

# Association of Blood Biomarkers of Inflammation With Acute Concussion in Collegiate Athletes and Military Service Academy Cadets

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## Abstract

### Background and Objectives

The objective was to characterize the acute effects of concussion (a subset of mild traumatic brain injury) on serum interleukin (IL)-6 and IL-1 receptor antagonist (RA) and 5 additional inflammatory markers in athletes and military service academy members from the Concussion Assessment, Research, and Education Consortium and to determine whether these markers aid in discrimination of concussed participants from controls.

### Methods

Athletes and cadets with concussion and matched controls provided blood at baseline and postinjury visits between January 2015 and March 2020. Linear models investigated changes in inflammatory markers measured using Meso Scale Discovery assays across time points (baseline and 0–12, 12–36, 36–60 hours). Subanalyses were conducted in participants split by sex and injury population. Logistic regression analyses tested whether acute levels of IL-6 and IL-1RA improved discrimination of concussed participants relative to brain injury markers (glial fibrillary acidic protein, tau, neurofilament light, ubiquitin c-terminal hydrolase-L1) or clinical data (Sport Concussion Assessment Tool-Third Edition, Standardized Assessment of Concussion, Balance Error Scoring System).

### Results

Participants with concussion (total, N = 422) had elevated IL-6 and IL-1RA at 0–12 hours vs controls (n = 345; IL-6: mean difference [MD] (standard error) = 0.701 (0.091),  $p < 0.0001$ ; IL-1RA: MD = 0.283 (0.042),  $p < 0.0001$ ) and relative to baseline (IL-6: MD = 0.656 (0.078),  $p < 0.0001$ ; IL-1RA: MD = 0.242 (0.038),  $p < 0.0001$ ), 12–36 hours (IL-6: MD = 0.609 (0.086),  $p < 0.0001$ ; IL-1RA: MD = 0.322 (0.041),  $p < 0.0001$ ), and 36–60 hours (IL-6: MD = 0.818 (0.084),  $p < 0.0001$ ; IL-1RA: MD = 0.317 (0.040),  $p < 0.0001$ ). IL-6 and IL-1RA were elevated in participants with sport (IL-6: MD = 0.748 (0.115),  $p < 0.0001$ ; IL-1RA: MD = 0.304 (0.055),  $p < 0.0001$ ) and combative-related concussions (IL-6: MD = 0.583 (0.178),  $p = 0.001$ ; IL-1RA: MD = 0.312 (0.081),  $p = 0.0001$ ). IL-6 was elevated in male (MD = 0.734 (0.105),  $p < 0.0001$ ) and female participants (MD = 0.600 (0.177),  $p = 0.0008$ ); IL-1RA was only elevated in male participants (MD = 0.356 (0.047),  $p < 0.0001$ ). Logistic regression showed the inclusion of IL-6 and IL-1RA at 0–12 hours improved the discrimination of participants with concussion from controls relative to brain injury markers ( $\chi^2(2) = 17.855$ ,  $p = 0.0001$ ; area under the receiver operating characteristic curve [AUC] 0.73 [0.66–0.80] to 0.78 [0.71–0.84]), objective clinical measures (balance and cognition;  $\chi^2(2) = 40.661$ ,  $p < 0.0001$ ; AUC 0.81 [0.76–0.86] to 0.87 [0.83–0.91]), and objective

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Coinvestigators are listed in Appendix 2 at the end of the article.

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## Glossary

AUC = area under the receiver operating characteristic curve; BESS = Balance Error Scoring System; BMI = body mass index; CARE = Concussion Assessment, Research, and Education; CRP = C-reactive protein; DoD = Department of Defense; GFAP = glial fibrillary acidic protein; IL = interleukin; LME = linear mixed-effects; LOD = limit of detection; MD = mean difference; MSA = military service academy; MSD = Meso Scale Discovery; mTBI = mild traumatic brain injury; NCAA = National Collegiate Athletic Association; NfL = neurofilament light; RA = receptor antagonist; SAC = Standardized Assessment of Concussion; SCAT = Sport Concussion Assessment Tool-Third Edition; TNF = tumor necrosis factor; UCH-L1 = ubiquitin c-terminal hydrolase-L1; VEGF = vascular endothelial growth factor; WTAR = Wechsler Test of Adult Reading.

and subjective measures combined ( $\chi^2(2) = 13.456, p = 0.001$ ; AUC 0.97 [0.95–0.99] to 0.98 [0.96–0.99]), although improvement in AUC was only significantly relative to objective clinical measures.

