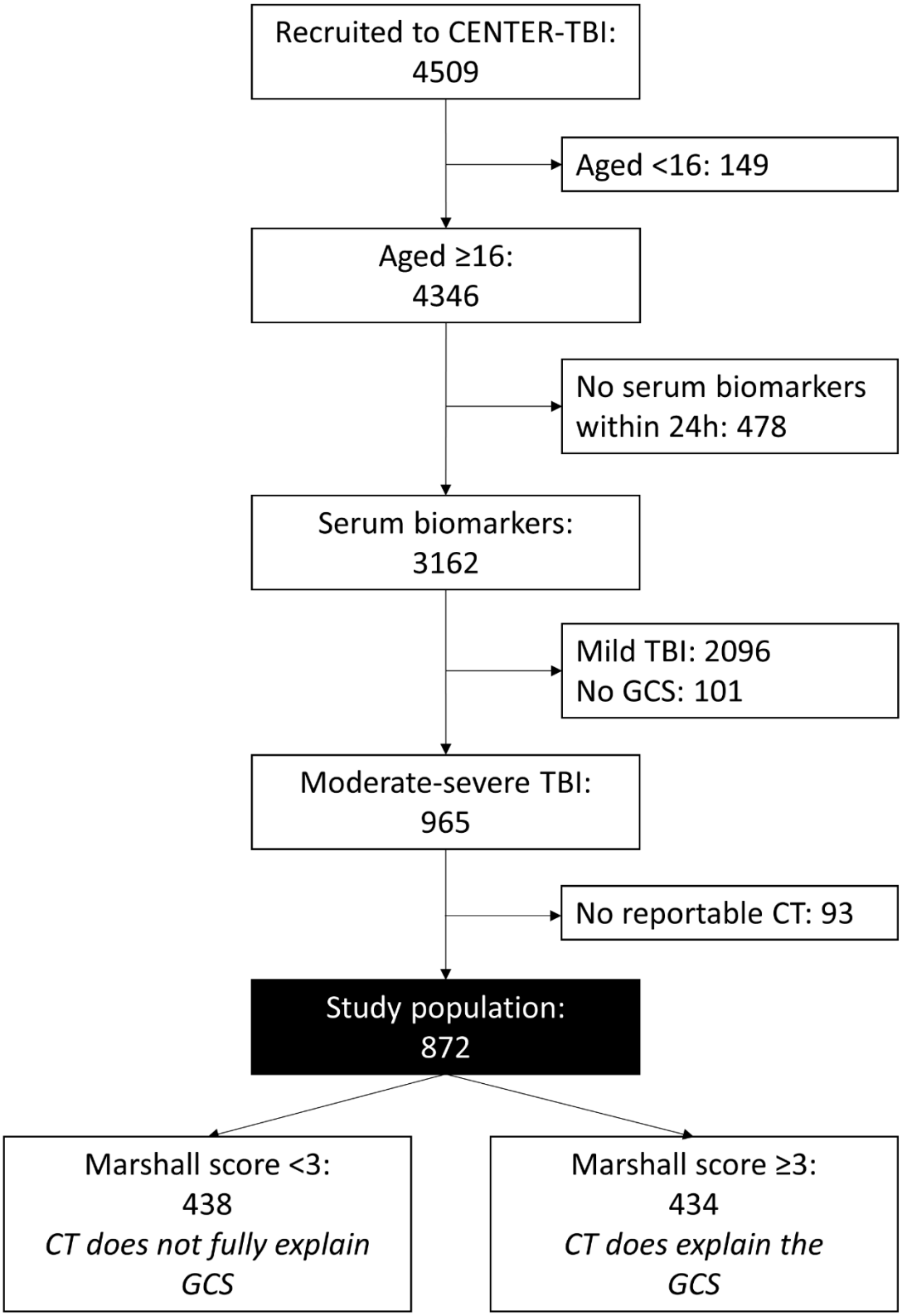
1. We fitted a regression model with the observed biomarker concentration as the dependent variable and time, $time^2$ and $time^3$ (as orthogonal polynomials) as the independent variables. We also added the following covariates as independent variables: Age, Sex, GCS, Motor score, Reactive pupils, Marshall score, presence of subarachnoid hemorrhage on CT, presence of epidural haematoma in CT, presence of petechial haemorrhages on CT, presence of cisternal compression on CT, presence of midline shift on CT, presence of majr extracranial haemorrhage, hypoxia, hypotension, care pathway and alcohol intoxication.

2. We used this regression model to calculate for each patient their predicted biomarker concentration (at their actual sampling time) and the predicted biomarker concentration at 14 hours. The difference, delta, between these two predicted values was calculated.

3. For each patient we added delta to their observed biomarker concentration.



Supplemental Figure S1. Workflow combining multiple imputation with bootstrapping. Ten imputed datasets imp_01 to imp_10 were generated from the original incomplete dataset. For each imputed dataset 1000 bootstrap samples boot_0001 to boot_1000 were created by resampling patients with replacement. Assessing the performance of a model on the same data that it was derived on will provide overly optimistic performance metrics. Bootstrapping was used to correct the model performance metrics for optimism. As an example, this process is shown for the area under the curve (AUC) but the same process was used for all performance metrics.



