Prospective Assessment of Acute Blood Markers of Brain Injury in Sport-Related Concussion

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Abstract

There is a pressing need to identify objective biomarkers for the assessment of sport-related concussion (SRC) to reduce the reliance on clinical judgment for the management of these injuries. The goal of the current study was to prospectively establish the acute effects of SRC on serum levels of S100 calcium-binding protein beta (S100B), glial fibrillary acidic protein (GFAP), and ubiquitin C-terminal hydrolase-L1 (UCH-L1). Collegiate and high school football players were enrolled and provided blood at pre-season. Injured athletes participated in follow-up visits at ~6 and 24–48 h following documented SRC ($n=32$). Uninjured football players participated in similar follow-up visits and served as controls ($n=29$). The median time between injury and blood collection was 2 h (6 h visit) and 22.5 h (24–48 h visit) in concussed athletes. Concussed athletes had significantly elevated UCH-L1 levels at the 6 h visit relative to pre-season levels ($Z=2.22$, $p=0.03$) and levels in control athletes ($Z=3.02$, $p=0.003$). Concussed athletes also had elevated S100B at 6 h relative to pre-season ($Z=2.07$, $p=0.04$) and controls ($Z=2.75$, $p=0.006$). Both markers showed fair discrimination between concussed and control athletes (UCH-L1 area under receiver operating characteristic curve [AUC] [95% CI]=0.74 [0.61–0.88], S100B AUC=0.72 [0.58–0.87]). Percent-change of UCH-L1 and S100B at 6 h relative to pre-season also showed fair discrimination (AUC=0.79 [0.66–0.92] and AUC=0.77 [0.64–0.90]). GFAP levels did not differ between groups or in concussed athletes relative to pre-season. This study provides prospective evidence of significant increases in serum levels of UCH-L1 and S100B during the early acute period following SRC, and lays the foundation for future studies examining the clinical potential for blood-based biomarkers in the early detection of concussion.

Keywords: GFAP; mild traumatic brain injury; S100B; UCH-L1

Introduction

THE DIAGNOSIS OF SPORT-RELATED CONCUSSION (SRC) is often challenging because of the subjective and nonspecific nature of self-reported symptoms and a variety of non-injury factors that may affect accurate symptom reporting (e.g., underreporting in athlete samples).¹ Therefore, there is a pressing need to identify objective biomarkers for the assessment of SRC to reduce the reliance on clinical judgment for the management of these injuries. In the broader domain of civilian traumatic brain injury (TBI), blood biomarkers have garnered great interest because of the relative ease with which these markers can be obtained and tested, as well as their cost effectiveness relative to other potential biomarkers (e.g., neuroimaging).^{2,3}

A variety of blood biomarkers that are linked with the neurophysiological consequences of brain injury, including cell damage and inflammation/gliosis, have been proposed. Several recent studies of mild and moderate TBI patients recruited from hospital emergency departments and trauma centers have demonstrated

promising results for ubiquitin C-terminal hydrolase-L1 (UCH-L1), glial fibrillary acidic protein (GFAP), and S100 calcium-binding protein beta (S100B). UCH-L1 is highly specific to and abundant in neurons and has shown promise as a marker of neuronal damage following brain injury. For example, acute increases in serum or plasma levels are observed in TBI patients of all severities and are associated with injury severity, outcome, and the presence of intracranial lesions.^{4–10} GFAP, a marker of glial damage, has also been consistently shown to be elevated following brain injury and to correlate with injury severity and outcome.^{4,7,9–14} Although found both within and outside the nervous system, S100B has also shown promise as a marker of astrocytic damage following brain injury.^{15,16} S100B is elevated following mild traumatic brain injury (mTBI) and has demonstrated some utility in detecting the presence of intracranial lesions, although with less success than GFAP.^{11,17–20}

To date, work investigating these blood biomarkers in SRC has been relatively limited. Two studies have prospectively assessed

