

# Acute elevation of serum inflammatory markers predicts symptom recovery after concussion

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## Abstract

### Objective

To test the hypothesis that acute elevations in serum inflammatory markers predict symptom recovery after sport-related concussion (SRC).

### Methods

High school and collegiate football players ( $n = 857$ ) were prospectively enrolled. Forty-one athletes with concussion and 43 matched control athletes met inclusion criteria. Serum levels of interleukin (IL)-6, IL-1 $\beta$ , IL-10, tumor necrosis factor, C-reactive protein, interferon- $\gamma$ , and IL-1 receptor antagonist and Sport Concussion Assessment Tool, 3rd edition (SCAT3) symptom severity scores were collected at a preinjury baseline, 6 and 24–48 hours postinjury, and approximately 8, 15, and 45 days following concussion. The number of days that athletes were symptomatic following SRC (i.e., duration of symptoms) was the primary outcome variable.

### Results

IL-6 and IL-1RA were significantly elevated in athletes with concussion at 6 hours relative to preinjury and other postinjury visits, as well as compared to controls ( $ps \leq 0.001$ ). IL-6 and IL-1RA significantly discriminated concussed from control athletes at 6 hours postconcussion (IL-6 area under receiver operating characteristic curve 0.79 [95% confidence interval (CI) 0.65–0.92], IL-1RA AUC 0.79 [95% CI 0.67–0.90]). Further, IL-6 levels at 6 hours postconcussion were significantly associated with the duration of symptoms (hazard ratio for symptom recovery = 0.61 [95% CI 0.38–0.96],  $p = 0.031$ ).

### Conclusions

Results support the potential utility of IL-6 and IL-1RA as serum biomarkers of SRC and demonstrate the potential of these markers in identifying athletes at risk for prolonged recovery after SRC.

### Classification of evidence

This study provides Class III evidence that serum levels of IL-6 and IL-1RA 6 hours postconcussion significantly discriminated concussed from control athletes.

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## Glossary

**AUC** = area under the receiver operating characteristic curve; **BIC** = Bayesian information criterion; **BMI** = body mass index; **CI** = confidence interval; **CRP** = C-reactive protein; **CV** = coefficient of variation; **ED** = emergency department; **HR** = hazard ratio; **IFN** = interferon; **IL** = interleukin; **IL-1RA** = interleukin-1 receptor antagonist; **LME** = linear mixed effects; **MSD** = Meso Scale Discovery; **mTBI** = mild traumatic brain injury; **SCAT3** = Sport Concussion Assessment Tool, 3rd edition; **SRC** = sport-related concussion; **TBI** = traumatic brain injury; **TNF** = tumor necrosis factor.

Sport-related concussion (SRC) occurs frequently in the United States, with rates cited as high as 3.8 million per year.<sup>1</sup> Clinical recovery is commonly based on the resolution of symptoms and is typically observed within 1 to 2 weeks of injury; however, a sizeable subset of athletes report more prolonged symptoms and recovery.<sup>2</sup> Thus, there is great interest in identifying objective biomarkers to not only aid in the diagnosis of SRC but also to predict which patients may be at risk for medical morbidity or delayed recovery. To date, most research has focused on identifying blood biomarkers that are relatively specific to brain injury. However, blood biomarkers capturing other aspects of the known neurometabolic cascade of concussion may hold promise as prognostic markers for poor recovery following mild traumatic brain injury (mTBI) including SRC.

Inflammation is one of the known physiologic responses to brain injury of all severities.<sup>3,4</sup> Numerous studies have demonstrated that the inflammatory effects of brain injury can be measured in the periphery (i.e., blood), with elevated cytokine profiles being documented in the blood of patients with moderate to severe traumatic brain injury (TBI). For example, increases in serum levels of inflammatory cytokines interleukin (IL)-1 $\beta$ , IL-6, IL-10, and tumor necrosis factor (TNF) have been documented in emergency department (ED) patients within 24 hours following injury.<sup>5-11</sup> Importantly, additional research has reported associations between inflammatory cytokines measured within 24 hours in ED patients with worse outcomes on the Glasgow Outcome Score,<sup>9,11,12</sup> as well as risk for mortality in intensive care unit patients.<sup>13</sup>

The acute inflammatory response following mTBI in humans has not been well-documented. Lagerstedt et al.<sup>14</sup> reported IL-10 levels at 6 hours following mTBI differentiated between CT-positive and CT-negative patients, though there was no comparison to a control group without mTBI. Gill and colleagues<sup>15</sup> reported acute and transient increases in IL-6 and TNF following moderate blast exposures in military personnel without diagnoses of mTBI. Two studies in overlapping samples of athletes observed downregulated mRNA expression of genes involved in the inflammatory response in peripheral blood mononuclear cells within 6 hours of SRC.<sup>16,17</sup> Finally, another study reported that elevated C-reactive protein (CRP) levels, which are typically increased in response to IL-6, in mTBI patients at hospital admission predicted persistent symptoms 3 months postinjury.<sup>18</sup>

In the current study, we investigated acute (i.e., hours) and subacute (i.e., days to weeks) effects of SRC on circulating concentrations of inflammatory markers in high school and collegiate football players. Importantly, all athletes participated in baseline, preseason visits to allow for the comparison of postinjury to preinjury biomarker levels. Inflammatory markers were selected based on previous work and included IL-6, IL-1 $\beta$ , IL-10, TNF, CRP, and interferon- $\gamma$  (IFN- $\gamma$ ). In addition, IL-1 receptor antagonist (IL-1RA) was investigated as a surrogate for IL-1 $\beta$  activity due to typically difficult to detect lower levels of IL-1 $\beta$  in serum.<sup>3</sup> We hypothesized that football players with SRC would have elevated inflammatory markers at the acute phase relative to preinjury levels and relative to noninjured football players, with subsequent recovery at the subacute time points. In addition, we hypothesized that acute increases in inflammatory markers would predict longer duration of symptoms recovery following SRC.