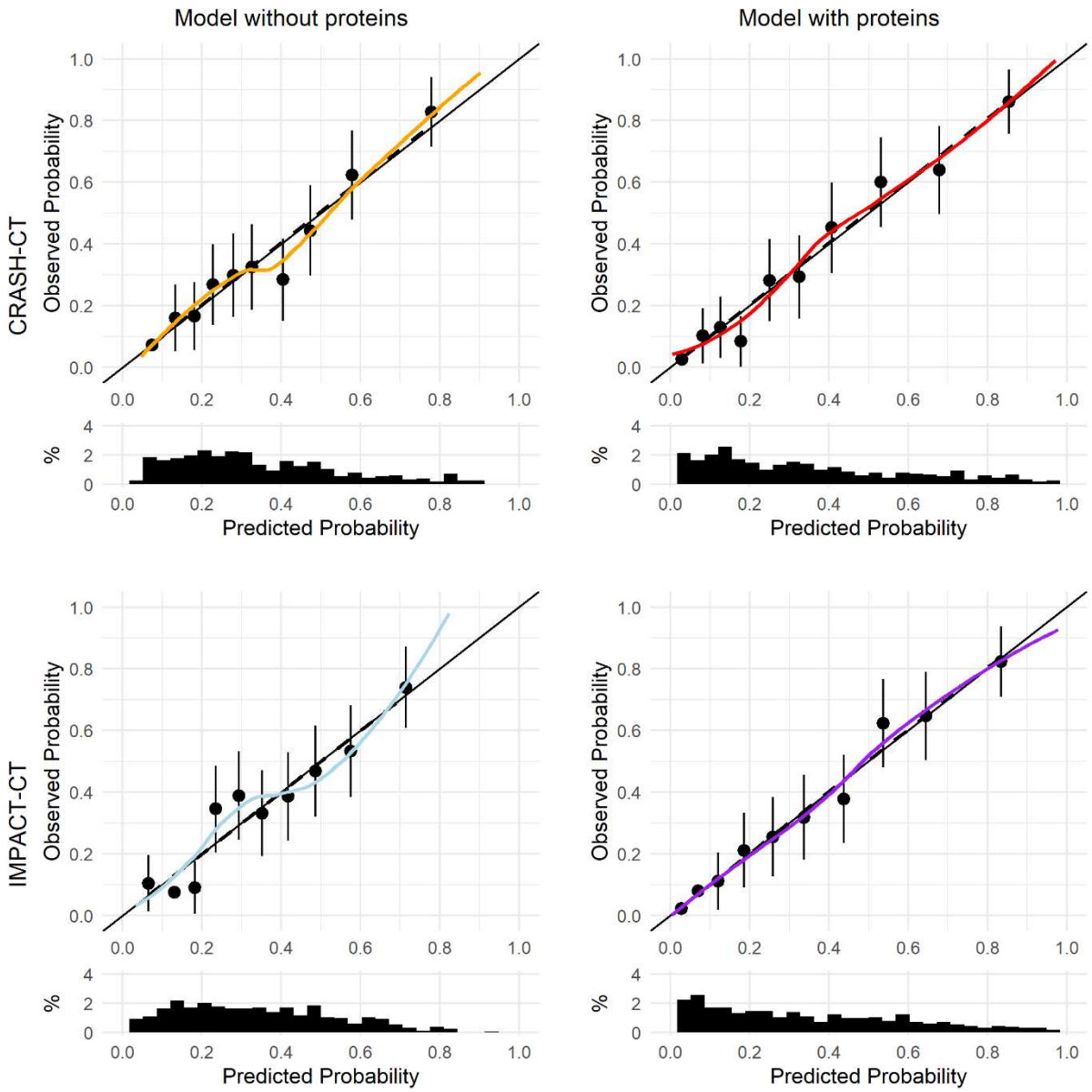
Supplemental Figure S2. Flowchart of patient inclusion

Marshall score	Outcome	N	Biomarker score
≥3	0	149	-0.064 (-0.069--0.058)
	1	285	0.128 (0.125- 0.132)
<3	0	285	-0.118 (-0.124--0.111)
	1	153	0.046 (0.038- 0.053)

Supplemental Table S1. Biomarker mean scores. The biomarker score is a weighted summary measure of GFAP, NFL, S100B and UCH-L1 concentrations, on a logit scale. N refers to the number of patients. Outcome was either favourable (0) or unfavourable (1).

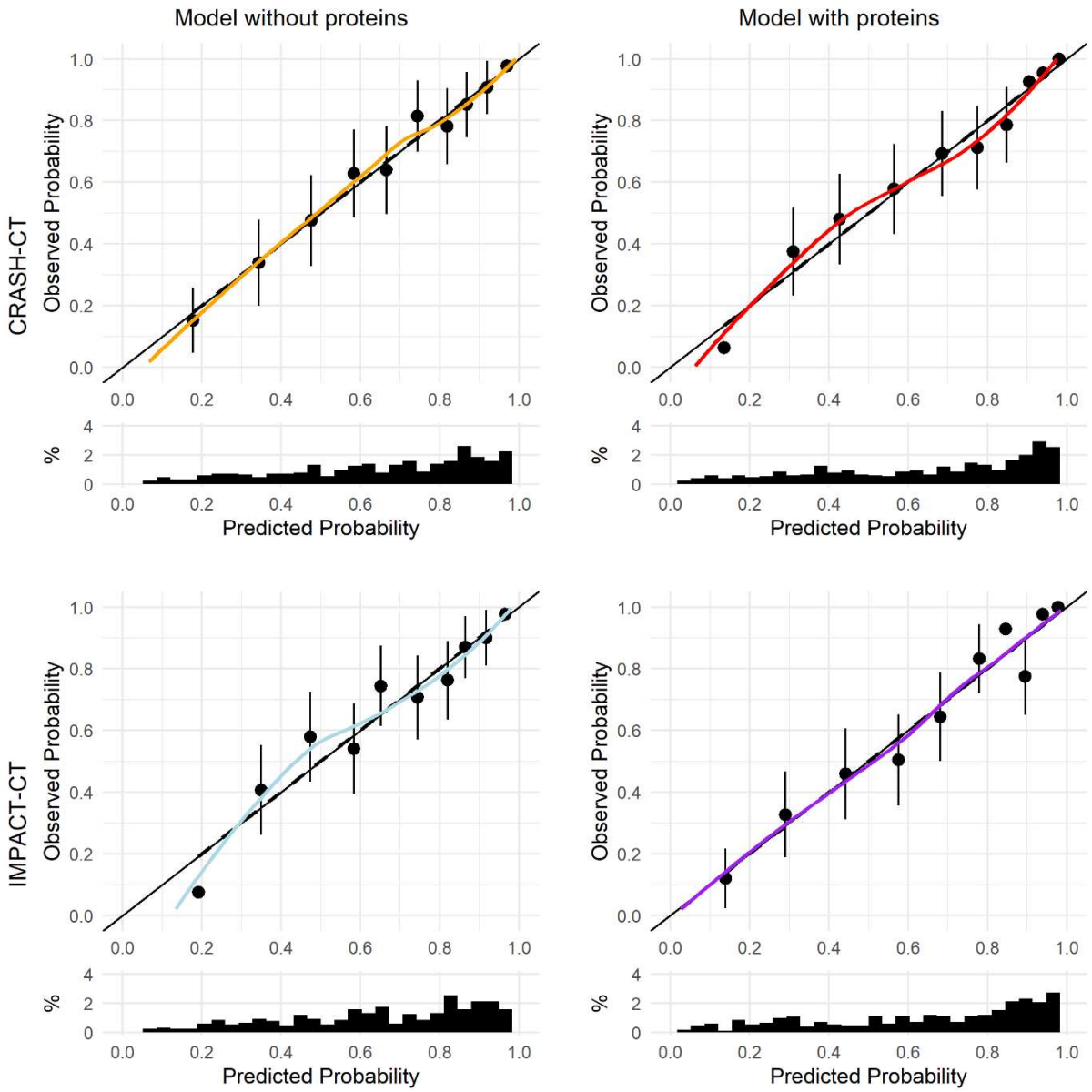
Model	Variable	Estimate	Std. Error	z value	q-value
CRASH-CT	Intercept	0.330	0.117	2.824	0.006
	Marshall<3	-0.681	0.163	-4.171	<0.001
	CRASH-CT	0.991	0.113	8.783	<0.001
	Marshall<3 : CRASH-CT	-0.145	0.157	-0.924	0.362
IMPACT-CT	Intercept	-0.065	0.132	-0.494	0.623
	Marshall<3	-0.001	0.182	-0.003	0.963
	IMPACT-CT	1.135	0.125	9.102	<0.001
	Marshall<3 : IMPACT-CT	-0.261	0.168	-1.550	0.136
Biomarker score	Intercept	0.498	0.111	4.498	<0.001
	Marshall<3	-0.937	0.157	-5.956	<0.001
	pred_bio	4.577	0.596	7.680	<0.001
	Marshall<3 : Biomarker score	1.030	0.934	1.102	0.280

