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ORIGINAL ARTICLE

Biomarkers Improve Clinical Outcome Predictors of Mortality Following Non-Penetrating Severe Traumatic Brain Injury

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Abstract
Objective This study assessed whether early levels of biomarkers measured in CSF within 24-h of severe TBI would improve the clinical prediction of 6-months mortality.
Methods This prospective study conducted at two Level 1 Trauma Centers enrolled adults with severe TBI (GCS $\leq$8) requiring a ventriculostomy as well as control subjects. Ventricular CSF was sampled within 24-h of injury and analyzed for seven candidate biomarkers (UCH-L1, MAP-2, SBDP150, SBDP145, SBDP120, MBP, and S100B). The International Mission on Prognosis and Analysis of Clinical Trials in TBI (IMPACT) scores (Core, Extended, and Lab) were calculated for each patient to determine risk of 6-months mortality. The IMPACT models and biomarkers were assessed alone and in combination.
Results There were 152 patients enrolled, 131 TBI patients and 21 control patients. Thirty six (27 %) patients did not survive to 6 months. Biomarkers were all significantly elevated in TBI versus controls ($p < 0.001$). Peak levels of UCH-L1, SBDP145, MAP-2, and MBP were significantly higher in non-survivors ($p < 0.05$). Of the seven biomarkers measured at 12-h post-injury MAP-2 ($p = 0.004$), UCH-L1 ($p = 0.024$), and MBP ($p = 0.037$) had significant unadjusted hazard ratios. Of the seven biomarkers measured at the earliest time within 24-h, MAP-2 ($p = 0.002$), UCH-L1 ($p = 0.016$), MBP

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($p = 0.021$), and SBDP145 (0.029) had the most significant elevations. When the IMPACT Extended Model was combined with the biomarkers, MAP-2 contributed most significantly to the survival models with sensitivities of 97–100 %.

Conclusions These data suggest that early levels of MAP-2 in combination with clinical data provide enhanced prognostic capabilities for mortality at 6 months.

Keywords Biomarkers · Severe traumatic brain injury · Mortality · Microtubual associated protein (MAP-2) · S100B · Alpha-spectrin breakdown products (SBDPs) · Ubiquitin C-terminal hydrolase (UCH-L1)

Introduction

Brain injury resulting from traumatic, ischemic and/or chemical etiology is an international health concern with significant morbidity and mortality [1]. Approximately 1.7 million people sustain a traumatic brain injury (TBI) annually [2]. In 2010, the Center for disease control (CDC) estimated the economic burden of TBI, including direct and indirect medical costs, to be approximately $76.5 billion. Furthermore, many injuries are under-reported and the true incidence and cost of TBI are likely much higher [1]. According to the CDC, a TBI is defined as being "…caused by a bump, blow or jolt to the head or a penetrating head injury that disrupts the normal function of the brain." [2]. Unlike other organ-based diseases where rapid diagnosis employing biomarkers can guide treatment of various diseases, no such rapid, definitive diagnostic tests exist for TBI at this time.

Research has shown evidence for the involvement of protein processes that contribute to secondary injury after brain trauma [3, 4]. Advances in protein identification and quantification technologies [5] have provided opportunities to measure neuronal damage. Together with clinical assessment, the quantitative evaluation of neuronal biomarkers measured within 24 h from either cerebrospinal fluid (CSF) and/or blood could assist in the determination of injury severity, provide specifics to anatomical and cellular pathology of the injury, and could alter clinical management. Although there are a number of biochemical markers that have been investigated in TBI, the most extensively studied among these are glial protein S-100 beta (β) [6–16], neuron-specific enolase (NSE) [17–22], and myelin basic protein (MBP) [20, 23–26] Other promising biomarkers include alpha-II-spectrin breakdown products [27–30], Ubiquitin C-terminal Hydrolase-L1 (UCH-L1) [31–34], and microtubule-associated protein (MAP-2) [35].

The International Mission on Prognosis and Analysis of Clinical Trials in TBI (IMPACT) used prospectively collected individual patient data from 11 studies in 8,509 patients with severe or moderate TBI, to develop prognostic models from data available at admission. External validation of the models was conducted on 6,681 patients from the Medical Research Council Corticosteroid Randomization after Significant Head Injury (MRC CRASH) trial [36]. The strongest predictors of outcome were age, pupillary reactivity and GCS motor score (IMPACT Core model score). The performance improved by adding CT characteristics, secondary insults such as hypotension and hypoxia to the Core (IMPACT Extended Model Score) and further improved by including the laboratory parameters of glucose and hemoglobin (IMPACT Lab Model Score).

We hypothesized that together with clinical attributes and radiographic evaluation, neuronal biomarkers would add to long-term mortality prediction in patients with severe TBI. This study assessed whether early levels of biomarkers measured in CSF within 24 h of injury would improve IMPACT prediction of mortality at 6 months in patients with severe TBI.

Methods

This prospective controlled cohort study enrolled a convenience sample adult patients ($\geq$18 years of age) with closed head injuries with a GCS score of $\leq$8. From March 2007–August 2011, patients were enrolled at two Level 1 trauma centers in Gainesville, Florida (Shands Hospital, University of Florida) and Houston, Texas (Ben Taub General Hospital, Baylor College of Medicine). Patients met inclusion criteria if they were $\geq$18 years old with a non-penetrating head injury and had a GCS $\leq$8 requiring the placement of an intraventricular catheter (IVC). Patients were excluded if they had a history of pre-existing end-stage organ disease or severe psychiatric illness. All patients had a computerized tomography (CT) scan done as part of their routine evaluation per hospital protocol.

