Blood Biomarkers for Brain Injury in Concussed Professional Ice Hockey Players

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IMPORTANCE Lack of objective biomarkers for brain damage hampers acute diagnosis and clinical decision making about return to play after sports-related concussion.

OBJECTIVES To determine whether sports-related concussion is associated with elevated levels of blood biochemical markers of injury to the central nervous system and to assess whether plasma levels of these biomarkers predict return to play in professional ice hockey players with sports-related concussion.

DESIGN, SETTING, AND PARTICIPANTS Multicenter prospective cohort study involving all 12 teams of the top professional ice hockey league in Sweden, the Swedish Hockey League. Two hundred eighty professional ice hockey players from 12 teams contesting during the 2012-2013 season consented to participate. All players underwent clinical preseason baseline testing regarding concussion assessment measures. Forty-seven players from 2 of the 12 ice hockey teams underwent blood sampling prior to the start of the season. Thirty-five players had a concussion from September 13, 2012, to January 31, 2013; of these players, 28 underwent repeated blood sampling at 1, 12, 36, and 144 hours and when the players returned to play.

MAIN OUTCOMES AND MEASURES Total tau, S-100 calcium-binding protein B, and neuron-specific enolase concentrations in plasma and serum were measured.

RESULTS Concussed players had increased levels of the axonal injury biomarker total tau (median, 10.0 pg/mL; range, 2.0-102 pg/mL) compared with preseason values (median, 4.5 pg/mL; range, 0.06-22.7 pg/mL) (P < .001). The levels of the astrogial injury biomarker S-100 calcium-binding protein B were also increased in players with sports-related concussion (median, 0.075 μg/L; range, 0.037-0.24 μg/L) compared with preseason values (median, 0.045 μg/L; range, 0.005-0.45 μg/L) (P < .001). The highest biomarker concentrations of total tau and S-100 calcium-binding protein B were measured immediately after a concussion, and they decreased during rehabilitation. No significant changes were detected in the levels of neuron-specific enolase from preseason values (median, 6.5 μg/L; range, 3.45-18.0 μg/L) to postconcussion values (median, 6.1 μg/L; range, 3.6-12.8 μg/L) (P = .10).

CONCLUSIONS AND RELEVANCE Sports-related concussion in professional ice hockey players is associated with acute axonal and astrogial injury. This can be monitored using blood biomarkers, which may be developed into clinical tools to guide sport physicians in the medical counseling of athletes in return-to-play decisions.
concussion in athletes practicing contact sports such as ice hockey, American football, and boxing is a growing problem worldwide. The term is often used interchangeably with mild traumatic brain injury (TBI). During recent years, many professional athletes have retired owing to chronic symptoms after repeated concussions. An international consensus statement defined concussion as a complex pathophysiological process affecting the brain, induced by traumatic biomechanical forces. Mild concussion causes no loss of consciousness, but many other symptoms such as dizziness, nausea, reduced attention and concentration, memory problems, and headache may occur. More severe concussion also causes unconsciousness. The pathophysiology underlying concussion is an acute disturbance of neuronal function together with damage to neuronal and glial cells, which may have both subacute and chronic consequences. Most concussions resolve within days to weeks, but 10% to 15% remain symptomatic more than 1 year after injury. The danger of developing chronic or even progressive symptoms has been linked to repeated concussions before the brain has recovered properly. Tools to detect and monitor injury to the central nervous system (CNS) due to concussion are therefore of great importance and could objectively inform decisions on when return to play (RTP) may be safe.

Cerebrospinal fluid (CSF) is in direct contact with the brain parenchyma and thus is a suitable biofluid to monitor biochemical changes in the CNS. Elevated levels of CSF biomarkers of axonal damage, eg, total tau (T-tau) and neurofilament light (NFL), are found after acute damage to the brain such as stroke and subarachnoid hemorrhage, and the levels of these biomarkers correlate with the severity of the brain damage. Cerebrospinal fluid T-tau has been measured in severe TBI, and its concentrations correlate with 1-year outcome. In the context of contact sports, CSF NFL and T-tau have been shown to increase in amateur boxers, even after bouts without any knockout. Further, the CSF levels of NFL and T-tau correlate with the number and severity of received head blows and return to normal levels after a period of rest from boxing. These findings indicate that CSF biomarkers may be used to identify brain damage in concussed athletes and as a guide for medical teams in RTP decisions. However, owing to the invasive nature of lumbar puncture, these results may be difficult to implement in the routine evaluation and follow-up of concussed athletes. Thus, analysis of brain injury biomarkers in blood would be preferable. Neuron-specific enolase (NSE) is a biomarker for neuronal injury, and an increase in serum levels of NSE was found in boxers who received repetitive trauma to the head. Similarly, increases in NSE and the glial cell biomarker S-100 calcium-binding protein B (S-100B) were found in serum of amateur boxers who received direct punches to the head compared with boxers who received body punches. Last, using a novel ultrasensitive method to measure tau in plasma, increased plasma tau concentrations were found in Olympic boxers after bout, with normalization of levels after a resting period.