## Methods

The primary research questions were the following: What are the acute effects of SRC on serum inflammatory markers? Can acute serum inflammatory markers discriminate concussed from control athletes (Class III level of evidence)? Are elevated inflammatory markers associated with symptom duration following SRC?

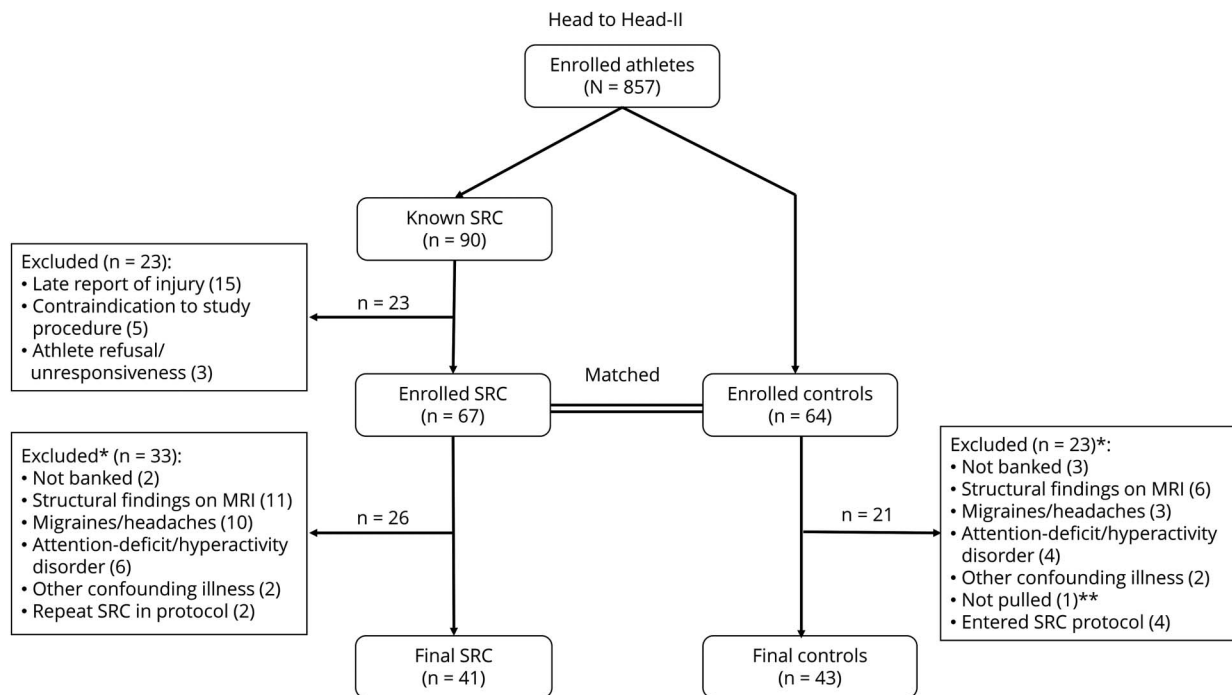
### Standard protocol approvals, registrations, and patient consents

The Medical College of Wisconsin institutional review board approved all aspects of this study. Minors provided written assent and parents of minors and adult participants provided written informed consent.

### Participants

A subset of high school and collegiate football players enrolled as part of a prospective study of SRC in athletes from southeastern Wisconsin (Project Head to Head II) were included in the current work. Football players ( $n = 857$ ) were recruited prior to the 2015 ( $n = 545$ ) or 2016 ( $n = 312$ ) football seasons (figure 1). A total of 90 concussions were documented in the time frame of this study; 67 participated in follow-up visits as part of Project Head to Head II based on the following exclusion criteria: history or suspicion of conditions known to cause cognitive dysfunction (e.g., moderate to severe TBI, epilepsy), injury that would preclude participation in the study protocol, current psychotic disorder or narcotic use, or any

**Figure 1** Flowchart of athlete recruitment



Athlete enrollment and selection from the parent project cohort (Head to Head II) and the current study. SRC = sport-related concussions. \*Excluded participants could meet more than one exclusion criterion. \*\*One control participant was not pulled for further analysis in error.

contraindication to study procedures. Athletes with concussion completed follow-up visits at approximately 6 hours and 24–48 hours postinjury, as well as approximately 8, 15, and 45 days postinjury. Identification and diagnosis of SRC was determined by team physicians or certified athletic trainers at participating institutions. Study investigators screened and triaged all injuries to confirm they met study requirements and definition. The study definition of concussion was based on the Centers for Disease Control and Prevention HEADS UP educational initiative, as previously described.<sup>19</sup>