Cerebrospinal fluid samples were obtained within 24 h of admission at 6, 12, 18, and 24 h post-injury by collection from the IVC reservoir that had been emptied 1 h prior to collection. Samples were then stored on ice for up to 12 h before being centrifuged and frozen at −80 °C as 1 ml serum aliquots for future analysis at Banyan Biomarkers Inc. (Alachua, FL, USA). The initial CT scan performed in the emergency department for each patient was reviewed by a single board certified neuroradiologist blinded to the patient's clinical examination and outcome. The principal scoring system used in the CT interpretation was the Rotterdam Score [36]. The Rotterdam CT score was developed for prognostic purposes in TBI to determine the risk for mortality. It is based on CT findings of basal cistern compression, midline shift, presence of an epidural

hematoma, and the presence of either intraventricular blood and/or traumatic subarachnoid hemorrhage. The primary outcome measure was mortality at 6 months post-injury. Because patients were unconscious upon eligibility determination, a 24-h waiver of consent was granted by the IRB. If informed consent could not be obtained from the patient's legally authorized representative within 24 h, samples were discarded and the patient withdrawn from the study. Control subjects were patients without TBI ($n = 21$) requiring CSF drainage for other medical conditions such as for routine anesthetic or surgical management (e.g. endovascular aortic aneurysm stent repair, selected orthopedic procedures) or chronic hydrocephalus. Based on our previous work with UCH-L1 [32], the minimum number of control patients required to detect a difference between control and TBI patients was 14 given a power a power of 80 % and a significance level (alpha) of 0.05.

The study was approved by the Institutional Review Boards of the University of Florida and Baylor College of Medicine as well as the University of Houston Committee for the Protection of Human Subjects.

Biomarker Analysis

A number of known and relatively novel brain injury protein biomarkers were selected for this study. They include neuronal cell body injury marker Ubiquitin C-terminal hydrolase-L1 (UCH-L1), breakdown products of axonally enriched αII-spectrin (SBDP150 and SBDP145 produced by necrosis-linked calpain protease and SBDP120 produced by the apoptosis-linked caspase-3 protease), dendritic injury marker microtubule-associated protein-2 (MAP-2), glial marker S100b and demyelination marker myelin basic protein (MBP) [37–39]. The table in the appendix describes the Lower (LLoD) and Upper (ULoD) limits of detection for each of the seven biomarkers. Imputed values were used for concentrations below the LLoD by calculating half of lowest level measured. Appendix 1 describes how biomarker analysis was performed in the lab on each of the seven biomarkers.

Data Analysis

Data were analyzed using descriptive statistics and were assessed for distribution and variance. The IMPACT scores were calculated for each patient (1) Core IMPACT score (age, pupillary reactivity and GCS motor score), (2) Extended IMPACT score (core + hypoxia, hypotension, CT findings), and (3) Lab IMPACT score (Extended + glucose and hemoglobin) to determine risk of mortality at 6 months [36]. Biomarkers were also examined independently for prediction of 6 month mortality. Correlations between the biomarkers were assessed using Spearman's rho. A comparison of biomarker levels between TBI and control subjects was performed using the Mann–Whitney U test. Data were assessed for equality of variance and distribution. Logarithmic transformations were conducted on non-normally distributed data. We examined the association between the biomarkers and the risk of 6 month mortality using multivariable proportional hazards (Cox) models. Variables included in the Cox proportional hazards models were the Core, Extended and Lab IMPACT scores, as well as the levels of each of the seven biomarkers measured within 24 h of injury. Different combinations of IMPACT scores and biomarkers were modeled. Area under the ROC curve (AUC) was calculated to determine performance of each model in predicting 6-months mortality. Analysis of the biomarkers included performance of each biomarker at 12-h post-injury and earliest CSF samples (mixture of samples obtained at 6, 12, 18 and 24 h post-injury). Significance was set at $p \leq 0.05$.

Results

There were at total 152 patients enrolled, 131 were TBI patients and 21 were control patients. Characteristics of the TBI patients are described in Table 1. Control patients were a mean age of 73 (SD8) (range 56–85), 68 % were male, and 5 % were Asian, 5 % Black, 5 % Hispanic and 85 % were white. The enrolled TBI patients were a mixture of those with isolated head injury and multiple traumas. In Table 2 types and severity of concomitant injuries, based on the abbreviated injury scale (AIS), are compared in those who survived and did not survive to 6 months. Concomitant injuries were equally distributed among the two groups with no statistically significant differences except for thoracic trauma.

Of the 131 TBI patients, 110 had CSF collected for biomarker analysis within 24 h of injury (Fig. 1). Thirty (27 %) patients did not survive to 6 months. Of these, 5 patients (17 %) died within 48 h of injury, fourteen (47 %) died between 48 h to a week, seven (23 %) died between 1-week and 1-month, and 4 patients (13 %) died between 1 to 3-months. No patients died between 3–6 months. Twenty one patients (70 %) of patients' deaths were directly associated with their TBI and the other 9 (30 %) had concomitant injuries or complications that may have contributed to their deaths in addition to the TBI. Two patients (7 %) died of brain death, 4 (13 %) died of traumatic/cardiac arrest, 6 (20 %) died from TBI complications, 13 (43 %) remained in a vegetative state and/or had care withdrawn, and 5 (17 %) died from non-neurological complications. Initial levels of biomarkers drawn within 24 h of injury are compared in those who did and did not survive to 6 months (Fig. 2).

Table 1 Patient characteristics

	TBI patients $N = 131$
Mean age (years) (SD)	38 (15)
Median age (range)	35 (18–83)
Gender (male/female) (% male)	102/29 (78 %)
Race	
Asian	1 (1 %)
Black	19 (15 %)
Hispanic	23 (18 %)
White	86 (66 %)
Other/unknown	2 (2 %)
Rotterdam score[a]	
1	1 (1 %)
2	29 (22 %)
3	44 (34 %)
4	28 (21 %)
5	25 (19 %)
6	4 (3 %)
Dichotomized post-resuscitation GCS score	
GCS 3–5	62 (47 %)
GCS 6–8	69 (53 %)
Post-resuscitation GCS motor score	
1	45 (34 %)
2	10 (8 %)
3	8 (6 %)
4	15 (12 %)
5	48 (37 %)
6	5 (4 %)
Pupils	
Both reactive	87 (66 %)
One reactive	12 (9 %)
None reactive	32 (24 %)
Prehospital hypoxia	53 (41 %)
Prehospital hypotension	13 (10 %)
Mortality at 6 months	36 (27 %)