The diagnosis of sports-related concussion is based on the evaluation of subjective symptoms after exclusion of overt gross structural brain damage. Thus, reliable biomarkers to assess TBI and recovery in concussed athletes are highly desirable. In this study, we examined concentrations of plasma T-tau and serum S-100B and NSE in professional ice hockey players who had a sports-related concussion. Postconcussion levels of T-tau, NSE, and S-100B were compared with preseason levels in a cohort of hockey players prior to the start of the hockey season.

Methods

Study Population

This is a prospective cohort study of concussion among professional ice hockey players from the Swedish Hockey League who competed during the first half of the 2012-2013 ice hockey season. The Swedish Hockey League is the top professional ice hockey league in Sweden with 12 teams, each consisting of 24 players. The study was approved by the Ethics Committee for Medical Research at the University of Gothenburg and by the Swedish Ice Hockey Association. Written informed consent was obtained from all participants. The teams’ physicians were present at all regular season games, documenting signs and symptoms of concussion and physical examination findings in the event of a concussion. Physicians also recorded the date when a player had completely recovered from his concussion and was able to return to unrestricted competition. During the preseason, the team physicians were provided with a concussion kit containing an injury protocol, the Rivermead Post Concussion Symptoms Questionnaire, instructions for blood tests, blood sampling equipment and tubes, and instructions for the local laboratory to handle blood samples.

All players were examined physically and with the Standardized Assessment of Concussion before the start of the season. Players from 2 of the contesting teams, Frölunda Hockey Club and Luleå Hockey, were sampled for baseline values prior to the start of ice hockey season and before the players went on ice. Players from Luleå Hockey were also sampled immediately after a friendly game without concussion incidents. In players who sustained head injury or concussion during the season, consecutive blood samples were collected at 1, 12, 36, and 48 hours as well as 144 hours after the injury or the date on which the player returned to unrestricted competition. The diagnosis of concussion was made according to the latest diagnostic guidelines on sports-related concussion, and players with concussion were managed according to these guidelines.

Biochemical Procedures

Blood samples were collected by venipuncture into gel-separator tubes for serum and ethylenediaminetetraacetic acid (EDTA) tubes for plasma and centrifuged within 20 to 60 minutes. Serum and plasma were separated, aliquoted, and stored at −80°C pending biochemical analysis. Samples for S-100B and NSE were analyzed on a Modular E170 instrument (Roche Diagnostics) with reagents from the same manufacturer. Plasma T-tau was measured with a novel immunoassay using digital array technology (Quanterix Corp) as previously described. The limit of detection for the assay is 0.02 pg/mL, which is more...
than 1000-fold more sensitive than conventional immunoas-
says for the protein. The assay uses Tau 5 monoclonal anti-
body for capture (Covance) and HT7 and BT2 monoclonal an-
tibodies for detection (Thermo Scientific Pierce). This
combination of antibodies reacts with both normal and phos-
phorylated tau with epitopes in the midregion of the mol-
ecule, making the assay specific for all tau isoforms. All samples
were analyzed at the same time using the same batch of re-
agents by board-certified laboratory technicians who were
blinded to clinical information. Intra-assay coefficients of varia-
tion were below 3% for S-100B and NSE and 11.5% for T-tau.

Statistical Analysis
For the paired observations, the Wilcoxon signed rank test was
used. For the TBI vs preseason comparison, the Mann-
Whitney U test was used, and for multiple group compari-
sions, the Kruskal-Wallis test was used. The Spearman rank cor-
relation coefficient (ρ) was used for analyses of correlation
between changes in various biomarker levels after concus-
sion. Linear regression analysis examined the prediction of con-
cussion severity by postconcussion biomarker levels. The cu-
bic spline interpolation for graphical representation of the
biomarker changes was conducted with SPSS version 20.0 sta-
tistical software (IBM Corp). The area under the receiver op-
erating characteristic curve (AUC) was calculated for all bio-
markers in concussed players vs biomarker changes after a
friendly game. Statistical significance was determined at
P < .05. All statistical calculations were performed using SPSS
version 20.0 and GraphPad Prism version 5.0 (GraphPad Inc).