Supplemental Table S2. Interaction between models and patient groups. Three models were fitted to check if prognostic tools (the CRASH-CT model, the IMPACT-CT model, the biomarker score) predict outcome differently in different patient groups (Marshall score <3 versus Marshall score ≥3). A positive interaction term shows outcome is better predicted in Marshall score <3 patients, a negative interaction term shows outcome is less well predicted in Marshall score <3 patients. q-values are p-values corrected for multiple comparisons.



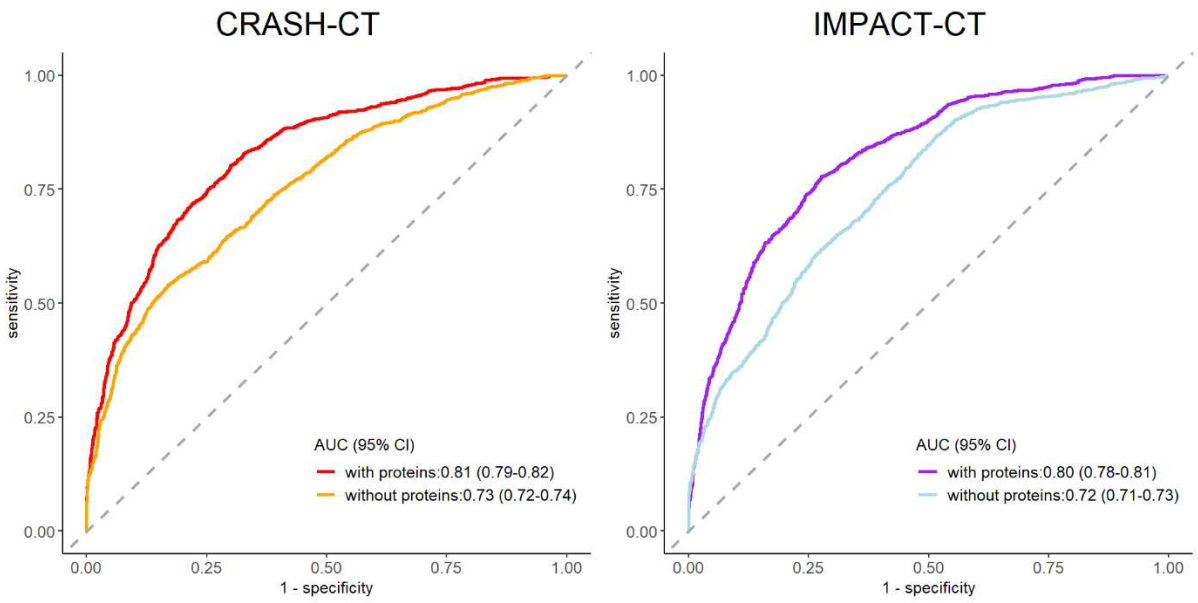
Supplemental Figure S3: Calibration curves for patients with Marshall score <3. Calibration plots were created by ordering patients from the lowest to the highest predicted probability of unfavorable outcome, then splitting patients into 10 groups, and then plotting each group's mean predicted probability of unfavorable outcome against the prevalence of that outcome actually observed in that group. Dots with bars = mean predicted probability of unfavorable outcome in each group with its 95% confidence interval. Solid line = calibration line that should be followed by a perfectly calibrated model. Dashed line = calibration line of the actual model, fitted using linear

regression. Colored line = calibration line of the actual model, fitted using locally estimated scatterplot smoothing.