Percentages are rounded and may not add up to 100 %

[a] The Rotterdam CT score was developed for prognostic purposes in TBI to determine the risk for mortality. It is based on CT findings of basal cistern compression, midline shift, presence of an epidural hematoma, and the presence of either intraventricular blood and/or traumatic subarachnoid hemorrhage

The temporal profile of the biomarkers in the first 24 h after injury, taken at 6 h intervals, is shown in Fig. 3. The biomarkers were all significantly elevated compared to controls ($p < 0.001$) and each showed different patterns of elevation. UCH-L1, S100B and SBDP150 decreased gradually over 24 h. MBP had a more labile pattern of a rapid rise followed by a rapid fall and a subsequent rise. MAP-2 and SBDP145

Table 2 Comparison of concomitant injuries in those who did and did not survive to 6 months

Abbreviated injury scale	Survived or lost to follow-up by 6 months $N = 95$	Did not survive by 6 months $N = 36$	p value
AIS face			
1	58 (61 %)	18 (50 %)	0.169
2	21 (22 %)	12 (33 %)	
3	12 (13 %)	6 (17 %)	
4	4 (4 %)	0 (0)	
5	0 (0)	0 (0)	
6	0 (0)	0 (0)	
AIS neck			
1	90 (95 %)	35 (97 %)	0.551
2	0 (0)	(0)	
3	5 (5 %)	1 (3 %)	
4	0 (0)	0 (0)	
5	0 (0)	0 (0)	
6	0 (0)	0 (0)	
AIS thorax			
1	51 (54 %)	10 (28 %)	0.005
2	10 (11 %)	7 (19 %)	
3	25 (26 %)	13 (36 %)	
4	7 (7 %)	5 (14 %)	
5	2 (2 %)	1 (3 %)	
6	0 (0)	0 (0)	
AIS abdomen			
1	75 (79 %)	27 (75 %)	0.758
2	10 (11 %)	6 (17 %)	
3	2 (2 %)	1 (3 %)	
4	6 (6 %)	2 (6 %)	
5	2 (2 %)	0 (0)	
6	0 (0)	(0)	
AIS spine			
1	91 (96 %)	35 (97 %)	0.693
2	4 (4 %)	1 (3 %)	
3	0 (0)	0 (0)	
4	0 (0)	0 (0)	
5	0 (0)	0 (0)	
6	0 (0)	0 (0)	
AIS upper extremity			
1	73 (77 %)	24 (67 %)	0.341
2	13 (14 %)	6 (17 %)	
3	8 (8 %)	5 (14 %)	
4	1 (1 %)	0 (0)	
5	0 (0)	1 (3 %)	
6	0 (0)	0 (0)	
AIS lower extremity			
1	69 (73 %)	24 (67 %)	0.535
2	14 (15 %)	6 (17 %)	
3	11 (12 %)	5 (14 %)	
4	1 (1 %)	0 (0)	
5	0 (0)	1 (3 %)	
6	0 (0)	0 (0)	

Abbreviated injury score code is on a scale of one to six with one being a minor injury and six being maximal

(1 = minor, 2 = moderate. 3 = serious, 4 = severe, 5 = critical, 6 = maximum)

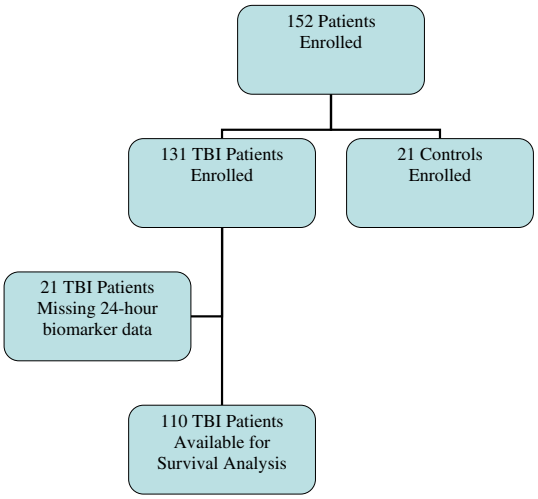

Fig. 1 Flow diagram

remained consistently elevated, with minor peaks and toughs. Interestingly, SBDP120 only began to rise after 12 h. In Fig. 4 the temporal profile of each biomarker is compared in patients who did and did not survive to 6 months. The peak (maximum) levels of UCH-L1, SBDP145, MAP-2, and MBP were significantly higher in those who did not survive to 6 months ($p < 0.05$). The correlations between the seven biomarkers are listed in Table 3. The highest correlation was found between MAP-2 and UCH-L1 ($rho = 0.81$).

We evaluated the biomarkers at a discrete time-point (12-h post-injury) and also at the earliest time-point the sample was available at in the first 24 h ("earliest" level was mixture of samples taken at 6, 12, 18 and 24 h post-injury). The rationale for the 12 h time-point was that many severe TBI patients are stabilized and admitted and have a ventriculostomy in place for fluid sampling by 12 h. The rationale for the "earliest" time-point is practice variation. Some clinicians may have access to samples earlier or later than 12 h so taking the "earliest" sample is most reflective of how the biomarker samples would be obtained in clinical practice. In our cohort there were 57 patients who had 12-h samples available for analysis and 110 patients with an "earliest time-point" available. The distribution of sample times in the "earliest time-point" group included 69 (63 %) enrollment samples (taken at the time of the ventriculostomy), 11 (10 %) samples taken at 6 h post-injury, 15 (14 %) samples at 12 h post-injury, 10 (9 %) samples at 18 , and 5 (5 %) samples at 24 h.