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S100B levels following SRC, reporting elevations in acute (1–3 h) post-injury versus pre-season levels^{21,22} and increases following combat sport matches and football games during which athletes sustained blows to the head.^{23–25} UCH-L1 levels also rise from pre- to post-football game, implying sensitivity to subconcussive head impact exposure.¹⁹ Therefore, there is a need to more systematically study the utility of UCH-L1, GFAP, and S100B for the assessment of SRC, which is often less severe than other forms of mTBI typically studied in the biomarker literature (e.g., where intracranial lesions are commonly reported). Moreover, the sport laboratory assessment model (SLAM) provides the unique opportunity to assess pre-injury biomarker concentrations in individuals at high risk for SRC, providing a truly prospective assessment of the effects of mTBI that is not feasible in non-SRC.²⁶

The goal of this study was to prospectively establish the acute effects of SRC on three candidate brain injury biomarkers. Serum levels of S100B, GFAP, and UCH-L1 were measured in football players at pre-season and at multiple time points following SRC. Matched, uninjured football player controls participated in the same protocol. We hypothesized that serum levels of S100B, GFAP, and UCH-L1 would be elevated during the acute phase post-concussion relative to concussed athletes' own pre-season levels, as well as levels in control athletes.

Methods

High school and collegiate football players were recruited from Project Head to Head-II, an ongoing prospective study of SRC in athletes from southeastern Wisconsin. Baseline, pre-season enrollment of 546 athletes occurred prior to the 2015 football season and included the collection of blood biospecimens from all athletes. Exclusion criteria included any contraindication to study procedures, injury that would preclude participation in the study protocol, current psychotic disorder, current narcotic use, and history or suspicion of conditions known to cause cognitive dysfunction (e.g., epilepsy, moderate-to-severe TBI). Adult participants and parents of minors provided written informed consent; minors completed written assent. The institutional review board at the principal investigator's institution approved the study.

Of the 546 athletes enrolled at baseline, 32 sustained a concussion during the 2015–2016 football season and were evaluated within 6 h, 24–48 h, and 8, 15, and 45 days post-concussion. Initial identification and diagnosis of concussion was determined by certified athletic trainers and/or team physicians at each institution. Study investigators then screened and triaged all injuries to confirm that they met study definition and requirements. The study definition of concussion was based on the Centers for Disease Control and Prevention HEADS UP educational initiative: *An injury resulting from a forceful bump, blow or jolt to the head that results in rapid movement of the head and causes a change in the athlete's behavior, thinking, physical functioning, or the following symptoms: headache, nausea, vomiting, dizziness/balance problems, fatigue, difficulty sleeping, drowsiness, sensitivity to light/noise, blurred vision, memory difficulty, and difficulty concentrating.* Clinical reads of MRI scans conducted by neuroradiologists on data collected at 24–48-h, 8 days, 15 days, and 45 days post-injury as part of the broader study protocol revealed no acute intracranial lesions in any concussed athlete.

Twenty-nine healthy football athletes matched on institution, sport team, sex, age, education, intellectual functioning, grade point average, race, and concussion history participated in similar follow-up visits as concussed athletes and served as controls. Two football athletes were injured while enrolled in the control protocol and their injured and control data are treated as independent data points. Given the focus of this study on performance of blood biomarkers during the acute injury phase, data from the pre-season,

6 h, and 24–48 h time points were analyzed. The demographic characteristics of each group and sample size at each time point can be found in Table 1.