## Discussion

IL-6 and IL-1RA (male participants only) are elevated in the early-acute window postconcussion and may aid in diagnostic decisions beyond traditional blood markers and common clinical measures. IL-1RA results highlight sex differences in the immune response to concussion which should be considered in future biomarker work.

## Introduction

Mild traumatic brain injury (mTBI), or concussion, is the most common form of traumatic brain injury, affecting millions of individuals each year.<sup>1,2</sup> There is great interest in identifying objective biomarkers to help inform the natural history of concussion and to aid in the diagnosis and clinical management, which is currently largely informed by subjective self-report of symptoms in addition to other clinical signs and tools. Recent work from the National Collegiate Athletic Association (NCAA) and Department of Defense (DoD) Concussion Assessment, Research, and Education (CARE) Consortium and other studies demonstrates that a variety of blood-based biomarkers thought to capture aspects of the known neurophysiologic consequences of concussion are elevated after concussion, such as glial fibrillary acidic protein (GFAP), ubiquitin c-terminal hydrolase-L1 (UCH-L1), tau, neurofilament light (NfL), and others.<sup>3–8</sup>

Growing evidence highlights peripheral markers of inflammation as promising candidates for blood-based biomarkers of concussion/mTBI. For example, interleukin (IL)-6 was elevated acutely after moderate blast exposure and mTBI in military personnel<sup>9,10</sup> and C-reactive protein (CRP) was elevated acutely in mTBI hospital patients with poor outcome<sup>11</sup> while a variety of other cytokines and chemokines, including tumor necrosis factor (TNF), IL-10, IL-8, and others, have been investigated in both saliva and blood.<sup>12–18</sup> Our group recently documented elevated IL-6 and IL-1 receptor antagonist (RA) in the early-acute window (<6 hours postinjury) after concussion in a prospective cohort of football players relative to preinjury baseline levels and relative to controls.<sup>19,20</sup> Although promising, additional studies in larger and more diverse cohorts with injuries from a variety of activities are needed to replicate and extend these findings, particularly given the wide variety of factors that potentially influence markers of inflammation.<sup>17,21</sup>

Accordingly, the goal of this work was to characterize the acute effects of concussion on a panel of serum inflammatory markers in male and female collegiate athletes and military service academy (MSA) members from the CARE Consortium. The panel of markers was selected based on previous reports of markers sensitive to either the acute or chronic effects of concussion and mTBI and included IL-6, IL-1RA, IL-10, IL-8, TNF, and CRP.<sup>9–16,19,20,22</sup> In addition, the vascular endothelial growth factor (VEGF) was also investigated based on recent data demonstrating its sensitivity to intracranial lesions in patients with mTBI and after chronic blast exposure.<sup>23,24</sup> Based on our previous work,<sup>9,19,20</sup> we hypothesized that IL-1RA and IL-6 would be elevated at the early-acute phase after concussion relative to uninjured controls and relative to preinjury levels. Secondary analyses considered whether the effects of concussion on serum inflammatory markers were similar across mechanism of injury (i.e., sporting event vs combative-related training) and sex because of sex differences in immune function and brain injury.<sup>25–27</sup> Finally, we hypothesized that IL-1RA and IL-6 at the early-acute window would significantly improve the discrimination of concussion relative to traditional brain-injury markers (i.e., tau, GFAP, UCH-L1, and NfL) and relative to objective and subjective clinical measures.