Survival analysis was conducted to assess the IMPACT models and biomarkers, both independently and in combination. At the 12-h post-injury there were 57 patients with biomarker data available. Of the three IMPACT Clinical Models (Core, Extended and Lab), the Extended Model had the highest unadjusted hazard ratio for mortality at 6 months 1.03 (95 % CI 1.01–1.05) ($p < 0.001$) and was selected for further analysis (Table 4). Of the seven biomarkers measured at 12 h post-injury ($N = 57$), the ones with the most significant unadjusted hazard ratios were MAP-2 ($p = 0.004$), UCH-L1 ($p = 0.024$), and MBP ($p = 0.037$) (Table 4). When the IMPACT Extended Model was combined with the biomarkers, MAP-2 was the

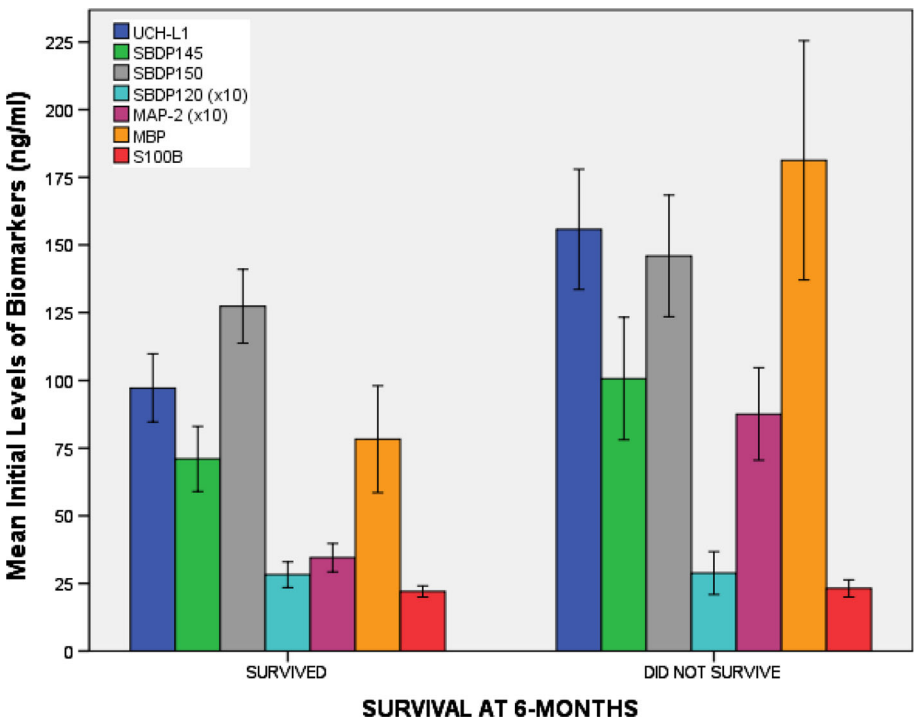

Fig. 2 Comparison of initial biomarker levels in patients who survived and did not survive over 6 months. *Bar graphs* represent mean values with standard errors. A comparison of the earliest 24-h level of each biomarker in patients who survived versus those who died within 6-months post-injury reveals that survivors had significantly lower biomarker levels than non-survivors

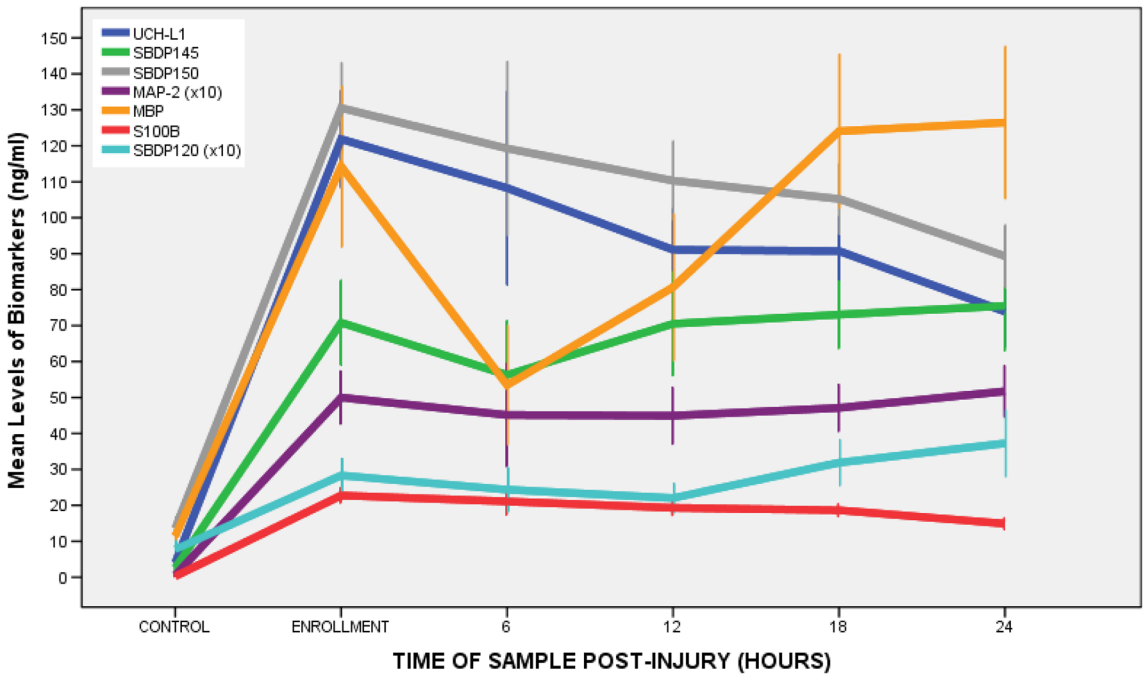

Fig. 3 Temporal profile of biomarkers over 24 h in TBI patients compared to control subjects. *Lines* represent mean values with standard errors. All biomarkers are significantly elevated in TBI patients compared to control subjects ($p < 0.001$). The number of available samples at each time-point was 21 controls, 23 at 6-h, 57 at 12-h, 83 at 18-h, and 89 at 24-h. MAP-2 and SBDP120 levels were multiplied by 10 in order to show their patterns more clearly on the graph