Behavioral battery

All participants completed a comprehensive interview and questionnaire at pre-season, which gathered concussion and health history and demographic information. The Sport Concussion Assessment Tool – 3rd Edition (SCAT3) symptom checklist was collected at each visit and used to characterize concussion symptom severity (range from 0 to 132; higher scores reflecting greater symptom burden).²⁷ The Wechsler Test of Adult Reading was administered at the pre-season visit to provide an estimate of intellectual functioning.²⁸

Biomarker data

Venous blood was collected using Red Top BD Vacutainer tubes, left to clot at room temperature for 30 min, and centrifuged at 1,500 relative centrifugal force (RCF) for 15 min. Serum was stored at –80°C until transportation to Banyan Biomarker Incorporated. Serum samples were analyzed in duplicate by examiners blinded to diagnosis by Banyan Biomarkers within 10 months of collection.²⁹ Concentrations of UCH-L1 and GFAP were determined using the enzyme-linked immunosorbent assay; an electrochemiluminescence immunoassay designed for diagnostic testing (Roche, Cobas

TABLE 1. SAMPLE CHARACTERISTICS

	Concussed athletes n=32	Contact sport control athletes n=29	p value
Age	18.16 (1.8)	18.52 (1.6)	0.41
Height (inches)	71.55 (3.0)	71.05 (2.3)	0.47
Weight (lbs)	206.15 (44.5)	220.17 (40.5)	0.20
Years of education	12.0 (1.7)	12.4 (1.6)	0.29
WTAR standard score	102.48 (14.5)	103.45 (13.0)	0.79
Prior concussions (median)	1.0	1.0	0.41
Years of football participation	8.59 (3.1)	8.45 (2.0)	0.83
Competition Level (%college)	71.9%	72.4%	0.96
ADHD+ (%)	3.1%	6.9%	0.50
Racial distribution			0.46
Black or African American	25.0%	17.2%	
White	71.9%	82.8%	
Other/Not reported	3.1%	0.0%	
Injury characteristics			
LOC+ (%)	9.68%	-	
PTA+ (%)	16.13%	-	
RGA+ (%)	3.23%	-	
Available blood data (pre, 6 h, 48 h)	32/23/30	28/29/29	
Available SCAT3 data (pre, 6 h, 48 h)	32/32/31	29/26/29	

Mean (SD) shown unless otherwise indicated.

WTAR, Wechsler Test of Adult Reading; ADHD, attention-deficit/hyperactivity disorder; SCAT3, Sport Concussion Assessment Tool 3; LOC, loss of consciousness; PTA, post-traumatic amnesia; RGA, retrograde amnesia.

TABLE 2. BIOMARKER LIMITS OF QUANTIFICATION AND DETECTION

	Below LOD ^a n (%)			Below LLOQ ^b n (%)			Above ULOQ ^c n (%)		
	Pre-season	6h	24–48h	Pre-season	6h	24–48h	Pre-season	6h	24–48h
GFAP ^d									
Concussed	19 (59.4)	10 (43.5)	13 (43.3)	9 (28.1)	7 (30.4)	8 (26.7)	-	-	-
Control	19 (67.9)	14 (48.3)	15 (51.7)	5 (17.9)	7 (24.1)	7 (24.1)	-	-	-
S100B ^e									
Concussed	-	-	-	-	-	-	-	-	-
Control	-	-	-	-	-	-	-	-	-
UCH-L1 ^f									
Concussed	-	-	-	-	-	-	1 (3.1)	4 (17.4)	1 (3.3)
Control	-	-	-	-	-	-	2 (7.1)	2 (6.9)	2 (6.9)

Total number of athletes assessed may differ between groups and time points. LOD, LLOQ, and ULOQ are measured in pg/mL.

^aLOD: GFAP 7.6 pg/mL; S100B 5 pg/mL; UCH-L1 6 pg/mL.

^bLLOQ: GFAP 22 pg/mL; S100B not applicable; UCH-L1 28 pg/mL.

^cULOQ: GFAP 421 pg/mL; S100B 39,000 pg/mL; UCH-L1 412 pg/mL.

^dNo GFAP samples were above the ULOQ at any time point.

^eAll S100B samples were within the limits of quantification at all time points.

^fNo UCH-L1 samples were below the LOD or LLOQ at any time point.