## Methods

### Participants

Data in NCAA athletes and MSA cadets were collected from the Advanced Research Core of the prospective CARE Consortium study from January 2015 to March 2020.<sup>28</sup> Athletes from 6 NCAA sites and cadets from 2 MSA sites were enrolled at a preseason baseline visit (N = 3,777). Participants who sustained a concussion, defined based on DoD evidence-based guidelines<sup>29</sup> and diagnosed by local medical staff, completed postinjury visits with blood collection. For athletes with concussion, contact and noncontact sport athletes

completed similar visits, serving as controls. Contact sport controls were exactly matched on institution, sport, and sex and algorithmically matched on race/ethnicity, Wechsler Test of Adult Reading (WTAR) scores at baseline, primary position, years of participation in primary sport, concussion history, and starter/nonstarter status. Noncontact sport controls were matched on institution and sex. For cadets, uninjured cadets matched by institution, sport, varsity/nonvarsity status, and sex completed similar visits at a 1 control-to-4 injured cadet ratio. Baseline blood samples were collected in contact sport controls and athletes with concussion; baseline blood samples were not collected in noncontact sport athletes or in all cadets. Participants could complete follow-up visits for multiple injuries or be originally enrolled as controls and subsequently complete follow-up visits for injuries. Analyses for this article were limited to only the first set of follow-up visits associated with concussion.

### Standard Protocol Approvals, Registrations, and Patient Consents

This study was approved by the institutional review board at the Medical College of Wisconsin (FWA#00000820) and by the US Army Medical Research and Development Command Human Research Protection Office; written informed consent was obtained from all participants.

### Time Points and Clinical Battery

The targeted time points for data collection for the CARE Consortium baseline and postinjury protocol have been previously described.<sup>3,4</sup> Specifically, follow-up visits were conducted at acute postinjury time point, 24–48 hours postinjury, the point of reporting being asymptomatic, and 7 days after unrestricted return to play. For the current study focused on the acute effects of concussion, time points were reclassified based on the time of blood collection since injury; for controls, the time of the first nonbaseline blood collection was considered as 0 hours postinjury. There was a trimodal distribution of time since injury across both participants with concussion and controls (eFigure 1, [links.lww.com/WNL/D290](https://links.lww.com/WNL/D290)). Based on this distribution, 3 postinjury time points were identified, in addition to the baseline time point: 0–12, 12–36, and 36–60 hours postinjury. Not all participants had blood samples at all identified time points.

A clinical battery consisting of the Sport Concussion Assessment Tool-Third Edition (SCAT) symptom checklist, the Standardized Assessment of Concussion (SAC), and the Balance Error Scoring System (BESS) were collected at each time point. Participants self-reported their “current biological sex,” with options of male or female.

### Blood Sample Collection and Analysis

Nonfasting venous blood was collected using 10-mL red-top serum and 10-mL EDTA plasma tubes, processed following recommended guidelines based on common data elements for TBI<sup>30</sup> and aliquoted into cryovials. Cryovials were stored locally at –27°C until shipping on dry ice to the CARE