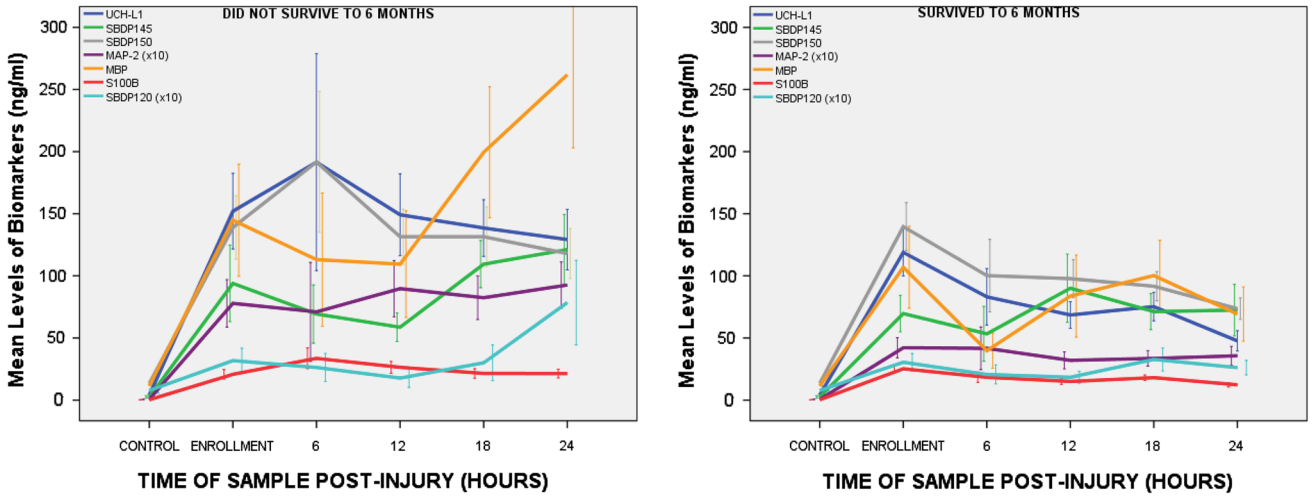

Fig. 4 Temporal profile of biomarkers over 24 h in all TBI patients who did and did not survive to 6 months. *Lines* represent mean values with standard errors. There are higher peaks of biomarkers in those who did not survive to 6 months compared to those who did survive

only biomarker that contributed significantly to the survival model (Table 5). IMPACT Extended Model and MAP-2 yielded adjusted hazard ratios of 1.04 (1.01–1.06) ($p = 0.001$) and 2.14 (1.31–3.50) ($p = 0.002$) respectively. There was no further benefit to including additional biomarkers.

At the "earliest" 24-h time-point there were 110 patients with biomarker data available for analysis. Concordant with the 12-h time-point, the Extended IMPACT Model had the highest unadjusted hazard ratio for mortality at 6 months 1.03 (95 %CI 1.02–1.05) ($p < 0.001$) and was selected for further analysis (Table 4). Of the seven biomarkers

Table 3 Correlation between the seven biomarkers

Biomarker combinations	Correlation coefficient (ρ)
UCH-L1–MAP-2	0.81
SBDP150–S100B	0.76
UCH-L1–SBDP150	0.72
SBDP150–MAP-2	0.70
UCH-L1–S100B	0.68
UCH-L1–SBDP145	0.67
UCH-L1–MBP	0.63
SBDP150–MBP	0.62
SBDP145–MBP	0.60
MAP-2–S100B	0.59
MAP-2–MBP	0.55
SBDP145–SBDP150	0.54
SBDP145–S100B	0.53
MBP–S100B	0.51
SBDP145–MAP-2	0.49
SBDP120–MBP	0.34
SBDP145–SBDP120	0.33
SBDP150–SBDP120	0.32
UCH-L1–SBDP120	0.29
SBDP120–S100B	0.25
SBDP120–MAP-2	0.20

measured at the earliest time post-injury (N = 110), the ones with the most significant unadjusted hazard ratios were MAP-2 ($p = 0.002$), UCH-L1 ($p = 0.016$), MBP ($p = 0.021$), and SBDP145 (0.029) (Table 4). When the IMPACT Extended Model was combined with the biomarkers, MAP-2 was the only biomarker that contributed significantly to the survival model (Table 5). IMPACT Extended Model and MAP-2 yielded adjusted hazard ratios of 1.03 (1.01–1.04) ($p = 0.001$) and 1.43 (1.04–1.95)($p = 0.026$) respectively. There was no further benefit to including additional biomarkers.

We explored cutoff points for IMPACT and MAP-2 derived from the ROC Curves for predicting mortality at 6 months. This exploratory analysis was intended to maximize the sensitivity. Using the 12 h time-point, the combination of an IMPACT Extended risk of death ≥24 % or MAP-2 level >1.0 ng/ml yielded a sensitivity of 100 %, a specificity of 19 % and a negative predictive value of 100 % (Table 6). Using the earliest 24 h level, the same combination of an IMPACT Extended risk of death ≥24 % and MAP-2 level >1.0 ng/ml yielded a sensitivity of 97 %, a specificity of 20 %, and a negative predictive value of 92 % (Table 6). Sensitivity of 12-h MAP-2 levels (without IMPACT) provided a sensitivity of 87 % and a specificity of 30 % (Table 6). Sensitivity of the earliest MAP-2 levels (without IMPACT) provided a sensitivity of 83 % and a specificity of 31 % (Table 6).