Results
Of a total 288 professional ice hockey players, 35 had a sports-
related concussion between September 13, 2012, and January
31, 2013. We included 28 of these players in the study. The re-
maining concussed players either declined to participate or had
an uncertain diagnosis of concussion. Three of the included
players had concussion with unconsciousness and 25 experi-
cenced symptoms such as headache, confusion, dizziness, or
nausea. Many players were free from symptoms less than 6 days
after injury, but in 15 players the symptoms lasted longer than
6 days. The players’ postconcussion biomarker results were
compared longitudinally as well as with preseason values from
47 players from 2 of the competing teams. There were no sig-
nificant correlations between biomarker levels at baseline and age (eFigure in Supplement).

The T-tau levels were significantly higher in postconcus-
sion samples (all times) compared with preseason samples
(P = .002) (Figure 1A). There was no significant increase in
the levels of S-100B and NSE after concussion compared with preseason samples (Figure 1B and C). However, the levels of both T-tau (P < .001) and S-100B (P < .001) were higher immediately after a concussion com-
pared with preseason samples (Table 1). There was no sig-
nificant difference in the levels of NSE (Table 1). Further, the
levels of S-100B and NSE, but not T-tau, increased after a
friendly game without concussion compared with baseline
(eTable in Supplement).

Table 1. Demographic Data and Blood Concentrations of Total Tau, Neuron-Specific Enolase,
and S-100 Calcium-Binding Protein B

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Preseason (n = 47)</th>
<th>Postconcussion* (n = 28)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>28 (19-38)</td>
<td>27 (19-40)</td>
<td>.40</td>
</tr>
<tr>
<td>T-tau, pg/mL</td>
<td>4.5 (0.06-22.7)</td>
<td>10.0 (2.0-171)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>S-100B, μg/L</td>
<td>0.045 (0.005-0.45)</td>
<td>0.075 (0.037-0.24)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>NSE, μg/L</td>
<td>6.1 (3.6-12.8)</td>
<td>6.5 (3.45-18.0)</td>
<td>.20</td>
</tr>
</tbody>
</table>

Abbreviations: NSE, neuron-specific enolase; S-100B, S-100 calcium-binding protein B; T-tau, total tau.
* The biomarker values are from 1 hour after concussion.
The highest levels of T-tau were measured during the first hour after concussion. These levels declined during the first 12-hour period ($P < .001$) (Figure 2A). Further declines between 12 and 144 hours in T-tau were not statistically significant ($P = .15$), and the level of T-tau remained elevated 144 hours after concussion compared with preseason samples ($P = .002$). There was a delayed increase in T-tau between 12 and 36 hours after concussion, with a trend for a second T-tau peak at 36 hours after concussion (Figure 3A).

Similar to T-tau, the levels of S-100B peaked during the first hour after concussion and declined during the first 12-hour period ($P < .001$) (Figure 2B). However, unlike T-tau, we detected no delayed elevation of S-100B between 12 and 36 hours after concussion (Figure 3B). The levels of NSE remained unchanged over time in concussed players (Figure 2C and Figure 3C).

**Severity of Concussion**

Assessing the severity of TBI is important in the management of sports-related concussion. Recently, the emphasis has been on the time it takes until a player is declared fit to return to unrestricted competition. We graded concussions according to the latest guidelines, which are based on the resolution of concussion symptoms. We graded the players with concussion into 4 categories: (1) those who were free from symptoms and had safe RTP within 6 days after a concussion ($n = 12$), (2) those who had safe RTP 7 to 10 days after concussion ($n = 9$), (3) those who had safe RTP more than 10 days after concussion ($n = 7$), and (4) those who also had loss of consciousness due to the head impact ($n = 3$). The level of T-tau 1 hour after concussion was not statistically significantly different between the concussion categories; however, there were trends toward higher levels in players who had symptoms for more than 10 days or had loss of consciousness (Figure 4A). The levels of S-100B 1 hour after concussion were significantly higher in players with loss of consciousness ($P < .05$) and those with RTP more than 10 days after concussion ($P < .01$) than in players with concussion symptoms resolving within 6 days (Figure 4B). There was no significant difference in the levels of NSE at 1 hour after concussion between these different concussion categories (Figure 4C).