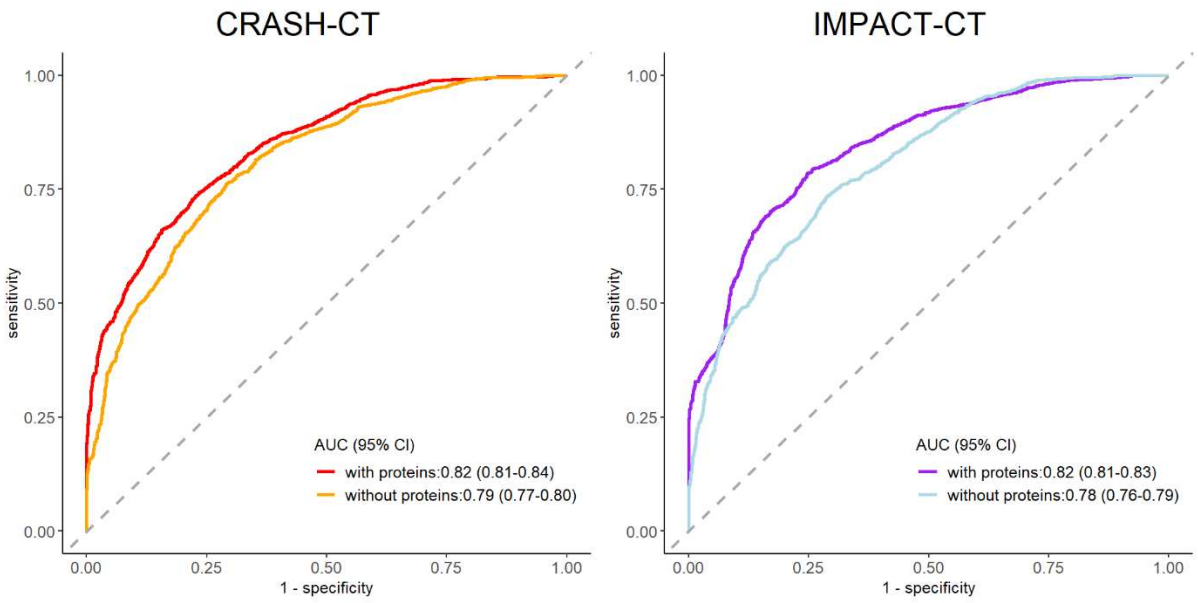


Supplemental Figure S4: Calibration curves for patients with Marshall score ≥3. Calibration plots were created by ordering patients from the lowest to the highest predicted probability of unfavorable outcome, then splitting patients into 10 groups, and then plotting each group's mean predicted probability of unfavorable outcome against the prevalence of that outcome actually observed in that group. Dots with bars = mean predicted probability of unfavorable outcome in each group with its 95% confidence interval. Solid line = calibration line that should be followed by a perfectly calibrated model. Dashed line = calibration line of the actual model, fitted using linear

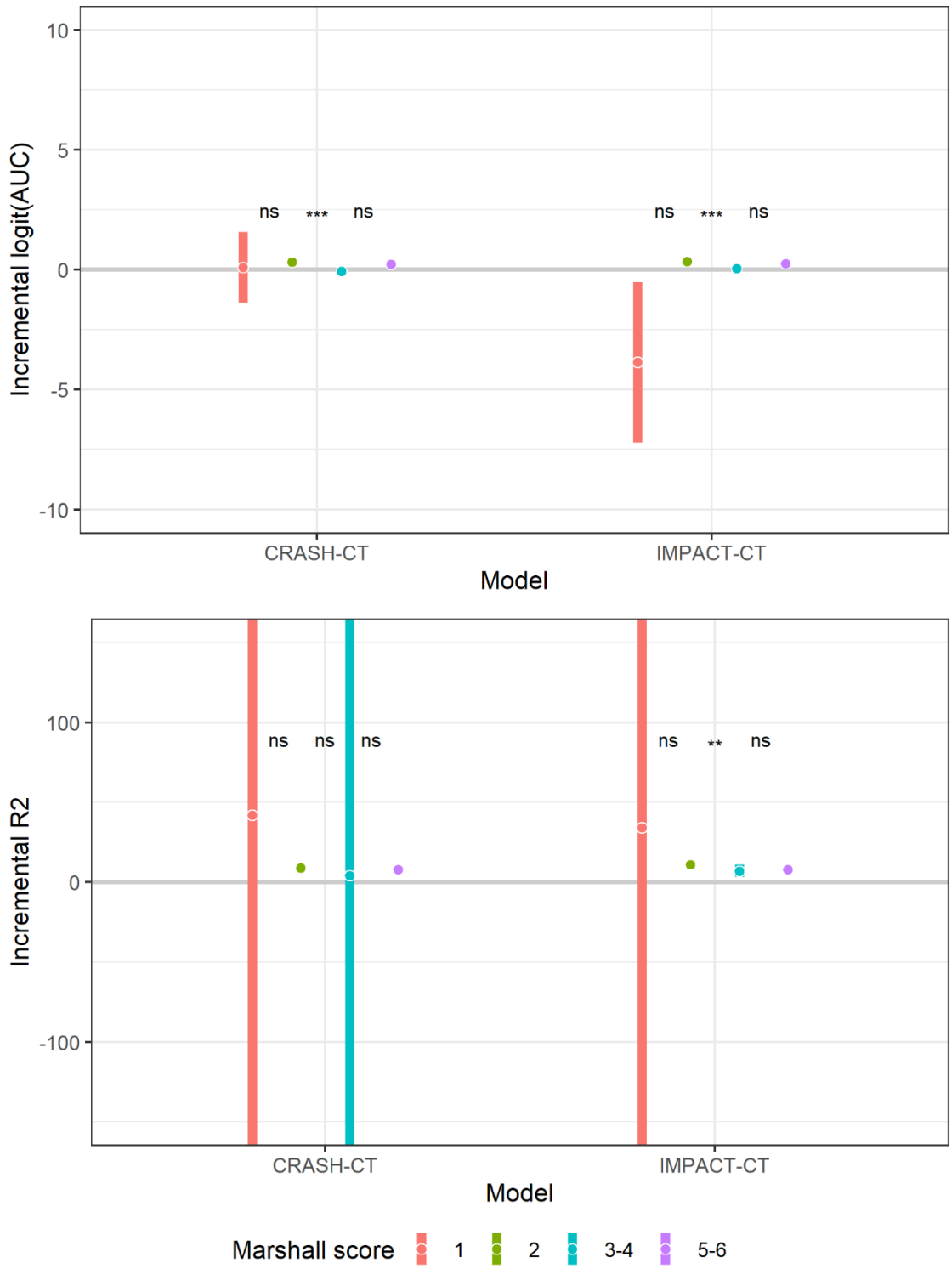
regression. Colored line = calibration line of the actual model, fitted using locally estimated scatterplot smoothing.