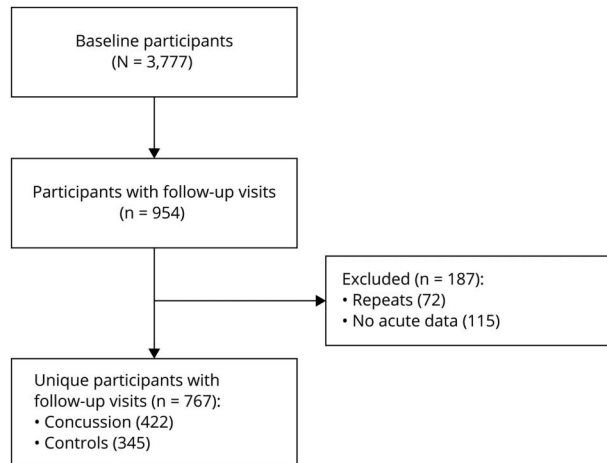
Consortium biorepository at Indiana School of Medicine for long-term storage at –80°C. For biomarker quantification, deidentified batches of samples were shipped on dry ice to Dr. Gill’s lab at the National Institute of Nursing Research of the NIH. Longitudinal samples from an individual were run on the same plate, and groups were randomly distributed across plates to minimize batch effects. Concentrations of IL-1RA, IL-6, IL-8, IL-10, TNF, CRP, and VEGF were measured in serum using a Meso Scale Discovery (MSD) QuickPlex SQ 120 instrument and MSD V-PLEX assays from a single lot following manufacturer’s instructions. Inflammatory markers below the lower limit of detection (LOD) were replaced by the lower LOD divided by the square root of 2<sup>31</sup>; inflammatory markers above the upper LOD were replaced by the upper LOD. Any duplicates with coefficient of variation of greater than 25% were excluded. Information regarding MSD assay performance can be found in eTable 1 ([links.lww.com/WNL/D290](https://links.lww.com/WNL/D290)). Finally, GFAP, UCH-L1, tau, and NFL were assayed in plasma using multiplexed single-protein array technology (Simoa; Quanterix Corp., Billerica, MA); performance characteristics and quality assessment procedures for these markers were previously described.<sup>3,4</sup> All samples were processed in duplicate, blinded to diagnosis.

### Statistical Analysis

Statistical analyses were conducted in R (version 4.2.1). Independent samples *t* tests, Wilcoxon rank sum tests, chi-square tests, and Fisher exact tests were conducted to compare baseline demographic variables between participants with concussion and controls. Linear mixed-effects (LME) models with subject treated as a random factor determined differences in clinical measures (SCAT symptom severity, BESS total score, and SAC total score) and inflammatory markers across time points as a function of group, implemented in the R function *lmer* from the *lme4* package. Models included time point (baseline, 0–12 hours, 12–36 hours, 36–60 hours), group (SRC/controls), and the group-by-time point interaction. Body mass index (BMI), age, and sex were selected a priori as covariates in all models. SCAT symptom severity (with addition of a constant) and biomarker values were natural log (ln) transformed to decrease skewness of their distributions. All control participants were combined into a single control group for the primary analyses. Not all participants had each marker at every time point; mixed models can include these participants with the missing at random assumption.

Two sets of subanalyses were conducted on split data sets. First, identical models as described above were run for MSA participants injured during combative training or boxing. Similar models were conducted for athletes injured during sport participation with contact and noncontact control athletes considered separately (i.e., group consisted of SRC, contact controls, and noncontact controls). The second set of subanalyses were conducted within male and female participants, separately, across all injury mechanisms using identical models to the primary analysis except without the inclusion of sex as a covariate. Simple main effect testing was

**Figure 1** Consort Diagram



conducted for significant interactions or main effects of group based on type III tests using the Satterthwaite method for estimating degrees of freedom, and post hoc testing with the Tukey method for pairwise comparison correction was conducted using the *emmeans* program.

Logistic regression analyses and likelihood ratio tests were used to determine whether, at the 0- to 12-hour time point, (1) the inclusion of IL-6 and IL-1RA (ln transformed) improved the discrimination of athletes and cadets with concussion from controls relative to the use of GFAP, UCH-L1, tau, and NfL (ln transformed) and (2) whether the inclusion of biomarkers improved the discrimination of athletes and cadets with concussion from controls relative to objective (BESS and SAC) and subjective (raw SCAT symptom severity scores) clinical measures. The Delong test was used to compare area under the receiver operating characteristic curve (AUC) calculated based on generated predicted probabilities. Sex, age, and BMI were included in each model.

An alpha of 0.05 was used for a priori markers IL-6 and IL-1RA. A Bonferroni-corrected alpha of 0.05 was used to account for multiple comparisons for the LME of the 5 secondary markers ( $p < 0.01$ ). All results are reported from 2-tailed tests.

