Dynamics of extracellular matrix proteins in cerebrospinal fluid and serum and their relation to clinical outcome in human traumatic brain injury

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Abstract

Background: Brevican, neurocan, tenascin-C and tenascin-R are extracellular matrix proteins present in brain that show increased expression in experimental animal models of brain injury. However, little is known about the dynamics of these proteins in human body fluids, such as cerebrospinal fluid (CSF) and serum, after traumatic brain injury (TBI). The aims of this study were to investigate if matrix proteins in CSF and serum are associated with functional outcome following traumatic brain injury, if their concentrations change over time and to compare their levels between brain injured patients to controls.

Methods: In total, 42 traumatic brain injury patients, nine healthy controls and a contrast group consisting of 38 idiopathic normal pressure hydrocephalus patients were included. Enzyme-linked immunosorbent assays (ELISAs) were used to measure the concentrations of proteins.

Results: Increased concentrations of brevican, tenascin-C and tenascin-R in CSF correlated with unfavourable outcome, with stronger outcome prediction ability compared to other biomarkers of brain tissue injury. CSF brevican, tenascin-R and serum neurocan gradually decreased with time ($p = 0.04$, $p = 0.008$, $p = 0.005$, respectively), while serum tenascin-C ($p = 0.01$) increased. CSF concentrations of brevican, neurocan and tenascin-R (only in time point 3) after TBI were lower than in the idiopathic normal pressure hydrocephalus group ($p < 0.0001$, $p < 0.0001$, and $p = 0.0008$, respectively). In serum, tenascin-C concentration was higher and neurocan lower compared to healthy controls ($p = 0.02$ and $p = 0.0009$).

Conclusions: These findings indicate that levels of extracellular matrix proteins are associated with clinical outcome following TBI and may act as markers for different pathophysiology than currently used protein biomarkers.

Keywords: brevican; neurocan; tenascin-C; tenascin-R; traumatic brain injury.

Introduction

Traumatic brain injury (TBI) results from a head trauma induced by external mechanical forces injuring the brain, affecting over 10 million people annually worldwide [1]. There are many causes of TBI, including motor vehicle accidents, firearms, falls and high-impact sports [1]. According to the World Health Organization, TBI will be one of the major cause of death and acquired disability by 2020 [1].
More severe TBI causes acute neuronal, glial and microvascular injury, and triggers a neurophysiological cascade that exacerbates cell death in the already affected brain [2].

The CSF is in close proximity to the brain extracellular matrix (ECM) providing a reflection of biochemical changes that occur within this organ [3]. Thus, it is believed that pathological changes in the brain ECM following TBI are reflected in the CSF as, e.g. altered protein levels. To date, there are a few promising CSF biomarkers (biological markers of tissue fate) for brain injury after TBI, including neurofilament light (NFL) [4–6], total tau (t-tau) [7–9], S100B [10, 11], neuron-specific enolase (NSE) [11] and glial fibrillary acidic protein (GFAP) [12]. CSF NFL, NSE and t-tau are suggested markers of neuronal damage [2, 13], while S100B and GFAP primarily have been suggested to be markers of glial cell damage [14, 15].

Measurement of biomarkers in peripheral blood represents a less invasive way of monitoring pathologic changes in the brain [16]. Potential blood biomarkers for brain tissue injury following TBI include S100B and GFAP reflecting primarily astroglial injury and NSE [17, 18], NFL [6, 19], myelin basic protein (MBP), ubiquitin C-terminal hydrolase-L1 (UCHL-1), phosphorylated neurofilament heavy (NFH) as well as t-tau as tentative markers of axonal and neuronal injury [20]. Pathologic brain changes following TBI are complex and there is a great need to characterise additional neurochemical indicators reflecting additional pathophysiological processes in TBI.

Outcome assessment after TBI can be done in different ways, where the most widely adopted measure of functional outcome is the five category Glasgow Outcome Scale (GOS) is primarily used to measure functional outcome [21]. Early prognostication of outcome after TBI is of importance for guiding treatment decisions and to provide patients and relatives with realistic long-term outlooks [22]. Biomarkers of biological and pathogenic processes may be used as a clinical tool in outcome prediction [23]. Even though serum NFL and S100B both have been proposed to be important clinical outcome predictors in TBI patients [6, 10, 19, 24], there is a lot of unexplained variance in the prediction models, suggesting a potential role for additional markers explaining different pathophysiology.