Supplemental Figure S5: Receiver operating characteristic curves for patients with Marshall score <3. Established models were used with and without the addition of protein biomarkers to predict unfavourable outcome. AUC = area under the curve, CI = confidence interval



Supplemental Figure S6: Receiver operating characteristic curves for patients with Marshall score ≥3. Established models were used with and without the addition of protein biomarkers to predict unfavourable outcome. AUC = area under the curve, CI = confidence interval



Supplemental Figure S7. Comparison of the incremental value of serum biomarkers between patient groups – subgroup analysis. The incremental value of adding serum biomarkers to established prognostic models (CRASH-CT and IMPACT-CT) was compared between patients with Marshall scores 1 versus 2, 2 versus 3-4 and 3-4 vs 5-6, using t-tests. Dot and bar plots show means

and 95% confidence intervals. Estimates were down-corrected for optimism via bootstrapping. q denotes the p-value corrected for multiple comparisons. ns = not significant, */**/** indicate q <0.05/ <0.01/ <0.001. AUC = area under the curve. The logit transformation ensures a fair comparison across models which had different baseline AUCs prior to the addition of serum biomarkers. R2 = Nagelkerke R^2 expressed as the percentage of the variation in outcome explained by the model. The upper and lower limits of the 95% confidence intervals are not shown for the some R2 values, as they approach plus and minus infinity, respectively.

Model	Variable	Estimate	Std. Error	z value	p-value
Refitted CRASH-CT	Intercept	-2.519	0.546	-4.609	<0.001
	Age (years)_crash	0.055	0.009	6.109	<0.001
	Glasgow Coma Score	0.143	0.040	3.550	0.001
	Reactive pupils (1)	0.205	0.537	0.381	0.653
	Reactive pupils (2)	-0.664	0.384	-1.729	0.104
	Major extra-cranial injury (present)	0.603	0.239	2.521	0.014
	Petechial haemorrhages (present)	0.795	0.253	3.138	0.002
	Cisternal compression (present)	2.574	1.083	2.359	0.022
	Subarachnoid haemorrhage (present)	0.257	0.249	1.033	0.354
	Midline shift (present)	-0.344	1.230	-0.282	0.783
	Unevacuated haematoma (present)	0.120	0.241	0.500	0.631
Refitted IMPACT-CT	Intercept	-2.171	0.587	-3.697	<0.001
	Age (years)	0.032	0.006	5.146	<0.001
	Motor score (2)	0.880	0.548	1.605	0.128
	Motor score (3)	-0.039	0.515	-0.076	0.882
	Motor score (4)	-0.179	0.356	-0.502	0.629
	Motor score (5 or 6)	-1.006	0.270	-3.723	<0.001
	Reactive pupils (1)	0.081	0.543	0.148	0.799
	Reactive pupils (2)	-0.790	0.386	-2.046	0.050
	Hypoxia (present or suspected)	-0.388	0.307	-1.262	0.238
	Hypotension (present or suspected)	0.755	0.292	2.585	0.012
	Marshall score (2)	1.388	0.427	3.248	0.002
	Subarachnoid haemorrhage (present)	-0.165	0.296	-0.552	0.532
	Extradural haematoma (present)	-0.698	0.387	-1.799	0.078
Refitted CRASH-CT plus biomarkers	Intercept	-2.099	0.594	-3.529	0.001
	Age (years)_crash	0.056	0.010	5.743	<0.001
	Glasgow Coma Score	0.140	0.044	3.156	0.002
	Reactive pupils (1)	0.321	0.571	0.560	0.546
	Reactive pupils (2)	-0.625	0.415	-1.509	0.156

Model	Variable	Estimate	Std. Error	z value	p-value
	Major extra-cranial injury (present)	0.190	0.263	0.719	0.485
	Petechial haemorrhages (present)	0.777	0.266	2.925	0.004
	Cisternal compression (present)	2.009	1.439	1.316	0.210
	Subarachnoid haemorrhage (present)	-0.117	0.274	-0.424	0.577
	Midline shift (present)	-0.358	1.309	-0.291	0.777
	Unevacuated haematoma (present)	-0.147	0.260	-0.567	0.584
	Biomarker panel logit(probability)	4.887	0.737	6.626	<0.001
Refitted IMPACT-CT plus biomarkers	Intercept	-1.828	0.635	-2.872	0.007
	Age (years)	0.034	0.007	5.001	<0.001
	Motor score (2)	0.772	0.575	1.340	0.204
	Motor score (3)	0.122	0.530	0.231	0.775
	Motor score (4)	-0.179	0.388	-0.461	0.657
	Motor score (5 or 6)	-0.958	0.293	-3.271	0.002
	Reactive pupils (1)	0.153	0.573	0.263	0.721
	Reactive pupils (2)	-0.831	0.415	-2.005	0.055
	Hypoxia (present or suspected)	-0.380	0.323	-1.173	0.272
	Hypotension (present or suspected)	0.308	0.314	0.982	0.335
	Marshall score (2)	1.090	0.471	2.307	0.027
	Subarachnoid haemorrhage (present)	-0.452	0.321	-1.404	0.188
	Extradural haematoma (present)	-0.947	0.415	-2.274	0.027
	Biomarker panel logit(probability)	4.962	0.725	6.846	<0.001

Supplemental Table S3. Model coefficients for patients with a Marshall score <3.

Model	Variable	Estimate	Std. Error	z value	p-value
Refitted CRASH-CT	Intercept	-1.113	0.723	-1.540	0.156
	Age (years)_crash	0.068	0.010	6.761	<0.001
	Glasgow Coma Score	0.206	0.043	4.806	<0.001
	Reactive pupils (1)	-0.871	0.508	-1.715	0.090
	Reactive pupils (2)	-1.276	0.379	-3.367	0.001
	Major extra-cranial injury (present)	0.470	0.259	1.814	0.073
	Petechial haemorrhages (present)	0.486	0.378	1.284	0.215
	Cisternal compression (present)	0.520	0.296	1.755	0.128
	Subarachnoid haemorrhage (present)	0.442	0.378	1.166	0.269
	Midline shift (present)	-0.040	0.266	-0.151	0.793
	Unevacuated haematoma (present)	-0.628	0.400	-1.567	0.130
Refitted IMPACT-CT	Intercept	-0.245	0.597	-0.407	0.640
	Age (years)	0.040	0.007	5.643	<0.001
	Motor score (2)	1.122	0.652	1.713	0.094
	Motor score (3)	0.701	0.640	1.087	0.295
	Motor score (4)	-0.660	0.396	-1.665	0.115
	Motor score (5 or 6)	-1.114	0.292	-3.818	<0.001
	Reactive pupils (1)	-0.694	0.512	-1.356	0.181
	Reactive pupils (2)	-1.395	0.383	-3.638	<0.001
	Hypoxia (present or suspected)	-0.081	0.382	-0.216	0.744
	Hypotension (present or suspected)	1.144	0.459	2.483	0.017
	Marshall score (4)	-0.192	0.727	-0.261	0.730
	Marshall score (5) or 6	-0.058	0.323	-0.181	0.829
	Subarachnoid haemorrhage (present)	0.504	0.376	1.338	0.206
	Extradural haematoma (present)	-0.651	0.275	-2.365	0.025
Refitted CRASH-CT plus biomarkers	Intercept	-0.635	0.757	-0.840	0.434
	Age (years)_crash	0.065	0.010	6.268	<0.001
	Glasgow Coma Score	0.172	0.045	3.816	<0.001
	Reactive pupils (1)	-0.537	0.533	-1.009	0.318
	Reactive pupils (2)	-1.060	0.394	-2.687	0.008