### Data Availability

Clinical, demographic, and Quanterix biomarker data are publicly available from the Federal Interagency Traumatic Brain Injury Research Informatics System. Additional deidentified biomarker data are available from the corresponding author on reasonable request with the execution of necessary data use agreements.

## Results

A total of 422 unique athletes and cadets with concussion and 345 uninjured controls were included in analyses (Figure 1). The number of participants with inflammatory biomarkers at

each time point and raw values for each measure are presented in eTables 2–4 ([links.lww.com/WNL/D290](https://links.lww.com/WNL/D290)). Sample characteristics and associated statistics are shown in Table 1. Participants with concussion were younger; mean difference (MD) (standard error) =  $-0.39$  (0.04),  $p = 0.002$ ; had higher BMI, MD = 0.99 (0.12),  $p < 0.001$ ; had lower baseline WTAR scores, MD =  $-1.32$  (0.37),  $p = 0.048$ ; and had more prior concussions compared with controls,  $Z = 74.1$ ,  $p < 0.001$ .

### Association of Concussion With Clinical Measures

F-statistics and effect sizes for all interactions and group effects are presented in eTables 5 and 6 ([links.lww.com/WNL/D290](https://links.lww.com/WNL/D290)). Marginal means are presented in eTable 7; eTable 8 has MD and standard errors for post hoc tests. There was a group-by-time point interaction for all clinical measures ( $ps < 0.0001$ ; Figure 2; eTables 5, 7, and 8). Participants with concussion had elevated symptom severity scores at all postinjury time points relative to baseline, including the 0- to 12-hour time point, MD = 1.799 (0.096),  $t(1,405) = 18.76$ ,  $p < 0.0001$ ; the 12- to 36-hour time point, MD = 1.569 (0.077),  $t(1,333) = 20.436$ ,  $p < 0.0001$ ; and the 36- to 60-hour time point, MD = 1.246 (0.077),  $t(1,333) = 16.295$ ,  $p < 0.0001$ . Symptom severity was also higher at the 0- to 12-hour time point, MD = 0.553 (0.107),  $t(1,488) = 5.181$ ,  $p < 0.0001$  and the 12- to 36-hour time point, MD = 0.323 (0.093),  $t(1,656) = 3.466$ ,  $p = 0.003$ , relative to 36–60 hours. Controls had greater symptom severity at baseline compared with the 0- to 12-hour time point, MD = 0.195 (0.068),  $t(1,116) = 2.856$ ,  $p = 0.023$ . Relative to controls, participants with concussion had greater symptom severity at baseline, MD = 0.442 (0.077),  $t(1,635) = 5.780$ ,  $p < 0.0001$ ; at 0–12 hours, MD = 2.436 (0.107),  $t(1,836) = 22.818$ ,  $p < 0.0001$ ; at 12–36 hours, MD = 2.025 (0.110),  $t(1,835) = 18.403$ ,  $p < 0.0001$ ; and at 36–60 hours, MD = 1.853 (0.126),  $t(1,811) = 14.691$ ,  $p < 0.0001$ .

Concussed participants also had worse balance deficits at the 0- to 12-hour time point relative to all other time points, including baseline, MD = 4.394 (0.600),  $t(1,274) = 7.319$ ,  $p < 0.0001$ ; the 12- to 36-hour time point, MD = 3.421 (0.682),  $t(1,380) = 5.017$ ,  $p < 0.0001$ ; and the 36- to 60-hour time point, MD = 4.152 (0.669),  $t(1,324) = 6.206$ ,  $p < 0.0001$ . Participants with concussion also had more balance deficits than controls at all visits; baseline, MD = 2.27 (0.507),  $t(1,428) = 4.482$ ,  $p < 0.0001$ ; the 0- to 12-hour time point, MD = 7.31 (0.696),  $t(1,772) = 10.499$ ,  $p < 0.0001$ ; the 12- to 36-hour time point, MD = 4.76 (0.705),  $t(1,773) = 6.748$ ,  $p < 0.0001$ ; and the 36- to 60-hour time point, MD = 4.08 (0.797),  $t(1,749) = 5.122$ ,  $p < 0.0001$ . Controls had more balance deficits at baseline relative to 12- to 36-hour time points, MD = 1.515 (0.558),  $t(1,187) = 2.714$ ,  $p = 0.034$ .