Control athletes for the parent project were selected using a prospective plan. Research assistants screened enrolled athletes who met criteria for the follow-up visits (described above) and attempted to identify individually matched controls for each injured athlete using the following matching criteria: level of play (i.e., college or high school), institution, team, age, estimated intellectual functioning (word reading performance at baseline), race, handedness, concussion history, and position. None of the control athletes was otherwise injured; there were no cases where a suspected concussion was determined to be a noninjury. The search for matched controls began as soon as possible following each injury given restrictions associated with scheduling and availability of athletes and staff. Given these restrictions, the selection of the matched control and start of the follow-up visits for controls did not always occur at the same time as the concussed athletes' visits, though they did occur within the same season. Following these procedures,

a total of 64 contact controls were enrolled in the parent project.

For the current study, additional exclusion criteria included history or current psychopathology (i.e., mood disorders), migraines or recurrent headaches, attention-deficit/hyperactivity disorder or memory difficulties, structural findings on MRI that required clinical follow-up as part of the parent study, history of potentially confounding illness/disease (e.g., meningitis), or declining to allow their data to be banked for future analyses beyond the scope of the Project Head to Head II. A total of 43 concussed football players and 47 noninjured control football players met the additional criteria for inclusion in the current study. This included 4 athletes who completed the control protocol and then subsequently the postinjury protocol and 2 athletes who enrolled in the postinjury protocol for repeat concussions. For these participants, only data from the first postinjury protocol were included in analyses, leaving a final total of 41 athletes with concussion and 43 controls. The final number of athletes with demographic information is reported in table 1.

### Clinical battery

Health history, sport and concussion history, and demographic information was collected from all participants at the preseason visit. The Wechsler Test of Adult Reading was administered at the preseason visit to provide an estimate of intellectual functioning.<sup>20</sup> A comprehensive clinical battery

**Table 1** Sample characteristics

Variable	SRC (n = 41)	Controls (n = 43)	Statistics
No. by visit (baseline/6 h/48 h/8 d/15 d/45 d)	41/24/38/37/33/32	43/42/42/39/38/35	
Age, y	17.80 (1.79)	18.14 (1.67)	$t(82) = -0.89, p = 0.38$
Race, white/nonwhite <sup>a</sup>	28/12	30/13	$\chi^2(1) = 0.00, p = 0.98$
Ethnicity, not Hispanic/Hispanic/not reported or unknown	35/2/4	37/2/4	FET, $p = 1.00$
Years of education	12.73 (1.70)	13.12 (1.66)	$t(82) = -1.05, p = 0.30$
Participants in college	29	31	$\chi^2(1) = 0.02, p = 0.89$
WTAR standard score	99.02 (14.89)	100.93 (13.63)	$t(82) = -0.61, p = 0.54$
Household socioeconomic status	45.69 (11.06)	46.32 (11.31)	$t(79) = -0.25, p = 0.80$
Body mass index	28.33 (5.02)	29.36 (5.54)	$t(82) = -0.90, p = 0.37$
Years of participation in football	7.28 (2.93)	8.09 (2.46)	$t(81) = -1.38, p = 0.17$
Median number of prior concussions (range)	1 (0–4)	0 (0–3)	$U = 767.00, p = 0.26$
Position			FET, $p = 0.12$
Offensive line	9	4	
Wide receiver/tight end	6	4	
Quarterback/full back/running back	6	9	
Defensive line	5	14	
Linebacker	10	6	
Cornerback/safety	5	4	
Kicker	0	2	
Institution/team			FET, $p = 0.56$
Team 1: College	12	10	
Team 2: HS	2	5	
Team 3: HS	3	1	
Team 4: HS	5	2	
Team 5: HS	2	4	
Team 6: College	7	11	
Team 7: College	4	2	
Team 8: College	6	8	
Handedness, right/left/ambidextrous	38/3/0	36/5/2	FET, $p = 0.49$

Abbreviations: FET = Fisher exact test; HS = high school; SRC = sport-related concussion; WTAR = Wechsler Test of Adult Reading.

Mean (SD) are shown unless otherwise indicated.

<sup>a</sup> Race not reported for one concussed athlete.

that included interviews, questionnaires, and cognitive testing was conducted at preseason and follow-up visits. For the current work, concussion severity was assessed using the Sport Concussion Assessment Tool, 3rd Edition, symptom severity score.<sup>21</sup> Injury and recovery information was collected at follow-up visits. The number of days that athletes were symptomatic following SRC (i.e., duration of symptoms) was used as the primary outcome variable.

### Serum biomarkers

Venous blood was collected using Red Top BD (East Rutherford, NJ) Vacutainer tubes, left to clot at room temperature for 30 minutes, and centrifuged at 1,500 relative centrifugal force for 15 minutes. Serum concentrations of IL-6, IL-1 $\beta$ , IL-1RA, IL-10, TNF, CRP, and IFN- $\gamma$  were measured in duplicate, blinded to concussion status, using a Meso Scale Discovery (MSD) (Rockville, MD) QuickPlex

SQ 120 instrument and MSD V-PLEX assays following manufacturer's instructions. The average coefficient of variation (CV) for each marker and the final number of samples included in analyses is found in table 2.

## Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics version 21 (Armonk, NY) and visualized using Prism 7 (La Jolla, CA). Independent samples *t* tests, Mann-Whitney *U* tests, and  $\chi^2$  tests were used to compare baseline variables between groups. Inflammatory markers were natural log-transformed to better approximate a normal distribution. Samples that were below the detection limit or with CV greater than 25% were excluded from analyses. Statistical analysis on IL-1 $\beta$  levels was not performed due to the small number of useable data points (<50% of total sample). Linear mixed-effects (LME) modeling was used to model changes in biomarkers and Sport Concussion Assessment Tool, 3rd edition (SCAT3) symptom severity scores within participants over time as a function of study group, with visit (i.e., baseline, 6 hours, 48 hours, 8 days, 15 days, 45 days), group (i.e., SRC vs control), and the group by visit interaction. A random intercept was modeled for each participant to allow inclusion of participants who did not complete all visits. Various models that included body mass index (BMI) or time since last physical exertion as covariates were compared using Bayesian information criterion (BIC) due to the known effects of exercise and adiposity on inflammatory markers.<sup>22,23</sup> Of all available samples, 31 (22 SRC) had missing data for the time since last exertion due to lack of response; these data points were imputed using multiple imputation under the missing at random assumption. Group, visit, BMI, and log-transformed biomarker levels (excluding IL-1 $\beta$ ) were included as predictors in the imputation process. The basic LME model including group, visit, and their interaction had the lowest BIC for all markers except IL-1RA and CRP, for which the model including BMI as a covariate had the lowest BIC. For

consistency and simplicity, the basic interactive model with no covariates is reported for all biomarkers. Sensitivity analyses confirmed the inclusion of BMI did not affect CRP or IL-1RA results (see Results). Interactions or main effects of group or visit were considered significant at  $p < 0.05$  with Bonferroni correction for 6 markers, corresponding to  $p < 0.008$ . Significant effects were followed up with simple effects testing, and if indicated, post hoc testing using Bonferroni correction at  $p < 0.05$ . Area under the receiver operating characteristic curve (AUC) was used to quantify the ability of markers to discriminate concussed from control athletes. Finally, time to recovery was analyzed using Cox proportional hazard model to determine if inflammatory markers were predictors of symptom duration in athletes with concussion after confirming the proportional hazard assumption was met. AUC and time to recovery analyses were limited to inflammatory markers that showed acute elevation following concussion (see Results).

## Data availability statement

Anonymized data used in preparation of the figures and tables will be shared upon request from qualified investigators for purposes of replicating procedures and results.

## Results

### Demographic comparisons and injury information

Postinjury visits occurred at an average of 4.43 hours (SD 4.13), 40.20 hours (SD 47.13), 8.17 days (SD 1.53), 16.31 days (SD 4.63), and 43.99 days (SD 7.59). Athletes with concussion and control athletes did not differ in demographic information, confirming the detailed matching criteria (table 1). Athletes with concussion reported an average duration of symptoms of 8.86 days (SD 7.24). Recovery data for 5 athletes with concussion were lost to follow-up. No athletes with concussion reported loss of consciousness, 2 reported post-traumatic amnesia, and 1 reported retrograde amnesia.

### SCAT3 symptom severity scores and inflammatory markers

Means and SDs for the main effects and interactions are reported in table 3. Post hoc testing showed that SCAT3 symptom severity scores were significantly higher at the 6- and 24- to 48-hour visits relative to all other visits in athletes with concussion ( $ps \leq 0.001$ ) (figure 2). Athletes with concussion also had significantly higher symptom severity scores relative to control athletes at the 6-hour and 24- to 48-hour visits ( $ps < 0.001$ ).

For inflammatory markers, significant effects were observed for IL-1RA and IL-6 (table 3; figure 2). Post hoc testing indicated that both IL-1RA and IL-6 were significantly elevated in athletes with concussion at the 6-hour visit relative to all other visits ( $ps \leq 0.001$ ). Of the injured athletes with measurable baseline and 6-hour data, 19 of 22 had elevated

**Table 2** Number of samples and biomarker assay performance

Marker	N	CV, mean (SD)	Useable, n (%)
IL-1RA	444	4.60 (4.10)	443 (99.8)
IL-6	444	10.98 (13.55)	392 (88.3)
TNF	444	6.06 (9.16)	425 (95.7)
IL-10 <sup>a</sup>	344	7.03 (10.64)	325 (94.5)
IFN- $\gamma$	444	9.81 (11.34)	383 (86.3)
CRP	444	2.13 (2.20)	443 (99.8)
IL-1 $\beta$	444	19.11 (22.62)	216 (48.6)

Abbreviations: CRP = C-reactive protein; CV = coefficient of variation; IFN = interferon; IL = interleukin; RA = receptor antagonist; TNF = tumor necrosis factor.

<sup>a</sup> Due to manufacturer error, IL-10 was not included on 3 inflammatory plates.