GFAP, glial fibrillary acidic protein; S100B, S100 calcium-binding protein beta; UCH-L1, ubiquitin C-terminal hydrolase-L1; LOD, limit of detection; LLOQ, lower limit of quantification; ULOQ, upper limit of quantification.

6000) was used to determine S100B concentrations. The limit of detection (LOD), lower limit of quantification (LLOQ), and upper limit of quantification (ULOQ) for each biomarker is presented in Table 2. Biomarker levels measured below LLOQ and the LOD were replaced with the LLOQ and LOD values, respectively, and values above the ULOQ were replaced with the ULOQ value.

Statistical analysis

Statistical analyses were performed using IBM SPSS version 21 (Armonk, NY) and visualized using Prism 6.0 (La Jolla, CA). Independent samples *t* tests, Mann–Whitney *U* tests, and χ^2 tests were used to compare demographic data between groups collected at the pre-season visit. Independent samples *t* tests were conducted to compare behavioral measures between control and concussed athletes at each time point. Paired samples *t* tests were conducted to determine whether behavioral measures changed over time in each group separately. Independent samples Mann–Whitney *U* tests were conducted to compare biomarkers between groups at each visit. Paired-samples Wilcoxon signed rank tests were used to compare biomarkers across each visit in each group separately. Area under receiver operating characteristic curve (AUC) was used to quantify the ability of markers to discriminate concussed and control athletes. The Benjamini and Hochberg method was used to control the false discovery rate (FDR) at $\alpha = 0.05$ for cross-sectional comparisons across all dependent variables of interest (biomarkers and symptom scores).³⁰ For longitudinal analyses, FDR correction was applied to control and concussed groups separately.

Results

Demographic and behavioral data

There were no differences in demographic variables between control and concussed athletes (p 's > 0.10; Table 1), confirming the detailed matching criteria used for this study. Pre-season symptom severity scores did not significantly differ between concussed (4.4[8.2]) and control athletes (1.6[2.0]; $p > 0.05$).

Concussed athletes had significantly higher symptom severity scores relative to control athletes at the 6 h time point (mean [SD] = 34.9[20.4] and 1.4[1.9], respectively, $t[5] = 8.32$, $p < 0.001$)

and at the 24–48-h time point (27.0[20.3] and 1.4[1.8], respectively, $t[58] = 6.76$, $p < 0.001$). There was no significant change in symptom severity scores over time in controls. In contrast, concussed athletes had significantly higher symptom severity scores at the 6 h time point ($t[31] = 7.94$, $p < 0.001$) and the 24–48-h time point ($t[30] = 5.45$, $p < 0.001$) relative to pre-season baseline, whereas scores at the 6 h visit were significantly higher than scores at the 24–48 h visit ($t[30] = 2.52$, $p = 0.017$).

Biomarker data

The median number of hours between injury and blood collection was 2 h (range = 1–24 h) at the 6 h visit and 22.5 h (range = 11–51 h) for the 24–48 h visit. The number and percentage of data points below the LOD or LLOQ or above the ULOQ are in Table 2. Scatter plots showing biomarker levels for each athlete as well as plots of group medians across time points are shown in Figure 1. Statistics for each biomarker are presented in Table 3. The correlations between biomarkers at each time point can be found in Table 4.

Concussed athletes had significantly elevated S100B levels (median = 90.32 pg/mL, interquartile range [IQR] = 50.22–119.2 pg/mL) at the 6 h visit relative to controls (47.70 [28.76–74.01] pg/mL) following FDR correction ($p = 0.006$). Concussed athletes also had significantly elevated UCH-L1 levels (204.35 [144.41–317.95] pg/mL) at the 6 h visit relative to controls (101.30 [73.15–230.66] pg/mL; $p = 0.003$). There were no differences between groups in GFAP at any time point or for UCH-L1 and S100B at the pre-season or 24–48 h visits.