Model	Variable	Estimate	Std. Error	z value	p-value
	Major extra-cranial injury (present)	0.397	0.273	1.457	0.151
	Petechial haemorrhages (present)	0.453	0.398	1.139	0.268
	Cisternal compression (present)	0.356	0.311	1.146	0.317
	Subarachnoid haemorrhage (present)	0.045	0.396	0.114	0.748
	Midline shift (present)	0.084	0.283	0.298	0.698
	Unevacuated haematoma (present)	-0.454	0.421	-1.078	0.300
	Biomarker panel logit(probability)	3.807	0.714	5.327	<0.001
Refitted IMPACT-CT plus biomarkers	Intercept	-0.051	0.631	-0.081	0.765
	Age (years)	0.038	0.007	5.188	<0.001
	Motor score (2)	1.507	0.673	2.230	0.030
	Motor score (3)	0.579	0.657	0.873	0.401
	Motor score (4)	-0.423	0.422	-1.004	0.351
	Motor score (5 or 6)	-0.854	0.308	-2.774	0.007
	Reactive pupils (1)	-0.436	0.526	-0.829	0.412
	Reactive pupils (2)	-1.240	0.394	-3.142	0.002
	Hypoxia (present or suspected)	-0.179	0.397	-0.454	0.649
	Hypotension (present or suspected)	0.820	0.477	1.711	0.097
	Marshall score (4)	-0.001	0.799	0.006	0.752
	Marshall score (5) or 6	0.118	0.339	0.347	0.732
	Subarachnoid haemorrhage (present)	0.073	0.402	0.183	0.718
	Extradural haematoma (present)	-0.559	0.293	-1.911	0.067
	Biomarker panel logit(probability)	3.900	0.717	5.440	<0.001

Supplemental Table S4. Model coefficients for patients with a Marshall score ≥3

	CRASH-CT		IMPACT-CT	
Proteins added	none	panel	none	panel
Marshall score <3				
Area under the curve	0.70 (0.67-0.74)	0.76 (0.74-0.79)	0.75 (0.73-0.77)	0.80 (0.78-0.83)
Variation explained (%)	32 (28-36)	45 (42-48)	47 (43-50)	58 (53-62)
Calibration intercept	0.11 (0.07-0.16)	0.11 (0.07-0.14)	0.16 (0.11-0.21)	0.14 (0.09-0.19)
Calibration slope	0.56 (0.43-0.69)	0.56 (0.47-0.65)	0.29 (0.25-0.34)	0.31 (0.27-0.35)
Likelihood ratio test q-value		0.004		0.003
Marshall score ≥3				
Area under the curve	0.77 (0.74-0.81)	0.86 (0.83-0.89)	0.68 (0.63-0.74)	0.83 (0.79-0.86)
Variation explained (%)	34 (29-38)	53 (46-60)	23 (16-30)	51 (44-57)
Calibration intercept	0.42 (0.22-0.63)	0.30 (0.18-0.42)	0.98 (0.87-1.09)	0.60 (0.49-0.70)
Calibration slope	0.63 (0.51-0.74)	0.65 (0.57-0.72)	0.27 (0.24-0.30)	0.38 (0.35-0.40)
Likelihood ratio test q-value		<0.001		<0.001

Supplemental Table S5. Model performance in patients aged ≥ 65 years. N = 71 for Marshall <3 and N = 139 for Marshall ≥3. CRASH-CT and IMPACT-CT are the established prognostic models. Their performance was assessed before and after adding the biomarker score. Figures are Mean (95% confidence interval). Results were obtained through bootstrapping within multiply imputed datasets, which reduces mean estimates and widens confidence intervals to ensure results are generalizable. q-values are p-values corrected for multiple comparisons.

	CRASH-CT		IMPACT-CT	
Proteins added	none	panel	none	panel
Marshall score <3				
Area under the curve	0.70 (0.66-0.74)	0.80 (0.76-0.84)	0.74 (0.69-0.78)	0.81 (0.79-0.83)
Variation explained (%)	26 (19-33)	43 (37-49)	33 (26-39)	47 (43-50)
Calibration intercept	-0.46 (-0.70--0.23)	-0.33 (-0.51--0.14)	-0.32 (-0.45--0.20)	-0.27 (-0.36--0.18)
Calibration slope	0.48 (0.28-0.67)	0.59 (0.42-0.76)	0.56 (0.52-0.60)	0.60 (0.57-0.63)
Likelihood ratio test q-value		<0.001		<0.001
Marshall score ≥3				
Area under the curve	0.80 (0.78-0.82)	0.85 (0.84-0.87)	0.80 (0.78-0.83)	0.86 (0.85-0.87)
Variation explained (%)	41 (38-45)	53 (49-57)	44 (40-49)	56 (52-60)
Calibration intercept	0.07 (0.05-0.08)	0.05 (0.04-0.07)	0.09 (0.07-0.11)	0.07 (0.05-0.09)
Calibration slope	0.85 (0.83-0.86)	0.83 (0.82-0.85)	0.75 (0.70-0.81)	0.75 (0.69-0.80)
Likelihood ratio test q-value		<0.001		<0.001