Brevican, neurocan, tenasin-C and tenasin-R are extracellular matrix (ECM) proteins expressed in the brain and produced by both neurons and glial cells [25, 26]. Brevican and neurocan belong to the chondroitin sulfate proteoglycans (CSPGs) family, the most abundant proteoglycan group in the CNS [27]. Tenasin-C and tenasin-R are large glycoproteins that serve as ligands to CSPGs in the CNS [25, 28]. Tenasin-R, CSPGs and hyaluronic acid contribute to the formation of perineuronal nets (PNNs), which are specialized ECM structures surrounding cell bodies and proximal neuronal dendrites [25]. ECM molecules such as proteoglycans and glycoproteins are responsible for stabilisation of synaptic connections and also play important roles in cell proliferation, migration and differentiation [25]. CSPGs and tenasin-R are also expressed in various pathological conditions, for example, constituting the major inhibitory component of glial scars [29, 30], which, however, do not include tenasin-C [31]. Glial scars may have both protective and detrimental effects on axonal regeneration. Hence, they limit the spread of the damage, but also form a barrier around the damaged area that repels axonal regeneration [29]. It is believed that CSPG together with tenasin-R are associated with these inhibitory processes playing an important role in axon guidance preventing axon growth and regeneration [27, 30]. A few studies have reported elevated levels of extracellular matrix compounds after brain injury. For example, brevican, neurocan, tenasin-R and tenasin-C were increased in nerve, brain and spinal cord scar tissues of several animal models of neuronal tissue injury [32–37]. In addition, increased serum levels of tenasin-C have been reported in human TBI [38] and the CSF tenasin-C concentration has been shown to positively correlate with subarachnoid haemorrhage severity [39]. Collectively, these findings suggest that ECM proteins participate in the pathophysiology of TBI. However, to what degree concentrations of brevican, neurocan and tenasin-R in CSF and serum are altered in TBI patients has not been explored.

Materials and methods

In the TBI cohort, 42 patients requiring neuro-critical care and intracranial monitoring for their injuries were included. They were recruited as a part of a prospective study between 2007 and 2010. Samples were drawn at three time points after TBI: time point 1 (1–5 days), time point 2 (4–8 days), time point 3 (6–12 days) (Table 1). The management of these TBI patients has been described in detail in a previous study [19]. Two freeze-thaw cycles were involved at start of the study.

Ventricular CSF and serum were collected through external ventricular drains (EVD) and arterial lines, respectively, in TBI patients. In total, CSF samples from 42 and serum from 40 TBI patients were included (Table 1). Functional outcome was defined by the GOS [21], assessed at 12 months following injury in the TBI patients. Favourable outcome was defined by GOS 4 and 5 categorizing moderate and low disability, respectively, while unfavourable outcomes were explained by GOS 1 and 3 representing death and severe disability, respectively. There were no patients in the cohort assessed as GOS 2.
Commercially available Human Brevican, Neurocan, Tenascin-C and Tenascin-R sandwich ELISA kits (RayBiotech, Norcross, GA, USA) were utilized to measure protein concentrations in CSF and serum except for tenascin-R that was not detectable in serum (unpublished results). Due to volume constraints serum brevican and serum except for tenascin-R that was not detectable in serum were utilized to measure protein concentrations in CSF and serum. In CSF, the dilution factors were: 1:400 for brevican, 1:10 for neurocan and 1:2 for both tenascins. In addition to the commercially available assays, an in-house ELISA was developed for brevican. Nunc-Immuno Polysorp microwell modules (Thermo Fisher Scientific, Waltham, MA, USA) were coated with mouse monoclonal anti-human brevican antibody (immunogen: aa23-911) (1 μg/mL, US Biological Life Sciences, Salem, MA, USA) in carbonate buffer, pH 9.6, overnight at +4 °C. After washing with PBS/0.05%Tween, wells were blocked with 0.5% Bovine Serum Albumin (Sigma-Aldrich, Saint Louis, MO, USA) in PBS/0.05%Tween for 1 h at room temperature. As a calibrator, recombinant brevican standard corresponding to aa23-911 (R&D, Minneapolis, MN, USA) was used in the 5–0.08 ng/mL concentration range. CSF samples together with CSF pools were diluted 1:8 in 0.5% Bovine Serum Albumin (Sigma-Aldrich, Saint Louis, MO, USA) in PBS/0.05%Tween. Next, after washing, they were incubated for 1 h at room temperature, gently shaking. For the detection – biotinylated monoclonal mouse anti-human brevican antibody (immunogen: aa23-911, with the ability to detect the detection – biotinylated monoclonal mouse anti-human brevican antibody (immunogen: aa23-911, with the ability to detect