Table 4 Unadjusted hazard ratio estimates the risk of mortality at 6 months using IMPACT clinical models and cSF biomarkers

	Unadjusted hazard ratio (95 %CI) mortality at 6 months	p value
CSF biomarkers measured at 12 h	$N = 57$	
Impact clinical models		
IMPACT Core	1.03 (1.01–1.05)	0.014
IMPACT Extended	1.03 (1.01–1.05)	0.001[a]
IMPACT Lab	1.03 (1.01–1.05)	0.005
Biomarkers		
UCH-L1 at 12 h	2.07 (1.10–3.91)	0.024
SBDP145 at 12 h	1.08 (0.78–1.52)	0.636
SBDP150 at 12 h	1.50 (0.84–2.70)	0.174
SBDP120 at 12 h	0.93 (0.63–1.37)	0.709
MAP-2 at 12 h	2.04 (1.26–3.30)	0.004
MBP at 12 h	1.40 (1.02–1.92)	0.037
S100B at 12 h	1.10 (0.74–1.63)	0.654
Csf biomarkers measured at earliest timepoint in 24 h	$N = 110$	
Impact clinical models		
Impact core	1.04 (1.02–1.05)	<0.001
Impact extended	1.03 (1.02–1.05)	<0.001[a]
Impact lab	1.03 (1.02–1.05)	<0.001
Biomarkers		
UCH-L1 at earliest time in 24 h	1.61 (1.09–2.37)	0.016
SBDP145 at earliest time in 24 h	1.42 (1.04–1.95)	0.029
SBDP150 at earliest time in 24 h	1.20 (0.89–1.62)	0.221
SBDP120 at earliest time in 24 h	0.90 (0.63–1.16)	0.412
MAP-2 at earliest time in 24 h	1.62 (1.19–2.20)	0.002
MBP at earliest time in 24 h	1.31 (1.04–1.65)	0.021
S100B at earliest time in 24 h	1.02 (0.81–1.28)	0.892

[a] Most significant model

Discussion

This study describes a cohort of non-penetrating severe TBI patients who underwent clinical and biomarker evaluation within 24 h of injury. Clinical IMPACT models were used to assess 6 month outcome using baseline clinical data [36]. IMPACT models were originally developed using a patient cohort of 8,509 patients with moderate and severe TBI and have subsequently been validated [40]. The early prediction of outcome in TBI allows for baseline risk establishment and clinical considerations for patient care.

Table 5 Adjusted hazard ratio estimates risk of mortality at 6 months using IMPACT clinical models and biomarkers

	Adjusted hazard ratio (95 % CI) mortality at 6 months	*p* value
Clinical model and biomarkers measured at 12 h	$N = 57$	
IMPACT extended	1.04 (1.01–1.06)	0.001
MAP-2 at 12 h	2.14 (1.31–3.50)	0.002
Biomarkers alone measured at 12 h		
MAP-2 at 12 h	2.03 (1.25–3.30)	0.004
Clinical model and biomarkers measured at earliest time in 24 h	$N = 110$	
IMPACT extended	1.03 (1.01–1.04)	0.001
MAP-2 at earliest time in 24 h	1.43 (1.04–1.95)	0.026
Biomarkers alone measured at earliest time in 24 h		
MAP-2 at earliest time in 24 h	1.58 (1.16–2.15)	0.004

Our hypothesis was that initial neuronal biomarkers measured in CSF would improve upon the clinical IMPACT prediction models of mortality. We found that, indeed, biomarkers contributed significantly to the clinical IMPACT models, particularly MAP-2. In fact, biomarkers had predictive ability, independent of the clinical models. In practice, this would be especially useful when certain pieces of clinical information and CT evaluation are not readily available at the time of assessment.

Depending on the hospital center or the patient's condition, CSF may be accessible at different times during the first 24 h after injury. Therefore, the analysis included biomarker evaluation at a discrete time-point (12-h post-injury) and at the earliest available sample time-point within 24 h ("earliest" level). The rationale for these two different types of analyses was to see how the biomarkers could be applied clinically. The results had better sensitivity using a single time-point analysis than the "earliest" time-point yet both provided important prognostic information to the clinical models. We suspect that because a number of CSF samples in the "earliest time point" group were obtained at or before 6-h post-injury, levels were not given as much time to become elevated as they were at 12-h, leading to reduced sensitivity. This underscores the need to evaluate biomarkers at multiple time-points within the first 24 h. The next step in determining optimal times for clinical use of each biomarker will be to formally assess temporal profiles by conducting biokinetic analyses. Much like biomarkers for other organ diseases, there is a pattern of release, peak and duration that is specific to each biomarker. For example, in myocardial ischemia troponin may not appear until 3–6 h after the start of ischemia. So, if troponin is measured at 1 h, the results will not be as accurate.

Although there are a number of biochemical markers that have been investigated in TBI, the most extensively studied among these are glial protein S-100 beta (β) [6–16], neuron-specific enolase (NSE) [17–22], and myelin basic protein (MBP) [20, 23–26] S100β is the major low affinity calcium binding protein in astrocytes [41] and it is considered a marker of astrocyte injury or death. Alpha-II-spectrin (280 kDa) is the major structural component of the cortical membrane cytoskeleton and is particularly abundant in axons and presynaptic terminals [42, 43]. It is also a major substrate for both calpain and caspase-3 cysteine proteases [44, 45]. Evaluation of these breakdown products in humans with severe TBI, are encouraging [27–30]. A promising candidate biomarker for TBI currently under investigation is Ubiquitin C-terminal Hydrolase-L1 (UCH-L1). UCH-L1 was previously used as a histological marker for neurons due to its high abundance and specific expression in neurons [46]. Clinical studies in humans with severe and mild TBI have confirmed that UCH-L1 is significantly elevated after injury and is associated with important clinical outcomes [31–34]. Another unexplored neurobiomaker is microtubule-associated protein (MAP-2). It is a dendritic marker of both acute damage and chronic neuronal regeneration after injury and can be detected in serum of survivors after severe TBI [35].