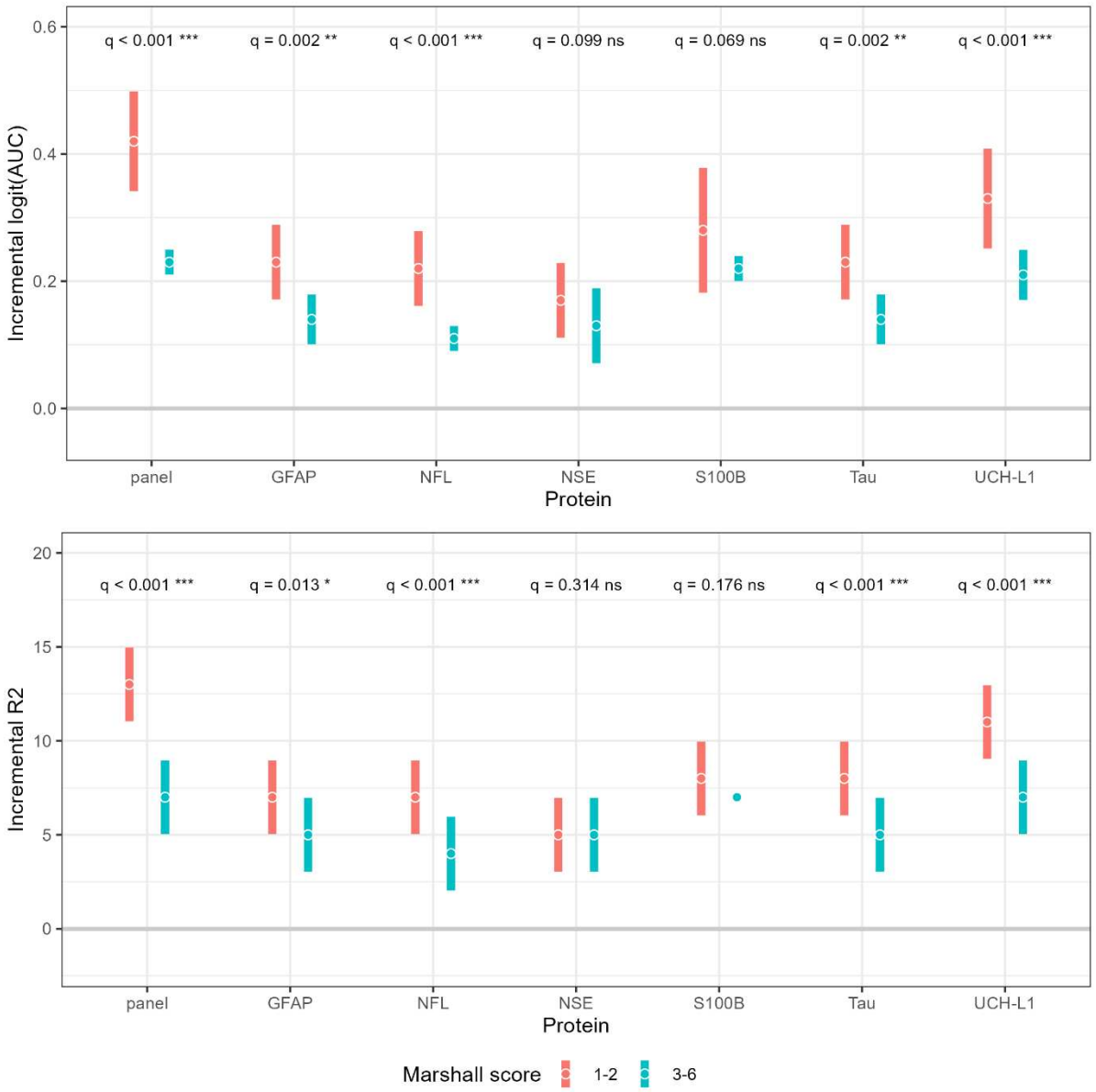
Supplemental Table S6. Model performance in patients with isolated TBI. N = 128 for Marshall <3 and N = 188 for Marshall ≥3. CRASH-CT and IMPACT-CT are the established prognostic models. Their performance was assessed before and after adding the biomarker score. Figures are Mean (95% confidence interval). Results were obtained through bootstrapping within multiply imputed datasets, which reduces mean estimates and widens confidence intervals to ensure results are generalizable. q-values are p-values corrected for multiple comparisons.

Variable	Overall	Marshall <3	Marshall >=3
(Intercept)	0.50	0.34	0.65
GFAP	0.03	0.01	0.04
NFL	0.09	0.09	0.02
NSE	0.00	0.00	0.00
S100B	0.12	0.09	0.11
Tau	0.00	0.00	0.00
UCH-L1	0.03	0.03	0.03

Supplemental table S7. Relative prognostic value of individual proteins – unadjusted for sample timing. The table shows the results of the lasso regression using acute serum biomarker concentrations to predict unfavourable six-month outcome. The higher the coefficient, the stronger the prognostic value of a biomarker. A coefficient of zero indicates that the protein does not add any further prognostic value if the other proteins are already available. The strength of the coefficients is color coded with darker shades indicating greater prognostic value. Biomarker concentrations are all log-transformed and adjusted for time of sampling so that individual proteins are directly comparable. All coefficients are standardized so that the two patient groups are directly comparable.