### Table 1: Demographics and biomarkers.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>TBI (n=42)</th>
<th>Controls (n=9)</th>
<th>iNPH (n=38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>32 (76%)</td>
<td>6 (67%)</td>
<td>28 (74%)</td>
</tr>
<tr>
<td>Female</td>
<td>10 (24%)</td>
<td>3 (33%)</td>
<td>10 (26%)</td>
</tr>
<tr>
<td>Age, median (IQR)</td>
<td>58 (22)</td>
<td>25 (5.0)</td>
<td>67 (11)</td>
</tr>
<tr>
<td>Trauma severity scoring, median (IQR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rotterdam CT</td>
<td>4 (2.00)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stockholm CT</td>
<td>3 (1.43)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIS</td>
<td>5 (1.00)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECM proteins, median (IQR), ng/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In CSF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brevican commercial</td>
<td>92.9 (106)</td>
<td>83.8 (77.6)</td>
<td>77.5 (65.8)</td>
</tr>
<tr>
<td>Neurocan</td>
<td>1.50 (1.51)</td>
<td>0.877 (0.772)</td>
<td>0.455 (0.243)</td>
</tr>
<tr>
<td>Tenascin-C</td>
<td>6.63 (7.95)</td>
<td>10.1 (9.55)</td>
<td>7.17 (11.8)</td>
</tr>
<tr>
<td>Tenascin-R</td>
<td>0.524 (1.34)</td>
<td>0.749 (1.40)</td>
<td>0.383 (0.310)</td>
</tr>
<tr>
<td>In serum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurocan</td>
<td>1.16 (0.990)</td>
<td>1.08 (0.787)</td>
<td>0.947 (0.471)</td>
</tr>
<tr>
<td>Tenascin-R</td>
<td>32.5 (27.6)</td>
<td>47.1 (30.5)</td>
<td>48.7 (26.1)</td>
</tr>
</tbody>
</table>

AIS, abbreviated injury scale; ECM, extracellular matrix; iNPH, idiopathic normal pressure hydrocephalus; IQR, interquartile range; TBI, traumatic brain injury.
the previous unpublished studies, the intra-variability for in-house assay targeting brevican was equal to 15%, while inter-assay CV% was lower than 17%.

**Statistical analyses**

Because CSF and serum sample measurements were non-normally distributed (examined by the Kolmogorov-Smirnov test), non-parametric tests were used. The differences between healthy controls, iNPH contrast group and TBI patients (each time point separately) were investigated using the Mann-Whitney U test. To compare the differences between the longitudinal measurements from TBI patients, a linear mixed effects model was applied on repeated measures of ECM proteins as dependent variables, time as a fixed factor and individuals as random factors. The mixed analysis included age and sex as covariates. The selection of model covariance structures was done by comparing diagonal and autoregressive models for each protein via the likelihood ratio test. Further post-hoc comparisons were made among the three time points by comparing estimated marginal means. The differences in the outcome for the TBI patients were analysed using the Mann-Whitney U test for individual biomarkers. Standardized effect size measurement between both controls and TBI (time point 1) groups was performed using Cohen’s d (η). While the effect size between time points was measured by partial eta squared (ηp). Correlations were assessed using Spearman’s rank correlation. Analysis of covariance was used to investigate the differences between control/ iNPH contrast and TBI groups accounting for the effect of age. Receiver operating characteristic (ROC) analysis was performed to predict the unfavourable outcome (severe disability and death) of TBI patients based on the ECM protein level (time point 1) and to evaluate the prognostic ability of the levels of these proteins compared to other biomarkers for the brain injury following TBI. Areas under the curve (AUC) together with sensitivities and specificities were obtained as measures of performance for the tests. The analyses were performed using SPSS software, version 25 or GraphPad Prism, version 7. All tests were two-sided and statistical significance was defined as p ≤ 0.05.