S100B and UCH-L1 discriminated concussed from control athletes at the 6 h visit with AUC of 0.72 and 0.74, respectively (Fig. 2a). GFAP at the 6 h visit did not discriminate between groups (AUC = 0.53). Combining UCH-L1 and S100B using logistic regression resulted in an AUC of 0.75 [95% CI: 0.62–0.88] at the 6 h visit.

Longitudinal analyses showed that GFAP levels at the 6 h (22.00 [7.60–22.59] pg/mL) and 24–48 h (7.6 [7.60–22.03] pg/mL) visits were significantly higher than pre-season GFAP levels (7.6 [7.60–

FIG. 1. Biomarker levels at each visit. Shown in the left panel are individual biomarker levels at each visit for each group. The horizontal line represents the group median at that time point. Shown in the right panel are group medians for each group across visits. GFAP, glial fibrillary acidic protein; UCH-L1, ubiquitin C-terminal hydrolase-L1; S100B, S100 calcium-binding protein beta.

22.00] pg/mL) in control athletes ($p=0.007$ and $p=0.021$, respectively). Concussed athletes also had significantly higher GFAP at the 24–48 h visit (22.00 [7.60–28.04] pg/mL) relative to 6 h (22.00 [7.60–25.40] pg/mL; $p=0.04$). For S100B, controls had significantly lower levels at the 6 h (47.70 [28.76–74.00] pg/mL) and 24–48 h visits (42.36 [28.08–63.18] pg/mL) relative to pre-season levels (60.64 [42.92–83.95] pg/mL; $p=0.005$ and $p<0.001$). In concussed athletes, S100B levels at the 6 h visit (90.32 [50.22–119.20] pg/mL) were significantly higher than pre-season levels (62.87 [43.58–91.74] pg/mL; $p=0.04$) and levels at the 24–48 h visit [48.28 [34.28–63.87] pg/mL; $p<0.001$], whereas levels were significantly lower at the 24–48 h relative to the pre-season visit ($p=0.01$). There were no significant differences across time in UCH-L1 levels in control athletes following FDR correction. In contrast, UCH-L1 levels in concussed athletes were elevated at the 6 h visit (204.35 [144.41–317.95] pg/mL) relative to pre-season (139.62 [82.01–234.66] pg/mL; $p=0.026$) and the 24–48 h visit (97.80 [56.49–154.36] pg/mL; $p<0.001$), whereas levels at the 24–48 h visit were significantly lower than pre-season levels ($p=0.004$).

Finally, the percent change at the 6 h visit relative to pre-season $[(6\text{ h} - \text{preseason})/\text{preseason}]$ was calculated.²¹ The median percentage change of UCH-L1 at the 6 h visit relative to the pre-season visit was 51.13% (IQR: 2.23–157.23%) in concussed athletes and –3.84% (IQR: –48.67–13.85%) in controls (Fig. 3). The median

percentage change of S100B at the 6 h visit was 18.51% (IQR: –13.19–60.01%) in concussed athletes and –19.00% (IQR: –46.27––5.82%) in controls. The percent changes in UCH-L1 and S100B at 6 h discriminated groups with AUC=0.79 [95% CI: 0.66–0.92] and AUC=0.77 [95% CI: 0.64–0.90], respectively (Fig. 2b). Combining the percent changes in UCH-L1 and S100B at 6 h slightly increased the AUC to 0.81 [95% CI: 0.69–0.94]. In contrast, the median percentage change in GFAP at the 6 h visit was 0% in concussed athletes (IQR: 0–189.47%) and 0% in control athletes (IQR: 0–12.49%) and yielded poor discrimination (AUC=0.58 [95% CI: 0.42–0.75]).

Exploratory correlations

Based on peak biomarker and symptom elevations during the early acute post-injury period, exploratory Spearman correlations were performed to determine the relationship between percent change or absolute levels of concussion symptoms and biomarker levels at the 6 h visit. There were no significant correlations (uncorrected for multiple comparisons).