Although these biomarkers were collected from CSF, many are or have become available for use in serum [34, 47, 48]. Recently, Czeiter et al. [47] evaluated three biomarkers, including glial fibrillary acidic protein (GFAP), Ubiquitin C-terminal hydrolase (UCH-L1) and αII-spectrin break down product of 145 kDa (SBDP145) in a cohort of 45 severe TBI patients. They compared the biomarkers only to the IMPACT Core model and showed that

Table 6 Performance of the combination of IMPACT extended model together with MAP 2 as well as the biomarkers alone

	Sensitivity	Specificity	NPV	PPV
IMPACT $\geq$ 24 % and MAP-2 (at 12 h) $>$ 1 ng/ml	100 % (75–100)	19 % (7–39)	100 % (46–100)	41 % (25–58)
MAP-2 (at 12 h) $>$ 1 ng/ml	87 % (58–98)	30 % (14–50)	80 % (44–96)	41 % (24–59)
IMPACT $\geq$ 24 % and MAP-2 (earliest) $>$ 1 ng/ml	97 % (81–100)	20 % (11–34)	92 % (60–100)	40 % (29–53)
MAP-2 (earliest) $>$ 1 ng/ml	83 % (65–94)	31 % (20–46)	77 % (54–91)	40 % (28–54)

Cutoff points for IMPACT and MAP-2 were derived from the ROC Curves for predicting mortality at 6 months

biomarkers improved outcome prediction. Accordingly, our results also show the value of adding biomarkers to clinical parameters. We explored seven distinct biomarkers in a larger cohort of patients, together with all three IMPACT clinical models (Core, Extended, and Lab). The biomarker that consistently improved prognostic performance in our large cohort was MAP-2, a biomarker that has not been assessed in any other acute clinical TBI trial to date. MAP-2 is a relatively novel biomarker in human TBI and is a major component of cytoskeleton family proteins. It is localized predominantly in dendrites and is associated with promoting microtubule assembly and stability [49, 50]. It has been shown to be altered following TBI in animal models and is associated with dendritic damage [51, 52].

We performed an exploratory analysis of cutoff points for IMPACT and MAP-2 based on the points from the ROC to determine sensitivity and specificity of the model using both discrete 12-h levels and earliest 24-h levels. The combination of IMPACT and MAP-2 yielded a sensitivity of 100 % for predicting mortality with a specificity of 19 %. Using the same cutoff points, we also assessed the performance of MAP-2 alone and found a sensitivity of between 83–87 % with a much higher specificity of 30 %. There is always a trade-off between sensitivity and specificity and we chose to maximize sensitivity at the expense of the specificity. With a high sensitivity the biomarkers would be useful in "ruling out" (i.e. low risk of) mortality if levels were below the threshold, suggesting good chances for survival at 6-months. The converse would not be true however. The importance of these findings is that clinicians could make management decisions with the help of a readily available biomarker, as is done with cardiac ischemia, renal dysfunction or liver dysfunction. In practice, this would be especially useful when certain pieces of clinical information and CT evaluation are not readily available at the time of assessment.

Limitations

While these data are encouraging, the authors recognize there are limitations to this study. Clinical management dictated when the ventriculostomy was placed and therefore, patients had a variable number of samples available for analysis at the different time points during the first 24 h. The most common surgically placed monitors for intracranial pressure monitoring in severe TBI patients are intraventricular catheters (ventriculostomy). However, intraparenchymal catheters are becoming an increasingly popular alternative to ventriculostomy for in many countries as they are easier to use, less invasive and can be inserted in the ICU by non-neurosurgeons [53]. Since the purpose of the study was to identify biomarkers that are related to outcome in CSF, intraventricular catheters were the logical choice for study purposes. More importantly, it was standard of care at the participating institutions. Now that we've identified promising biomarkers in CSF, the same biomarkers are being examined in blood, where they could have more widespread application. As an exploratory analysis these results provide a starting point for larger studies that can validate these results. This is an important step forward in the management of patients with brain injuries, similar to the use of blood lactate levels in predicting mortality in critically ill patients [54, 55].

Although the overall mortality rate in our study seems higher than average there is a lot of variability in mortality rate in TBI studies, depending on initial injury severity. In 2011, Lingsma et al. examined between-center differences in outcome after moderate and severe TBI as part of the International Mission on Prognosis and Clinical Trial Design in TBI (IMPACT), and found mortality rates in the different studies ranged from 17 % to 44 % with an average of 27 % [56]. Based on published data, the patients in our study are fairly typical for a severe TBI study, and the outcomes are fairly typical for these patients as well even if they are at the higher end of what is reported.

There is a significant age difference between TBI subjects and control subjects in our study. Control subjects were patients without TBI requiring CSF drainage for other medical conditions such as for routine anesthetic or surgical management (e.g. endovascular aortic aneurysm stent repair, selected orthopedic procedures) or chronic hydrocephalus. Accordingly, they had a tendency of being older. However, it has been shown that older adults have higher levels of neuronal biomarker at baseline [57]. Therefore, older controls are actually a more robust control group as there levels are higher.

Additionally, there is a need to further explore these biomarkers in subpopulations of patients with TBI who have concomitant diagnoses of dementia, Parkinson's disease, stroke or prior TBI. Based on the current results, we cannot recommend a change in patient management. However, if validated, biomarker levels could be used to determine severity of injury, stratify patients into clinical trials and gauge effectiveness of therapy. Accordingly, they would be helpful in discussing prognosis with families and making decisions about futility of care earlier. Additionally, biokinetic analyses of these data will be crucial in quantifying the temporal patterns of each biomarker.

Conclusions

These data suggest that early CSF levels of MAP-2 in combination with clinical data provide enhanced

prognostic capabilities for mortality at 6 months in patients with severe TBI. These findings have implications for improved clinical decision-making early after injury. Further validation of these findings in a larger cohort of patients will be required before clinical implementation.

Conflict of interest Drs. Gabrielli, Hannay, Heaton, Robertson, Robicsek, and Schmalfuss have no competing financial interests.

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Appendix 1

See Table 7.

Ubiquitin C-terminal Hydrolase (UCH-L1)

UCH-L1 sandwich ELISA (swELISA) was performed in accordance with previously published studies [29, 32–34, 58–60]. Both mouse monoclonal antibody (capture antibody) and rabbit polyclonal antibody (detection antibody) were made in-house at Banyan Biomarkers Inc. against recombinant human UCH-L1 full-length protein and protein A purified. Plates were coated with capture antibody in 0.05 M sodium bicarbonate, pH 9.6 overnight at 4 °C. Blocking and washing buffer was Tris buffered saline with 0.05 % Tween-20 (v/v) (TBST). Antigen standard (UCH-L1 standard curve: 0.78–200 ng/mL; unknown samples: 10 µL of CSF) were incubated with detection antibody overnight and then added to the plate for 2 h. After washing, secondary anti-rabbit-IgG HRP (GE Healthcare) was added and incubated for 1 h. Plates were developed with substrate solution Ultra-TMB ELISA (Pierce# 34,028), stopped with acidic solution and read at 450 nm with a spectrophotometer (Molecular Device SpectraMax 190). The interassay CV was 2–8 % while intraassay CV was 2–11 % within the dynamic range. The limit of detection (LOD) was 0.03 ng/mL.