**Data availability**

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

**Results**

**ECM proteins in CSF**

There were no changes in CSF brevican concentrations measured by a commercially available assay within outcome classification (Figure 1A), however, for the in-house assay, higher concentrations of CSF-brevican were associated with lower GOS levels (Figure 1B). In the in-house assay, ROC analysis showed that brevican in CSF have high discriminant capacity between unfavourable and favourable outcomes (AUC = 0.86, p = 0.0008, Table 2). For the commercial assay there was no difference between longitudinal samples from TBI patients (Figure 1D), while this change was observed using the in-house assay (p = 0.04) (Figure 1E). Effect perceptible in size (ηp > 0.1) was shown over the different time points of TBI patients using the in-house assay (Figure 1E). In addition, the CSF brevican concentration was significantly decreased in TBI patients at each time point compared with iNPH group in both assays (Table 1). Brevican measured using a commercial assay showed large effect size (η > 0.8) between TBI patients (time point 1) and iNPH group.

There were no changes in CSF neurocan concentration within outcome classification or within longitudinal samples (Figure 1C, F). However, concentrations in TBI patients (in all three time points) were significantly lower compared to the iNPH group (Table 1). When comparing TBI patients (time point 1) to the iNPH group, neurocan in CSF showed a large effect size (η > 0.8).

CSF tenascin-C concentration in TBI patients was positively associated to worse outcome (Figure 1G) and ROC analysis showed that it could discriminate between the two outcome groups (AUC = 0.87, p = 0.0008, Table 2). However, there was no difference in CSF tenascin-C concentration within longitudinal samples or between TBI and iNPH groups (Figure 1I and Table 1).

CSF tenascin-R concentration was higher in patients with unfavourable vs. favourable outcome (p = 0.001, Figure 1H) and differentiated the two groups with an AUC of 0.85 (p = 0.001, Table 2). Tenascin-R concentration decreased over time (p = 0.008) following the brain injury and its levels in TBI patients were significantly lower compared to the iNPH group (time point 3, p = 0.0008) (Figure 1J and Table 1).

**ECM proteins in serum**

Neurocan concentration in serum could not discriminate between favourable and unfavourable outcomes in TBI patients (Figure 2A). However, there were significant differences between longitudinal samples, where neurocan decreased with time (p = 0.005, Figure 2C). In addition, the concentration of neurocan in serum from TBI patients at each time point was lower as compared to healthy controls (Figure 2C).

There were no differences in tenascin-C levels in relation to outcome (Figure 2B), though concentrations gradually increased over time after TBI (p = 0.01, Figure 2D). The concentrations of tenascin-C in serum from TBI patients (all three time points) was elevated compared to healthy
When comparing TBI patients (time point 1) to the healthy control group, tenascin-C in serum showed a large effect size ($\eta > 0.8$).

Higher tenascin-C CSF/serum ratios were associated with an unfavourable clinical outcome ($p = 0.01$, Supplementary Figure 2B), whereas neurocan CSF/serum ratios did not separate the two TBI outcome groups (Supplementary Figure 2A).

There is no correlation between tenascin-C in CSF and serum (Supplementary Figure 1).
Other biomarkers for brain injury

The ROC analysis of other biomarkers reflecting brain injury following TBI showed that both CSF NFL and serum S100B have a high discriminatory power for accurately separating the favourable and unfavourable outcome groups (AUC = 0.77, p = 0.005 and AUC = 0.70, p = 0.03, respectively) (Table 2).

**Table 2:** Summary of ROC analysis of ECM proteins and other biomarkers for brain injury following TBI in CSF and serum.