Discussion

This study represents the first of this biomarker panel in SRC and provides prospective evidence of acute increases in serum levels of UCH-L1, a neuronal injury marker, during the early acute period

TABLE 3. STATISTICS FOR BLOOD BIOMARKERS

		Pre-season	6 h	24–48 h
<i>Cross-sectional</i>				
GFAP	<i>U</i> (<i>p</i> value) ^a	481.5 (0.56)	355.5 (0.67)	483.5 (0.43)
	AUC (95% CI)	0.54 (0.39–0.68)	0.53 (0.37–0.69)	0.56 (0.41–0.70)
S100B	<i>U</i> (<i>p</i> value) ^a	454 (0.93)	483 (0.006)*	500 (0.32)
	AUC (95% CI)	0.51 (0.36–0.66)	0.72 (0.58–0.87)	0.58 (0.43–0.72)
UCH-L1	<i>U</i> (<i>p</i> value) ^a	452 (0.95)	497 (0.003)*	388 (0.48)
	AUC (95% CI)	0.50 (0.36–0.65)	0.74 (0.61–0.88)	0.55 (0.40–0.70) ^b
		Pre-season vs. 6 h	Pre-season vs. 24–48 h	6 h vs. 24–48 h
<i>Longitudinal</i>				
GFAP				
Control	<i>Z</i> (<i>p</i> value) ^c	2.69 (0.007)*	2.31 (0.021)*	–0.89 (0.37)
Concussed	<i>Z</i> (<i>p</i> value) ^c	1.08 (0.28)	1.89 (0.06)	2.05 (0.04)*
S100B				
Control	<i>Z</i> (<i>p</i> value) ^c	–2.80 (0.005)*	–4.51 (<0.001)*	–1.20 (0.23)
Concussed	<i>Z</i> (<i>p</i> value) ^c	2.07 (0.04)*	–2.56 (0.01)*	–3.59 (<0.001)*
UCH-L1				
Control	<i>Z</i> (<i>p</i> value) ^c	–1.32 (0.19)	–1.95 (0.05)	–0.94 (0.35)
Concussed	<i>Z</i> (<i>p</i> value) ^c	2.22 (0.026)*	–2.89 (0.004)*	–3.63 (<0.001)*

^aMann–Whitney *U* test.^bSmaller value indicative of positive test because of lower levels in concussed subjects relative to controls.^cWilcoxon signed rank test.**p* < 0.05 following FDR correction.

AUC, area under receiver operating characteristic curve; GFAP, glial fibrillary acidic protein; S100B, S100 calcium-binding protein beta; UCH-L1, ubiquitin C-terminal hydrolase-L1; FDR, false discovery rate.

(i.e., median 2 h) following SRC relative to both pre-injury baseline levels and to levels in contact sport controls. S100B levels were also elevated at 6 h following SRC relative to pre-season levels and contact sport controls, consistent with previous literature in concussed athletes.^{21,22} Both the absolute values and percent change of UCH-L1 and S100B yielded fair (AUC > 0.7) discrimination between concussed and control athletes at the 6 h visit. These results highlight the potential utility of these markers in aiding the assessment of SRC at the early acute time frame and in informing the underlying pathophysiology of SRC.

The observation of elevated UCH-L1 levels at the 6 h visit is consistent with previous findings in mTBI from the civilian neurotrauma setting. Increased serum levels of UCH-L1 have been

observed in large samples of predominantly mTBI patients relative to controls in the early acute time frame following injury, with UCH-L1 levels peaking at ~6–8 h post-injury.^{4,5,7,8} These previous studies, however, have largely focused on combined TBI samples, often including patients with intracranial lesions or more severe gradients of TBI. Civilian non-sport samples also frequently have more significant peripheral injuries and comorbid pre-injury health conditions, and rely on cross-sectional versus longitudinal comparisons, introducing more potential scientific confounds. Therefore, this study extends previous work to a more homogenous, mildly injured SRC sample and in doing so offers preliminary support for the potential of UCH-L1 to detect SRC versus routine football participation.