αII-Spectrin Breakdown Products 150 kDa (SBDP150), 145 kDa (SBDP145), 120 kDa (SBDP120)

SBDP150, SBDP145 and SBDP120 swELISAs were constructed similarly to those described previously [29]. Briefly, a 96-well plate was coated with 100 μL/well capture antibody (5ug/ml purified goat polyclonal anti-SBDP150 [28, 61] or 10ug/ml rabbit anti-SBDP145 or 10ug/ml anti-SBDP120 [62] overnight at 4 °C. Antigen used was partially purified human brain αII-spectrin for SBDP150 or recombinant glutathione-S-transferase– αII-spectrin (including the SBDP145 cleavage site in repeat 13–18) fusion protein cleaved with either calpain-1 (1: 40 ratio for 10 min at 4C) for SBDP145 production or with caspase-3 (1 : 20 ratio for 4 h at room temperature) for SBDP120. After blocking buffer (Startingblock T20-PBS), SBDP150 calibrator (10 × dilution factor, 1.17 ng/ml–300 ng/ml), SBDP145 calibrator (10X dilution factor, 1–500 ng/ml) and SBDP120 Calibrators (3X dilution factor, 0.9–120 ng/mL) or samples were added (CSF, 10 µL for SBDP150, SBDP145; 34 uL for SBDP120) with diluent (total volume 100 uL) to the wells. After washing, plates were incubated with affinity purified detection antibody (mouse monoclonal anti-αII-spectrin antibody (Biomol FG6090 or equivalent). If amplification was needed, biotinyl-tyramide solution (Perkin Elmer Elast Amplification Kit) was added, washed and followed by Streptavidin-HRP (1:500) in PBS with 0.02 % Tween-20 and 1 % BSA. Lastly, the wells were developed with chemiluminescent substrate solution (SuperSignal ELISA Femto, Pierce) for 1 min and read by a luminescence microplate reader (GloRunner DXL Luminometer, Turner BioSystems). The interassay and intraassay CV were $<$3–14 % within the assay dynamic range. The LOD was 1.54 ng/mL for SBDP150, 0.98 ng/mL for SBDP145 and 0.474 ng/mL for SBDP120.

Table 7 Lower (LLoD) and upper (ULoD) limit of detection for all biomarkers

Assay:	MAP2 CSF	MBP CSF	SBDP150 CSF	SBDP145 CSF	SBDP120 CSF	S100β CSF	UCH-L1 CSF
Upper limit of detection (ng/ml)	22.320	669.570	450.000	500.000	183.000	42.000	507.000
Lower limit of detection (ng/ml)	0.054	0.13	1.542	0.98	0.474	0.032	0.03
Imputed concentration for values lower than the LLoD (ng/ml)	0.027	0.065	0.771	0.490	0.237	0.016	0.015

Imputed values were used for concentrations below the LLoD. Half of lowest level made up the value of the LLoD

Microtubule Associated Protein 2 (MAP-2)

MAP-2 sandwich ELISA was performed using 10 uL CSF for quantitative determination. Mouse MAb anti-MAP2A/2B (clone M13, Zymed #13-1,500) was used as capture antibody (5 ug/well) to coat the plate. Biofluid samples (10 uL CSF, or recombinant antigen as GST-fusion protein with residue 1,078–1,551 of MAP-2 at 0.10–6.67 ng/mL) were added with diluent (100 uL total) to microtiter plate wells. After 2 h incubation and washing, HRP-labeled mouse monoclonal anti-MAP-2 (clone AP20; BD Bioscience; #552,320) antibody was added. After washing, plates were developed with substrate solution Ultra-TMB ELISA (Pierce# 34,028), stopped with acidic solution and read at 450 nm with a spectrophotometer (Molecular Device SpectraMax 190). The interassay and intraassay CV were <15 % within the assay dynamic range. Limit of detection (LOD) was determined to be 0.054 ng/mL.

S100B

S100B sandwich ELISA was performed using 5–10 uL CSF for quantitative determination. Mouse monoclonal anti-S100b was used as capture antibody (3ug/well) to coat the plate. After blocking buffer, biofluid samples (5–10 uL CSF) or standard protein (S100beta, human brain protein, Fitzgerald, at 0.0039 ng/ml–0.5 ng/ml) were added. After 30 min incubation and washing, detection polyclonal antibody was used and incubated for 1 h (1 ug/ml and 100 ul/well), followed by HRP-conjugated anti-rabbit-HRP (Jacksonville ImmunoResearch lab) for 30 min. After washing, plates were developed with substrate solution Ultra-TMB ELISA (Pierce# 34,028), stopped with acidic solution, and read at 450 nm with a microplate spectrophotometer (Molecular Device SpectraMax 190). The interassay and intraassay CV were <10 % within the assay dynamic range with a limit of detection (LOD).

Myelin Basic Protein (MBP)

MBP assay was based on commercial MBP ELISA for CSF (iPOC) according to manufacturer's instructions. Briefly, 50 uL of calibrator (0.13–36 ng/mL) or 4 uL CSF samples with diluent (to 50 uL) was used and incubated with plate with capture antibody (goat polyclonal anti-MBP) for 2 h. After washing, 50 uL detection mouse monoclonal antibody to MBP was added (50 uL) and incubated for 30 min, followed by HRP-enzyme-conjugated secondary donkey anti- mouse IgG antibody. After washing, 50 uL of chromogenic TMB substrate was used for 15 min. 100 uL stop solution was added and absorbance at 450 nm was measured with a spectrophotometer (Molecular Device Spectramax 190). The interassay and intraassay CV were <10 % within the assay dynamic range. The limit of detection was determined to be 0.13 ng/ml.

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