<table>
<thead>
<tr>
<th>Body fluid</th>
<th>Biomarkers</th>
<th>AUC</th>
<th>Cut off, ng/mL</th>
<th>Specificity, %</th>
<th>Sensitivity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF</td>
<td>ECM proteins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brevican commercial</td>
<td>0.68</td>
<td>52.1</td>
<td>58</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>Brevican in-house</td>
<td>0.86&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.61</td>
<td>100</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>Neurocan</td>
<td>0.49</td>
<td>2.25</td>
<td>17</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Tenascin-C</td>
<td>0.87&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.680</td>
<td>100</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>Tenascin-R</td>
<td>0.85&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.19</td>
<td>92</td>
<td>80</td>
</tr>
<tr>
<td>Other biomarkers</td>
<td>S100B</td>
<td>0.74</td>
<td>12.0</td>
<td>63</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>NSE</td>
<td>0.73</td>
<td>58.8</td>
<td>75</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>NFL</td>
<td>0.77&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.47</td>
<td>75</td>
<td>76</td>
</tr>
<tr>
<td>Serum</td>
<td>ECM proteins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neurocan</td>
<td>0.60</td>
<td>1.10</td>
<td>50</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>Tenascin-C</td>
<td>0.65</td>
<td>26.2</td>
<td>50</td>
<td>81</td>
</tr>
<tr>
<td>Other biomarkers</td>
<td>S100B</td>
<td>0.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.30</td>
<td>100</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>NSE</td>
<td>0.57</td>
<td>12.1</td>
<td>47</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>NFL</td>
<td>0.67</td>
<td>0.198</td>
<td>52</td>
<td>63</td>
</tr>
</tbody>
</table>

<sup>a</sup>Statistical significance in biomarker concentrations between favourable and unfavourable outcomes in TBI. <sup>b</sup>p ≤ 0.05. <sup>c</sup>p ≤ 0.01. <sup>d</sup>p ≤ 0.001.

AUC, area under the curve; ECM, extracellular matrix; NFL, neurofilament light; NSE, neuron specific enolase; ROC, receiving operator characteristic; S100B, S100 calcium-binding protein B.

**Figure 2:** Association of ECM proteins in serum to TBI outcome with comparison of their level in TBI patients (three time points = TP1, TP2, TP3) to healthy controls. Neurocan (A, C) and tenascin-C (B, D) ELISA assays were utilized to detect ECM protein concentrations in serum. Levels are represented as median with interquartile range. Statistical significance: comparing to control group: *, p ≤ 0.05, ***, p ≤ 0.001, ****, p ≤ 0.0001 and in longitudinal analysis: ##, p ≤ 0.01.

**Discussion**

To the best of our knowledge, this is the first report describing ECM proteins as potential CSF biomarkers for clinical outcome prediction in TBI. The results presented show that brevican, tenascin-C and tenascin-R in CSF predict clinical outcome in TBI to a similar degree as currently known biomarkers, i.e. S100B, NSE and NFL [6, 10, 11, 18, 19, 24]. In serum, ECM proteins (neurocan and tenascin-C) showed stronger prognostic ability compared to NSE, but not to NFL or S100B. There is a great need to develop additional biomarkers for outcome prediction and for elucidating different aspects of TBI pathophysiology. Our results suggest that ECM proteins may be similarly accurate outcome predictors as previously established biomarkers of TBI, making them promising novel biomarker candidates for TBI assessment. Moreover, some of the ECM proteins are detected not only in CSF, but also in serum, which makes them potential predictive markers of outcome following TBI in both body fluids. However, serum S100B and NFL were better markers of outcome compared to the available ECM proteins in serum which could be caused by potential degradation in serum.

Brevican in-house assay, tenascin-C and tenascin-R levels in CSF correlate with each other (rho: 0.61–0.87, p ≤ 0.001, Supplementary Figure 1), which suggest that
they might measure same pathological processes in the brain. Their correlations with CSF-NFL levels (rho: 0.53–0.73, p ≤ 0.05, Supplementary Figure 1) further support that ECM proteins might be related to axonal damage. CSF-S100B levels correlate with both tenascins (rho: 0.59–0.64, p ≤ 0.05, Supplementary Figure 1), which suggest that these glycoproteins might also measure astroglial injury. No correlation between neurocan and currently known biomarkers (Supplementary Figure 1) suggests that neurocan levels might represent different pathophysiology than previously used structural biomarkers.