**Table 3** Main effects and interaction for Sport Concussion Assessment Tool, 3rd edition (SCAT3) symptom severity scores and inflammatory biomarker levels, mean (SD)

Measures	Baseline	6 hours	48 hours	8 days	15 days	45 days	Group effect <i>p</i> value	Visit effect <i>p</i> value	Group by visit effect <i>p</i> value
<b>Symptom severity scores</b>							<0.001	<0.001	<0.001
<b>SRC</b>	2.93 (5.25)	25.62 (16.88)	20.45 (18.49)	3.16 (5.73)	0.85 (1.80)	0.50 (1.22)			
<b>Controls</b>	2.29 (4.84)	1.20 (1.58)	1.55 (2.71)	0.77 (2.19)	0.87 (2.59)	1.31 (3.54)			
<b>IL-1RA</b>							0.30	<0.001	<0.001
<b>SRC</b>	5.57 (0.50)	6.09 (0.67)	5.57 (0.58)	5.45 (0.44)	5.38 (0.30)	5.52 (0.39)			
<b>Controls</b>	5.54 (0.50)	5.47 (0.51)	5.48 (0.49)	5.53 (0.60)	5.47 (0.48)	5.52 (0.60)			
<b>IL-6</b>							0.01	<0.001	<0.001
<b>SRC</b>	-0.83 (0.77)	0.01 (1.15)	-0.69 (0.92)	-0.78 (0.92)	-0.62 (0.73)	-0.77 (0.63)			
<b>Controls</b>	-0.92 (0.65)	-0.93 (0.51)	-0.86 (0.62)	-1.04 (0.61)	-0.87 (0.64)	-1.04 (0.53)			
<b>TNF</b>							0.30	0.48	0.02
<b>SRC</b>	0.43 (0.55)	0.31 (0.77)	0.51 (0.60)	0.64 (0.39)	0.57 (0.58)	0.64 (0.48)			
<b>Controls</b>	0.71 (0.47)	0.56 (0.42)	0.65 (0.40)	0.55 (0.49)	0.55 (0.39)	0.48 (0.47)			
<b>IL-10</b>							0.17	0.75	0.92
<b>SRC</b>	-1.65 (0.69)	-1.58 (0.94)	-1.56 (0.56)	-1.55 (0.65)	-1.49 (0.54)	-1.48 (0.73)			
<b>Controls</b>	-1.37 (0.74)	-1.45 (0.69)	-1.48 (0.57)	-1.41 (0.69)	-1.28 (0.69)	-1.34 (0.57)			
<b>IFN-<math>\gamma</math></b>							0.27	0.53	0.30
<b>SRC</b>	0.55 (0.87)	0.78 (1.88)	1.03 (1.34)	0.94 (0.92)	0.82 (0.78)	0.82 (0.86)			
<b>Controls</b>	0.92 (0.88)	0.85 (0.75)	0.77 (0.82)	0.98 (1.14)	1.02 (0.98)	0.97 (1.20)			
<b>CRP</b>							0.73	0.10	0.27
<b>SRC</b>	13.14 (1.44)	13.51 (1.57)	13.54 (1.46)	12.98 (1.37)	13.17 (1.17)	13.11 (1.53)			
<b>Controls</b>	13.31 (1.20)	13.44 (1.53)	13.36 (1.56)	13.43 (1.38)	13.38 (1.24)	13.20 (1.38)			

Abbreviations: CRP = C-reactive protein; IFN = interferon; IL = interleukin; RA = receptor antagonist; SRC = sport-related concussion; TNF = tumor necrosis factor.

Natural log-transformed values of inflammatory markers are presented (pg/mL).