Although UCH-L1 levels were stable across visits in the uninjured football players at the group level in the current study, there was variability in UCH-L1 across time points in individual uninjured football players. Specific factors contributing to this variability in uninjured athletes are undetermined at this time. Recent work, however, has reported pre- to post-game elevations of UCH-L1 in the absence of concussion, presumably caused by repetitive head hits below the threshold for a concussion diagnosis that are common in football (i.e., subconcussive head impacts).¹⁹ Nevertheless, current results demonstrate that the effects of SRC at the acute phase far outweigh sources of individual variability not related to injury.

Our observation of elevated serum levels of S100B at the 6 h visit versus pre-season and uninjured control levels is consistent with previous studies in athletes. For example, elevated S100B levels were reported in male hockey players at 1 h post-concussion compared with baseline levels of S100B in a separate control group.²² In addition, one recent study found elevated serum levels

TABLE 4. CORRELATION BETWEEN BLOOD BIOMARKERS

		<i>Pre-season</i>	<i>6 h</i>	<i>24–48 h</i>
UCH-L1 with GFAP				
Concussed	0.32		0.65*	0.58*
Control	0.66*		0.79*	0.77*
UCH-L1 with S100B				
Concussed	0.08		0.07	0.08
Control	0.15		0.33	0.27
S100B with GFAP				
Concussed	−0.11		−0.26	−0.06
Control	−0.14		0.12	0.26

Spearman correlation coefficients are shown for each comparison.

**p* < 0.05, uncorrected.

GFAP, glial fibrillary acidic protein; S100B, S100 calcium-binding protein beta; UCH-L1, ubiquitin C-terminal hydrolase-L1.

FIG. 2. Discrimination of concussed from control athletes at the early acute period. Shown are receiver operating characteristic (ROC) curves for glial fibrillary acidic protein (GFAP), ubiquitin C-terminal hydrolase-L1 (UCH-L1), and S100 calcium-binding protein beta (S100B) at the 6 hour visit (A), and the ROC curve for the percent signal change at the 6h visit relative to baseline (B). Solid line represents the ROC curve for the relevant biomarker; dotted line represents the reference line. The area under the curve (AUC) and the 95% CI are shown for each curve.

of S100B at 3 h post-concussion in athletes, with recovery of S100B to pre-season levels by 2 days post-injury.²¹ Interestingly, S100B levels in the current study were significantly decreased at 24–48 h post-injury relative to pre-season levels. S100B levels are elevated with physical activity,¹⁶ and, therefore, the decrease at 24–48 h could reflect the fact that injured athletes were not active at this time, per recommended return-to-play guidelines. Decreased S100B levels, however, were also observed in control athletes at the 6 and 24–48 h follow-up visits relative to pre-season. Previous work has attributed change of S100B levels to various factors besides frank concussion, including subconcussive head impacts, physical exertion, and non-brain injury.^{16,19,23,24,31,32} Nevertheless, this study provides both cross-sectional and longitudinal evidence of elevated S100B levels measured at 6 h following SRC.

Contrary to our hypothesis, GFAP levels were not significantly increased following SRC relative to controls. Several previous

studies have reported elevations in GFAP following brain injury, although the majority of this work has been in more severely injured samples (e.g., those including any-severity TBI and subjects with intracranial lesions).^{5,7,12} Moreover, previous studies have also shown that GFAP can discriminate TBI patients with and without intracranial lesions.^{4,11} The observation that GFAP concentrations were often below the LOD or LLOQ in both control and injured athletes is consistent with the hypothesis that GFAP biomarker elevations are primarily associated with brain lesions not typically associated with SRC (e.g., petechial hemorrhages). There were, however, significant longitudinal differences of GFAP levels in both control athletes (6 h and 24–48 hours vs. pre-season) and concussed athletes (24–48 h vs 6 h). The extent to which GFAP levels are affected by other factors than concussion, such as exercise or repetitive head impacts below the threshold of frank concussion, remains to be determined.