Another finding of this study is that there is a statistically significant temporal change in the concentrations of neurocan and tenascin-C in serum (Figure 2C, D) in addition to brevican and tenasin-R in CSF (Figure 1E, J) after injury. A decrease in concentration is observed for brevican and tenasin-R which is likely due to a clearance mechanism of the different proteins after an injury-induced initial increase. Tenasin-C in serum behaves differently – its level increases with time in a way that is not seen in CSF. Notably, brevican, neurocan and tenasin-R are brain enriched [41–43], while tenascin-C is not only present in the brain, but also highly expressed in muscle tissues [44, 45]. The different change of tenasin-C level over time compared to other ECM proteins might be explained by the origin of this analyte in the periphery rather than CNS, which is also supported by the higher concentrations measured in serum compared to CSF and the lack of correlation between these fluids for tenasin-C (Supplementary Figure 1). Extracranial injuries, which were present in 31% of the TBI patients, could cause release of tenasin-C from muscle tissue to the blood. Thus, increased concentration of tenasin-C in serum of TBI patients could be a result of its leakage from muscles to serum. The CSF/serum ratios of tenascin-C were higher than in controls with the same origin as CSF from TBI patients (EVD), iNPH CSF sampled from EVD were included in the study as a contrast group. It is important to note that this study group might not reflect the same ECM protein changes in CSF as healthy controls. Both TBI and iNPH groups share similar pathophysiological changes involving microglia and astroglial activation, where various ECM proteins are being released [27, 47]. Also, the elevation of ECM-proteins in iNPH patients found here indicates that these proteins can also reflect more chronic disease states of the brain.

If high concentrations of the ECM proteins are a consequence of injury, it would be expected for the iNPH contrast group to have lower concentrations compared to TBI, which is not observed. This inconsistency can be attributable to the fact that iNPH is a disorder and therefore does not necessary reflect homeostatic concentrations in healthy individuals: hypothetically, iNPH pathophysiology could affect CSF levels of ECM proteins. However, at this time it cannot be excluded that preanalytical factors connected to the different cohorts contribute to this observation.

The use of two different assays for brevican can be justified by the fact that even though the antibodies employed are directed against brevican, the two assays seem to detect different species of the protein (different fragments, isoforms or splice variants) based on the moderate correlation between the assays (rho: 0.66, Supplementary Figure 1). The different relations were also observed between these two assays compared to other proteins. CSF brevican measured by commercially available assay correlates with neurocan (rho: 0.69, p ≤ 0.001, Supplementary Figure 1), whereas this relation was not observed for brevican in-house. Moreover, in-house brevican correlated with NFL levels in CSF (rho: 0.53, p ≤ 0.005, Supplementary Figure 1), whereas this correlation was not observed for the commercial assays. This indicates that the two brevican assays might detect two various brevican species possibly related to different pathological processes.

The study has a number of limitations that should be acknowledged including the numerous gaps in the follow-up measurements of longitudinal data, analysis of various biomarkers on different subsets of the samples, the small cohort of healthy controls, overlapping time points in repeated measurements and lack of CSF collected through EVDs for healthy controls. In fact, previous study has indicated certain differences in the protein composition of CSF sampled from ventricles and the lumbar cistern [46]. Due to the inability of finding CSF samples from healthy controls with the same origin as CSF from TBI patients (EVD), iNPH CSF sampled from EVD were included in the study as a contrast group. It is important to note that this study group might not reflect the same ECM protein changes in CSF as healthy controls. Both TBI and iNPH groups share similar pathophysiological changes involving microglia and astroglial activation, where various ECM proteins are being released [27, 47]. Also, the elevation of ECM-proteins in iNPH patients found here indicates that these proteins can also reflect more chronic disease states of the brain.

The control and iNPH group were gender-matched to the TBI patients. We were not able to age-match controls to TBI patients, so the TBI group has a wider age range. However, if controlled for age, the differences between TBI patients and healthy controls in neurocan (all time points) and tenascin-C (time point 3) concentrations in serum as well as all observed differences of ECM protein concentrations in CSF between the TBI patient and iNPH cohorts, remain significant. In the future, we will seek verification of the results presented in other, independent datasets to investigate their clinical relevance.

In conclusion, this study demonstrates significant changes in ECM proteins following TBI. Large effect sizes reveal that the results are not only statistically, but also substantially significant in magnitude. CSF levels of
brevican, tenascin-R and tenascin-C might discriminate the various clinical outcomes in TBI patients similar to other CSF biomarkers, including S100B, NSE and NFL. This indicates the ability of these ECM biomarkers for clinical outcome prediction in TBI making them promising biomarker candidates for TBI assessment.

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**Employment or leadership:** In case of non-competing interests, unrelated to this work, KB has served as a consultant or at advisory boards for Alzheon, CogRx, Biogen, Novartis, and Roche Diagnostics, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg. HZ has served on scientific advisory boards for Roche Diagnostics, Samumed, CogRx and Wave and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg.

**Honorarium:** None declared.

**Competing interests:** The authors report no competing financial interests. The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

**References**


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