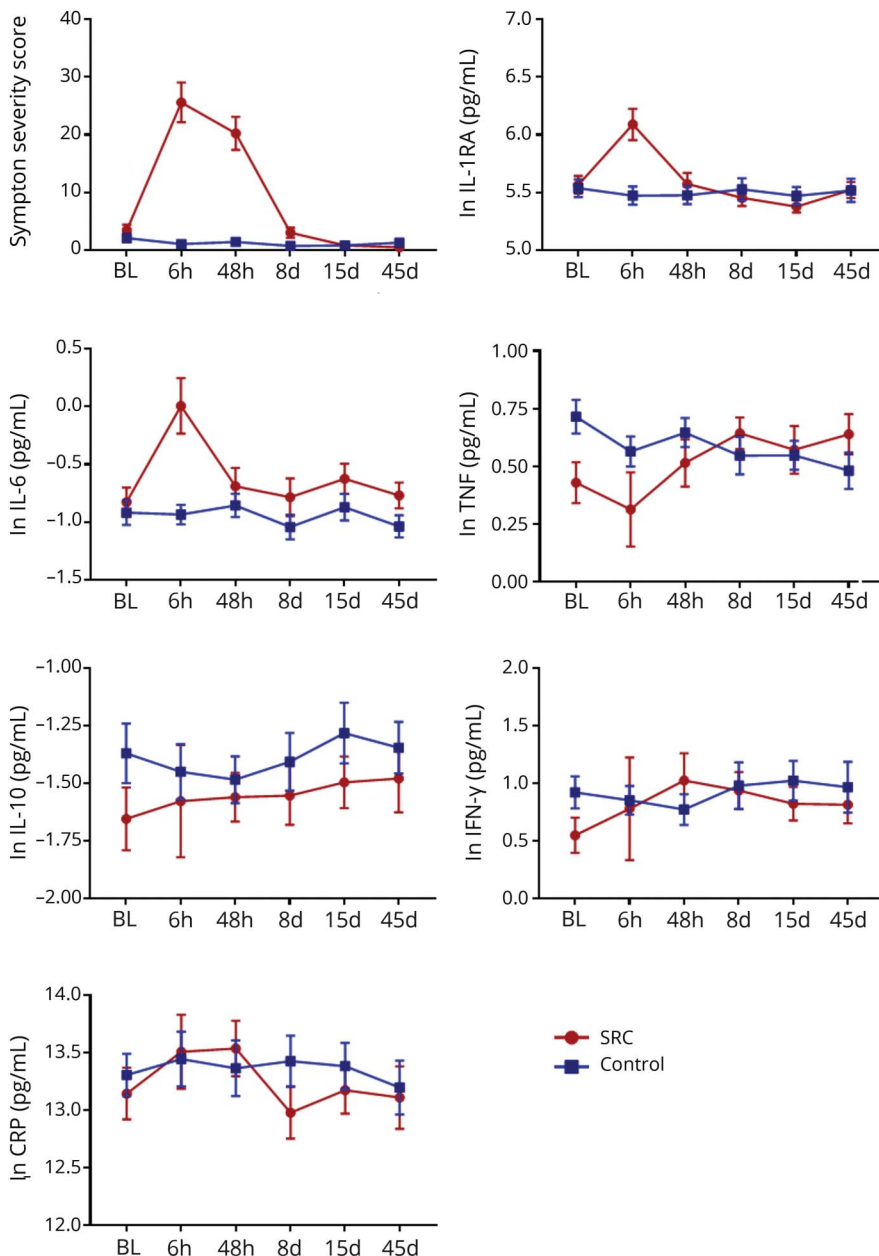
*p* < 0.05 significant following multiple comparison correction.

IL-6 and 21 of 24 had elevated IL-1RA at the 6-hour visit relative to their own baseline. In addition, both IL-1RA and IL-6 levels at the 6-hour visit were significantly higher in athletes with concussion compared to controls (*ps* < 0.001). The AUC for discriminating concussed from control athletes at the 6-hour visit was 0.79 (95% CI 0.67–0.90) for IL-1RA and 0.79 (95% CI 0.65–0.92) for IL-6 (figure 3).

There were no significant main effects or interactions for TNF, IFN- $\gamma$ , CRP, or IL-10 following multiple comparison correction (table 3; figure 2).

Sensitivity analyses for IL-1RA and CRP showed that the inclusion of BMI as a covariate did not affect results. There were no significant main effects or interactions for CRP

**Figure 2** Symptom severity scores and inflammatory markers



Shown are the Sport Concussion Assessment Tool, 3rd edition (SCAT3) mean symptom severity scores and the natural log-transformed (ln) levels of inflammatory markers for athletes with sport-related concussion (SRC) and controls at preseason baseline (BL), 6-hour (6 h), 24- to 48-hour (48 h), 8-day (8 d), 15-day (15 d), and 45-day (45 d) visits. Error bars are standard error of the mean. CRP = C-reactive protein; IFN = interferon; IL = interleukin; RA = receptor antagonist; TNF = tumor necrosis factor.

( $p > 0.10$ ). As in the primary analyses, there was a significant group by visit interaction for IL-1RA ( $p < 0.001$ ), driven by increased IL-1RA levels in athletes with concussion at the 6-hour visit relative to all other visits as well as relative to controls ( $p < 0.001$ ).

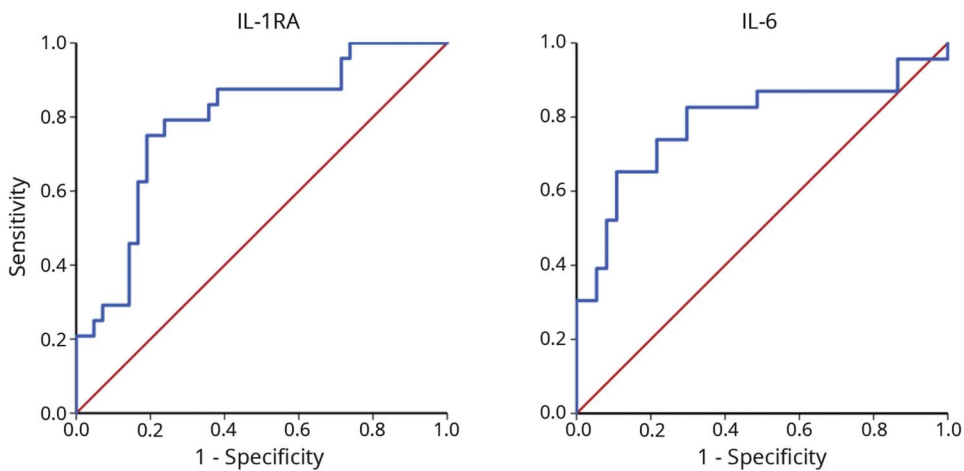
### Prediction of symptom recovery

Testing for associations between inflammatory markers and outcome in athletes with concussion (i.e., the duration of postconcussion symptoms in days) was limited to IL-1RA and IL-6 at the 6-hour visit. Eight of the 17 (47%) athletes with concussion who had elevated IL-6 levels at 6 hours postconcussion relative to their own baseline and complete