The concentration of T-tau 1 hour after concussion correlated with the number of days it took for concussion symptoms to resolve ($\rho = 0.60$; 95% CI, 0.23 to 0.90; $P = .002$) (Figure 4A). Similar to T-tau, there was a significant correlation between the concentration of S-100B and the time to resolution of concussion symptoms ($\rho = 0.75$; 95% CI, 0.50 to 0.90; $P < .001$) (Figure 4B). The concentration of NSE did not correlate with the resolution of concussion symptoms ($\rho = 0.20$; 95% CI, −0.20 to 0.55; $P = .30$) (Figure 4C). Using linear regression, only T-tau concentrations at 1 hour after concussion predicted the number of days it took for the concussion symptoms to resolve and the players to return to unrestricted competition (Figure 4D-F).

As postconcussion sampling at 1 hour may not always be practical, we examined the relationship between biomarker elevation 144 hours after concussion and persistence of postconcussive symptoms (PCS). The levels of T-tau 144 hours after concussion correlated with the number of days it took for concussion symptoms to resolve ($\rho = 0.60$; 95% CI, 0.23 to 0.90; $P = .002$) (Figure 4A). Similar to T-tau, there was a significant correlation between the concentration of S-100B and the time to resolution of concussion symptoms ($\rho = 0.75$; 95% CI, 0.50 to 0.90; $P < .001$) (Figure 4B). The concentration of NSE did not correlate with the resolution of concussion symptoms ($\rho = 0.20$; 95% CI, −0.20 to 0.55; $P = .30$) (Figure 4C). Using linear regression, only T-tau concentrations at 1 hour after concussion predicted the number of days it took for the concussion symptoms to resolve and the players to return to unrestricted competition (Figure 4D-F).
The spline curves represent the concentration time course of total tau (T-tau) (A), S-100 calcium-binding protein B (S-100B) (B), and neuron-specific enolase (NSE) (C) in relation to preconcussion levels, estimated on the basis of preseason values from 2 complete teams, from 1 hour after concussion until players return to unrestricted competition. The vertical lines represent the time of brain injury.
after concussion remained significantly elevated in players with PCS for more than 6 days as well as vs T-tau levels after a friendly game (Table 2). However, there was no significant difference in the levels of S-100B and NSE 144 hours after concussion in players with PCS for less than 6 days and players with persistent PCS or in biomarker changes after a friendly game (Table 2).

Diagnosis Accuracy of the Biomarkers

The AUC for T-tau 1 hour after concussion vs T-tau after a friendly game (AUC = 0.80; 95% CI, 0.65-0.94) was greater than that for S-100B (AUC = 0.67; 95% CI, 0.52-0.83) and NSE (AUC = 0.55; 95% CI, 0.37-0.70) (Figure 5A). The AUC for T-tau improved to 0.91 (95% CI, 0.81-1.00) when comparing T-tau concentrations 1 hour after concussion in players with PCS for more than 6 days vs T-tau concentrations after a friendly game, while the AUCs for S-100B and NSE remained almost unchanged (Figure 5B). Further, the AUC for T-tau 144 hours after concussion in players with PCS for more than 6 days vs T-tau concentrations after a friendly game was also higher (AUC = 0.76; 95% CI, 0.58-0.94) than that for S-100B and NSE (Figure 5C).

Discussion

The major finding of this study was that the plasma levels of T-tau increased in ice hockey players with sports-related concussion. The highest concentrations of T-tau were measured immediately after the injury, and the levels declined during the first 12 hours followed by a second peak between 12 and 36 hours. Importantly, T-tau concentrations at 1 hour after concussion predicted the number of days it took for the concussion symptoms to resolve and the players to have safe RTP. The biomarker with greatest diagnostic accuracy was T-tau, with an AUC of 0.91 when comparing T-tau concentrations in samples collected 1 hour after concussion

Table 2. Blood Concentrations of Total Tau, Neuron-Specific Enolase, and S-100 Calcium-Binding Protein B 144 Hours After Concussion vs Biomarker Changes After a Friendly Game

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>After Friendly Game, Median (Range)</th>
<th>PCS &lt;6 d</th>
<th>PCS &gt;6 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-tau, pg/mL</td>
<td>5.8 (0.06-12.45)</td>
<td>6.5 (0.93-88.0)</td>
<td>9.5 (4.0-164.8)</td>
</tr>
<tr>
<td>S-100B, μg/L</td>
<td>0.06 (0.0-0.10)</td>
<td>0.05 (0.03-0.07)</td>
<td>0.06 (0.03-0.23)</td>
</tr>
<tr>
<td>NSE, μg/L</td>
<td>8.3 (3.8-12.7)</td>
<td>6.5 (3.2-14.1)</td>
<td>5.4 (3.2-13.8)</td>
</tr>
</tbody>
</table>

Abbreviations: NSE, neuron-specific enolase; PCS, postconcussive symptoms; S-100B, S-100 calcium-binding protein B; T-tau, total tau.