FIG. 3. Percentage change in serum markers relative to pre-season baseline. Shown is the percent change in glial fibrillary acidic protein (GFAP), ubiquitin C-terminal hydrolase-L1 (UCH-L1), and S100 calcium-binding protein beta (S100B) for each athlete at the 6h and 48h visits relative to levels at the pre-season baseline.

The discovery and validation of blood biomarkers for the assessment of concussion is based on the assumption that markers of brain injury can be reliably measured in the blood. The most commonly assumed route is through a disrupted blood–brain barrier (BBB). BBB disruption has been reported following TBI and football participation without frank concussion, and both S100B and UCH-L1 have been proposed as measures of BBB integrity.^{25,33–35} Clearance from the glymphatic system represents an alternative method by which these markers are transported to the blood.³⁶ Sources outside of the central nervous system, however, cannot be ruled out. For example, although S100B is highly expressed in astrocytes and is thought to reflect glial injury, it is also expressed in non-brain tissue.¹⁵ In contrast, UCH-L1 is highly abundant in and specific to neurons, and is, therefore, indicative of acute neuronal damage or dysfunction following brain injury.³⁷ A recent study reported that UCH-L1 levels were not able to distinguish CT-negative mTBI from orthopedic injury.³⁸ The assay used in that study, however, had substantially higher LLOQ than the assay used in this work (UCH-L, 0.3 ng/mL vs. 28 pg/mL; GFAP, 0.16 ng/mL vs. 22 pg/mL), making comparisons across studies difficult. Previous work using the same assay as the current study showed that UCH-L1 and GFAP distinguished between mild TBI and orthopedic injuries.^{8,39} Additional work is needed to pinpoint other factors that may be associated with elevations in blood levels of these markers.

Finally, the observation of an acute increase in self-reported symptom severity relative to pre-season levels in the current sample is consistent with large-scale studies of SRC.⁴⁰ Interestingly, although both symptom measures and the concentrations of S100B and UCH-L1 were most affected at the 6 h time point, we observed no significant relationships between these measures at this time point. These initial data suggest that symptom reporting and blood biomarker levels reflect different as yet undetermined aspects of SRC. Given the small sample available for correlational analyses, however, further research is needed to clarify the relationship between these blood markers and both concurrent postconcussive symptoms and later symptom recovery following SRC.

Limitations

To our knowledge, this study represents the largest study to assess this array of biomarkers in athletes across multiple post-injury time points. Nevertheless, the sample size is modest relative to comparative studies in samples of civilian mTBI patients recruited from emergency rooms. This limitation is offset by the assessment of pre-season biomarker levels allowing for a truly prospective characterization of the acute effects of mTBI in these athletes (i.e., pre- to post-injury). This study only includes male football athletes playing at the high school and collegiate levels; the generalizability of these findings to female athletes, non-football athletes, and athletes of other ages is uncertain.

Conclusion

Early measurement of UCH-L1 and S100B may have potential as diagnostic markers for SRC. Given the large variability of these measures observed in uninjured football athletes, however, additional work is needed before the clinical potential of these markers is realized (e.g., before meaningful sensitivity or specificity thresholds can be established). Work is ongoing to replicate these results, as well as comparing biomarker levels in concussed athletes with those in both contact and non-contact sport athletes to enable the differentiation of the effects of concussion as well as the effects

of head impact exposure from nonexposure on candidate biomarkers.^{19,23,24,31} Future work will correlate fluid biomarker data with clinical measures and changes in brain structure and function to inform a more complete understanding of the underlying neurobiological effects and time course of physiological recovery after SRC.

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Author Disclosure Statement

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