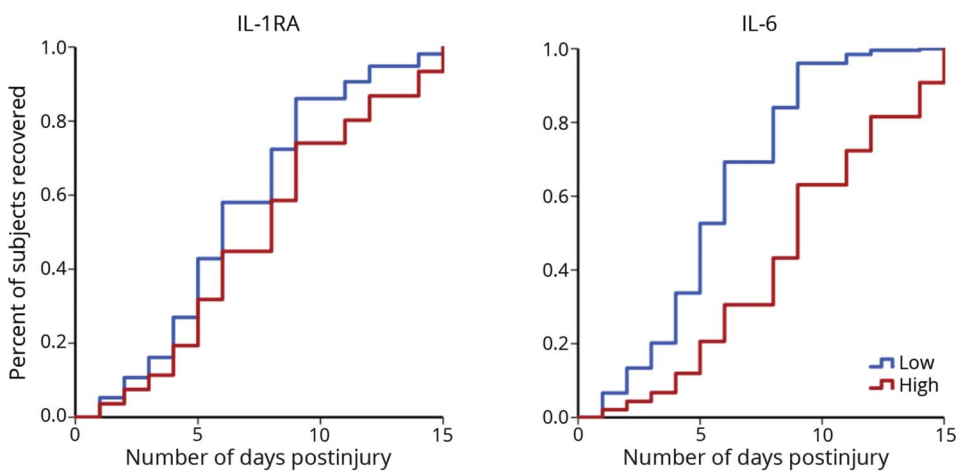
data regarding symptom recovery were still symptomatic at the 8-day visit, while 8 of the 18 (44%) athletes with concussion with elevated IL-1RA were still symptomatic at the 8-day visit. All injured athletes with elevated IL-6 or IL-1RA were symptom free by the 15-day visit. Time-to-event analyses were conducted to determine if acute IL-6 or IL-1RA levels predicted the number of days that athletes were symptomatic. IL-6 levels at 6 hours postconcussion were significantly associated with symptom duration (mean natural log-transformed IL-6  $-0.08$  [SD 1.12], mean duration of symptoms 7.10 [SD 3.82] days, HR 0.61 [95% CI 0.38–0.96],  $p = 0.03$ ), with a 1-unit increase in natural log-transformed IL-6 associated with 39% lower hazard of

**Figure 3** Receiver operating characteristic (ROC) curves and cumulative incidence of symptom recovery

**A.** Discrimination of athletes with concussions from controls



**B.** Cumulative incidence of symptom recovery



(A) ROC curve for interleukin (IL)-6 and IL-1 receptor antagonist (RA) at the 6-hour visit. Red line represents reference line. (B) Cumulative incidence of concussion symptom recovery by IL-6 and IL-1RA at the 6-hour visit in athletes with concussion. For visualization purposes, IL-6 and IL-1RA levels are represented as low or high based on median split.

recovery (i.e., higher IL-6 levels were associated with slower recovery rates; figure 3). IL-1RA levels at 6 hours postconcussion were not significantly associated with speed of recovery (mean natural log-transformed IL-1RA 6.03 [SD 0.66], mean duration of symptoms 7.14 [3.73] days, HR 0.62 [95% CI 0.31–1.23],  $p = 0.17$ ).

## Discussion

This prospective study longitudinally measured serum levels of inflammatory markers from preseason through multiple postinjury visits after SRC. Consistent with previous large-scale studies,<sup>24</sup> athletes with concussion had elevated symptom severity scores during the acute postinjury phase after SRC (i.e., 6 and 24–48 hours), with athletes with concussion reporting symptom recovery 8.79 days following injury, on average. Athletes with concussion also had elevated levels of IL-6 and IL-1RA at the early acute time point (i.e., 6 hours postinjury) relative to preinjury levels and relative to carefully

selected noninjured football athletes who served as matched controls. Importantly, elevated IL-6 levels at 6 hours postconcussion were associated with longer symptom recovery in athletes with concussion. These results demonstrate the sensitivity of serum inflammatory markers to SRC and highlight the potential of these markers in identifying athletes at risk for prolonged recovery following SRC.

IL-6 is a pleiotropic cytokine that exerts both pro- and anti-inflammatory effects as part of the innate immune response and is also involved in adaptive immunity.<sup>25</sup> The effects of brain injury on IL-6 have been extensively studied, with acute elevations documented in the brain following experimental brain injury and in CSF of patients following severe TBI.<sup>3,26</sup> Previous studies have also documented acute elevations in IL-6 in serum or plasma following moderate to severe TBI in pediatric ED patients<sup>5,9</sup> as well as in adult TBI patients compared to controls.<sup>6,8,10</sup> Prior work in severe head injury has also demonstrated the prognostic potential of IL-6, with reported associations between acute IL-6 levels and negative



outcomes,<sup>9,13,27</sup> though null and even opposite results have been reported as well.<sup>5,12</sup>

Alterations in IL-6 have also been documented in a limited number of studies of less severe brain injury. Gill et al.<sup>15</sup> reported increased serum levels of IL-6 in military personnel following moderate blast exposure with no diagnosis of mTBI relative to military personnel with low/no blast exposure. Moreover, they found that IL-6 levels were positively correlated with average peak pressure of the moderate blast exposure. Other recent work investigated changes in the gene expression of peripheral blood mononuclear cells within 6 hours following SRC and reported that IL-6 was a main hub of a network of differentially expressed genes following SRC that was associated with inflammatory response.<sup>17</sup> The current study extends these findings, and the findings in moderate and severe TBI, to demonstrate that acute elevations of IL-6 in serum are associated with prolonged symptom duration following SRC.