* Compared with biomarker values after a friendly game.
with T-tau concentrations in samples collected 1 hour after a friendly game. Further, high T-tau levels at 144 hours after concussion correlated with persistence of PCS. Although there was no significant increase in serum levels of S-100B in postconcussion samples (all times) compared with preseason samples, S-100B levels at 1 hour after concussion were significantly higher compared with preseason values. There was no significant difference in the levels of NSE after concussion compared with preseason levels, and the levels remained unchanged over time following concussion.

Total tau is one of the most CNS-specific proteins with high expression in unmyelinated cortical axons. To our knowledge, this is the first study to measure T-tau in serial plasma samples from professional athletes with sports-related concussion. Recently, an ultrasensitive digital immunobassay for the quantification of tau in serum was developed, and T-tau levels were measured in patients who were resuscitated following cardiac arrest. In these patients, the levels of T-tau correlated strongly with clinical outcome, with the highest levels found in patients with poor outcome. Similar to the patients with cardiac arrest, using the same method, we also noticed a biphasic release of T-tau in concussed hockey players. Bimodal kinetics for biomarkers reflecting tissue damage have also been found in other disorders, such as acute myocardial infarction in which a bimodal increase in serum troponin is found. The bimodal increase in plasma tau after mild concussion in hockey players might in a similar way represent an initial release of cytosolic tau followed by a later release of microtubule-bound tau from injured axons.

In the context of TBI, previous studies of T-tau have been on ventricular CSF, showing increased levels of T-tau in patients with severe TBI, and the levels in ventricular CSF correlate with the severity of injury and clinical outcome. However, these studies compared the levels of T-tau in ventricular CSF with levels in controls of lumbar CSF, which are not comparable owing to the gradient difference between ventricular and lumbar CSF. The finding that T-tau is increased in plasma following sports-related concussion suggests that this indeed is associated with axonal injury. In this study, the levels of T-tau normalized when the players returned to unrestricted competition, suggesting that T-tau may be a potential biomarker to monitor the course of recovery in athletes with TBI. Further, the levels of T-tau after concussion correlated with the duration of PCS, which implies that T-tau levels immediately following concussion may be used to predict the severity of the TBI.

In this study, S-100B levels also increased immediately after a concussion compared with the preseason levels, and the levels of S-100B at 1 hour after concussion correlated with the duration of PCS. The S-100B concentration reflects astroglial injury, and previous studies have shown that serum levels of S-100B correlate with scores on the Glasgow Coma Scale and neuroradiologic findings at hospital admission. However, a limitation of S-100B is that it is also expressed in extracerebral cell types, and, as in previous studies, the levels of S-100B increased after a friendly game without concussion compared with baseline.

The levels of NSE remained unchanged over time following concussion as well as compared with the preseason values. Neuron-specific enolase is a marker of general neuronal injury. In the context of TBI, higher levels of NSE have been reported in CSF from nonsurvivors compared with survivors following severe TBI, and increased levels correlate with TBI severity scores in both children and adults. The major limitation of NSE in CSF is that it is highly sensitive to in vitro lysis of erythrocytes from blood contamination of the samples. Further, similar to S-100B, the levels of NSE increased after a friendly game without concussion compared with baseline. The findings of this study argue against NSE as a sensitive marker of neuronal injury in athletes with sports-related concussion.
The main limitations of this study were the relatively small sample size, which restricts the possibilities of examining biomarker levels in relation to different forms and severities of concussion, and that the biomarkers analyzed might also not be optimal. Total tau is highly CNS specific; however, CSF studies show that NFL may be a more sensitive marker than T-tau for TBI. Further, we did not have access to preseason samples for all ice hockey players, which would have made it easier to evaluate the longitudinal change in biomarker levels after concussion.

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Conflict of Interest Disclosures: Drs Shahim and Zetterberg had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Shahim, Tegner, Blennow, Zetterberg. Acquisition of data: Shahim, Tegner, Wilson, Randall, Kalbberg. Analysis and interpretation of data: All authors. Drafting of the manuscript: Shahim, Tegner, Blennow, Zetterberg. Critical revision of the manuscript for important intellectual content: Tegner, Wilson, Randall, Skillbäck, Pazooki, Kalbberg, Blennow, Zetterberg. Statistical analysis: Shahim, Skillbäck, Blennow, Zetterberg. Obtained funding: Tegner, Blennow, Zetterberg. Administrative, technical, or material support: Tegner, Wilson, Randall, Skillbäck, Pazooki, Kalbberg, Blennow. Study supervision: Tegner, Blennow, Zetterberg.

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