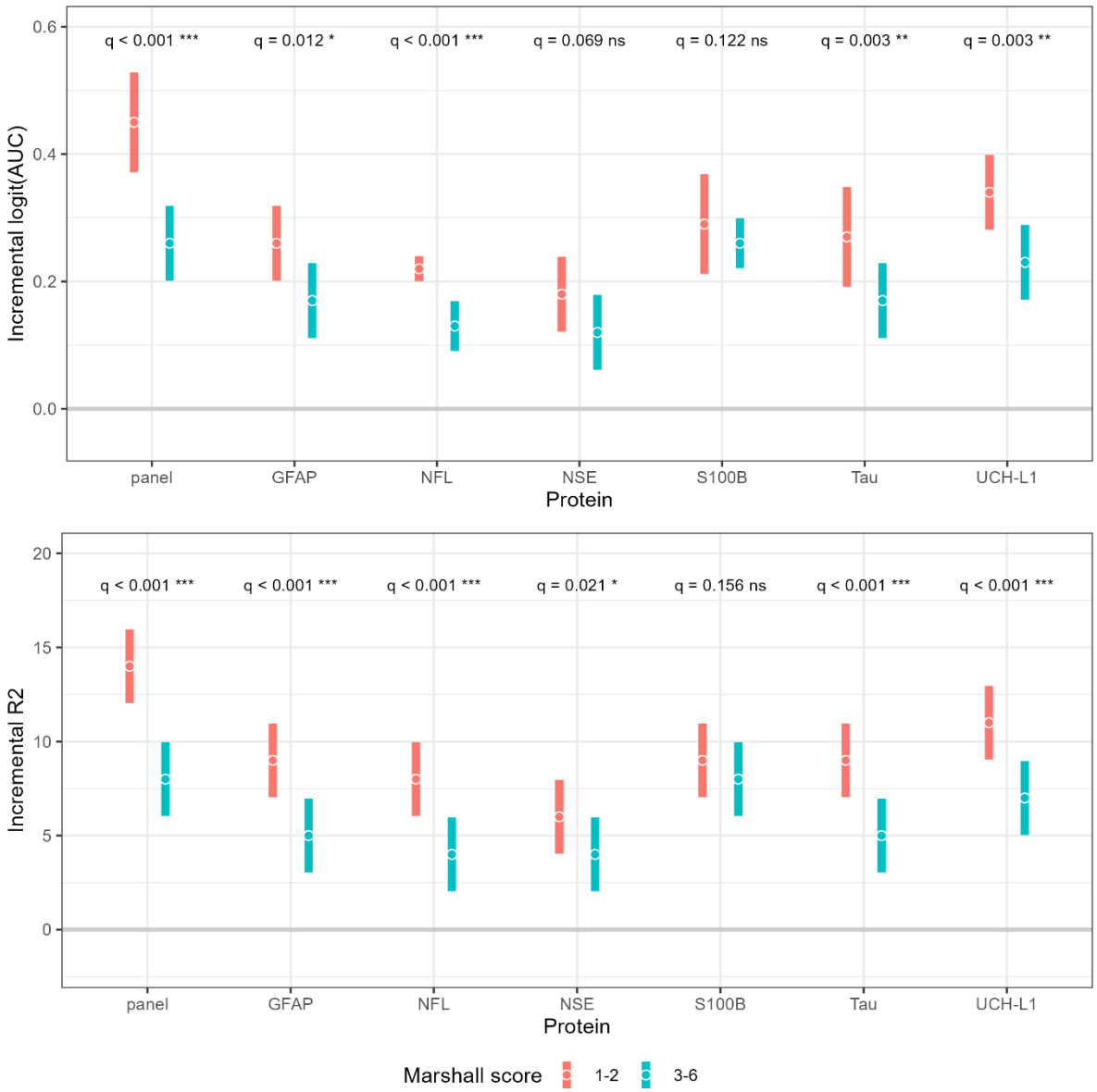
	CRASH-CT		IMPACT-CT	
Proteins added	none	panel	none	panel
Marshall score <3				
Area under the curve	0.73 (0.72-0.74)	0.81 (0.79-0.82)	0.72 (0.71-0.73)	0.80 (0.78-0.82)
Variation explained (%)	26 (24-27)	39 (37-41)	24 (22-25)	38 (35-41)
Calibration intercept	-0.16 (-0.21--0.11)	-0.11 (-0.14--0.07)	-0.08 (-0.11--0.06)	-0.05 (-0.07--0.03)
Calibration slope	0.73 (0.67-0.79)	0.80 (0.77-0.84)	0.84 (0.82-0.85)	0.87 (0.86-0.89)
Likelihood ratio test q-value		<0.001		<0.001
Marshall score ≥3				
Area under the curve	0.79 (0.77-0.80)	0.83 (0.81-0.84)	0.78 (0.76-0.79)	0.82 (0.81-0.83)
Variation explained (%)	36 (33-39)	43 (40-47)	35 (32-38)	43 (40-46)
Calibration intercept	0.04 (0.03-0.06)	0.04 (0.02-0.05)	0.07 (0.06-0.08)	0.06 (0.05-0.07)
Calibration slope	0.89 (0.88-0.90)	0.90 (0.89-0.91)	0.84 (0.83-0.86)	0.85 (0.84-0.87)
Likelihood ratio test q-value		<0.001		<0.001

Supplemental Table S8. Model performance in all patients – unadjusted biomarker concentrations. CRASH-CT and IMPACT-CT are the established prognostic models. Their performance was assessed before and after adding the biomarker score. Figures are Mean (95% confidence interval). Results were obtained through bootstrapping within multiply imputed datasets, which reduces mean estimates and widens confidence intervals to ensure results are generalizable. q-values are p-values corrected for multiple comparisons.



Supplemental Figure S8. Comparison of the incremental value of serum biomarkers between patient groups for CRASH-CT – unadjusted biomarker concentrations. The incremental value of adding serum biomarkers to established prognostic models (CRASH-CT and IMPACT-CT) was compared between patients with a Marshall score <3 and those with a Marshall score ≥3, using a t-test. Dot and bar plots show means and 95% confidence intervals. Estimates were down-corrected for optimism via bootstrapping. q denotes the p-value corrected for multiple comparisons. ns = not significant, */**/** indicate q <0.05/ <0.01/ <0.001. AUC = area under the curve. The logit transformation ensures a fair comparison across models which had different baseline AUCs prior to

the addition of serum biomarkers. R2 = Nagelkerke R^2 expressed as the percentage of the variation in outcome explained by the model.



Supplemental Figure S9. Comparison of the incremental value of serum biomarkers between patient groups for IMPACT-CT – unadjusted biomarker concentrations. The incremental value of adding serum biomarkers to established prognostic models (CRASH-CT and IMPACT-CT) was compared between patients with a Marshall score <3 and those with a Marshall score ≥3, using a t-test. Dot and bar plots show means and 95% confidence intervals. Estimates were down-corrected for optimism via bootstrapping. q denotes the p-value corrected for multiple comparisons. ns = not significant, */**/** indicate q <0.05/ <0.01/ <0.001. AUC = area under the curve. The logit transformation ensures a fair comparison across models which had different baseline AUCs prior to

the addition of serum biomarkers. R2 = Nagelkerke R^2 expressed as the percentage of the variation in outcome explained by the model.