The effects of brain injury on IL-1 activity have also been extensively studied. Preclinical models and work in moderate and severe TBI patients have repeatedly shown an increase in IL-1 $\beta$  expression acutely following injury.<sup>3</sup> Few studies have specifically measured IL-1RA, an endogenous inhibitor of IL-1 activity that can act as an acute phase protein.<sup>28</sup> One study reported significant elevations of IL-1RA levels in brain microdialysates that were inversely correlated with intracranial pressure and associated with more favorable outcome in mostly severe TBI patients.<sup>29</sup> The current findings of elevated IL-1RA levels provide the first indirect evidence of increased IL-1 activity in the early acute phase following SRC or mTBI.

Substantial recent efforts have been made to identify blood biomarkers based on the known neurophysiologic consequences of brain injury (e.g., cell injury, gliosis) to aid in the diagnosis of mTBI and SRC, as well as in the prediction of patients at risk for prolonged recovery following mTBI and SRC. As previously mentioned, much of this work has focused on identifying markers that are, ideally, relatively specific to brain injury. For example, we have previously reported in an overlapping sample an increase in ubiquitin C-terminal hydrolase-L1 and S100 calcium-binding protein  $\beta$  at 6 hours postconcussion, with AUCs of 0.72 and 0.74, respectively.<sup>19</sup> Inflammatory markers are not specific to brain injury (i.e., they are elevated in other conditions and diseases), and thus their clinical potential in diagnosing SRC in isolation is limited. However, inflammation may still play a causal role in injury severity and recovery. Moreover, current results suggest that they may hold promise as complementary diagnostic markers of concussion (AUCs 0.78) and prognostic markers of prolonged symptom recovery. Clinically, a biomarker panel that includes markers covering multiple aspects of the neurometabolic cascade of concussion is likely to have the greatest diagnostic and prognostic value. Follow-up studies with larger sample sizes are needed to sufficiently determine the sensitivity and specificity of inflammatory markers alone

and in combination with other, more specific brain injury markers.

This prospective cohort study represents a crucial first step in determining the potential utility of inflammatory markers in the clinical management of concussion. Inflammatory markers were chosen based on prior brain injury literature. There are, however, numerous other inflammatory markers that could be investigated. Analyses of alternative inflammatory markers as well as markers capturing other potentially relevant biological pathways that are related to inflammation (e.g., kynurenine metabolites) or more specific to brain injury are ongoing.<sup>19,30</sup> Future work in this cohort will investigate the combined diagnostic and prognostic potential of blood markers capturing multiple aspects of the neurometabolic cascade, as well as their relationship with other potential biomarkers (i.e., neuroimaging metrics). Additional work comparing inflammatory and other blood markers to non-football athletes as a secondary control group is also ongoing to account for the potential effects of repetitive head impacts that occur during football without diagnosed concussion.

The current work cannot determine whether measured IL-6 and IL-1RA originated from the brain or the periphery. Prior work has demonstrated that mTBI and even football participation without frank concussion can lead to compromised blood-brain barrier, highlighting the possibility that these markers reflect neuroinflammation.<sup>31,32</sup> Alternatively, recent work has demonstrated that brain injury can result in changes to peripheral immune function likely via the sympathetic nervous system. Ritzel et al.<sup>33</sup> found that controlled cortical impact in mice resulted in an acute increase in myeloid cells, specifically neutrophils and monocytes, in bone marrow as well as mobilization of these cells into circulation. Additional work is needed to determine the response of peripheral markers of inflammation to mTBI and SRC, their relationship with neuroinflammation, and their physiologic consequences following SRC. In addition, this work focused on male football athletes in high school and college. Thus, the extent to which the results generalize to female or youth athletes is unclear. The majority of athletes in the current study showed fairly typical recovery following their injury, which is consistent with the prospective nature of the study. The extent to which the current results extend to athletes who develop post-concussion syndrome is unclear. Finally, the number of athletes with SRC in this study may not be large enough to adequately determine the precision, accuracy, sensitivity, and specificity of inflammatory markers as clinical biomarkers. Large-scale follow-up studies are warranted.

Circulating levels of IL-6 and IL-1RA are increased in the early acute phase following SRC in high school and college football athletes, with IL-6 levels predicting the duration of symptoms reported by athletes with concussion. These markers may have promise as diagnostic markers of SRC as well as prognostic markers for recovery after SRC. Moreover, these findings highlight the IL-6 and IL-1 pathways as possible

targets for treatment development, consistent with recent attempts to determine the efficacy of recombinant human IL-1RA as treatment for severe TBI in humans.<sup>34</sup>

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## Disclosure

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## Appendix (continued)

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