For the SAC, participants with concussion had more cognitive deficits compared with baseline at the 0- to 12-hour time point, MD =  $-1.521$  (0.198),  $t(1,406) = -7.679$ ,  $p < 0.0001$ ; the 12- to 36-hour time point, MD =  $-1.017$  (0.157),  $t(1,314) = -6.497$ ,  $p < 0.0001$ ; and the 36- to 60-hour time point, MD

**Table 1** Sample Characteristics

	No.	Overall (N = 767) <sup>a</sup>	Controls (n = 345) <sup>a</sup>	Concussion (n = 422) <sup>a</sup>	p Value <sup>b</sup>
Age, y	767	19.35 (1.14)	19.50 (1.20)	19.23 (1.09)	0.002
Sex, male/female	767	610/157 (80/20)	283/62 (82/18)	327/95 (77/23)	0.120
BMI, kg/m <sup>2</sup>	767	25.79 (4.34)	25.22 (3.98)	26.25 (4.56)	<0.001
Race	767				0.950
White		517 (67)	236 (68)	281 (67)	
Black		156 (20)	69 (20)	87 (21)	
Multiple races		51 (6.6)	22 (6.4)	29 (6.9)	
Other, unknown, or not reported		43 (5.6)	18 (5.2)	25 (5.9)	
Ethnicity	767				0.130
Hispanic		49 (6.4)	28 (8.1)	21 (5.0)	
Non-Hispanic		641 (84)	287 (83)	354 (84)	
Unknown or not reported		77 (10)	30 (8.7)	47 (11)	
ADHD	767	47 (6.1)	19 (5.5)	28 (6.6)	0.520
Learning disorder	767	11 (1.4)	4 (1.2)	7 (1.7)	0.760
WTAR standard score	625	108.84 (11.90)	109.86 (12.23)	107.96 (11.55)	0.048
Length of sport participation	646	10.39 (3.92)	10.58 (3.86)	10.20 (3.98)	0.220
No. of prior concussions, median (IQR)	767	0.00 (0.00–1.00)	0.00 (0.00–1.00)	0.00 (0.00–1.00)	<0.001
Athlete type	767				
Noncontact control		149 (19)	149 (43)	0 (0)	
Contact control		180 (23)	180 (52)	0 (0)	
Sport-related concussion		328 (43)	0 (0)	328 (78)	
Non-sport		110 (14)	16 (4.6)	94 (22)	

Abbreviations: ADHD = attention deficit hyperactivity disorder; BMI = body mass index; IQR = interquartile range; WTAR = Wechsler Test of Adult Reading.

<sup>a</sup> Mean (SD); n/N (%).

<sup>b</sup> Welch 2-sample *t* test; Pearson  $\chi^2$  test; Fisher exact test; Wilcoxon rank sum test.

= -0.503 (0.157),  $t(1,340) = -3.211$ ,  $p = 0.007$ . In addition, participants with concussion also had more cognitive deficits at the 0- to 12-hour time point, MD = -1.017 (0.219),  $t(1,474) = -4.644$ ,  $p < 0.0001$  and the 12- to 36-hour time point, MD = -0.514 (0.190),  $t(1,635) = -2.706$ ,  $p = 0.035$ , relative to the 36- to 60-hour time point. Participants with concussion also had more cognitive deficits relative to controls at the 0- to 12-hour time point, MD = -1.567 (0.220),  $t(1,817) = -7.128$ ,  $p < 0.0001$ ; the 12- to 36-hour time point, MD = -1.131 (0.225),  $t(1,816) = -5.023$ ,  $p < 0.0001$ ; and the 36- to 60-hour time point, MD = -0.620 (0.257),  $t(1,792) = -2.407$ ,  $p = 0.016$ .

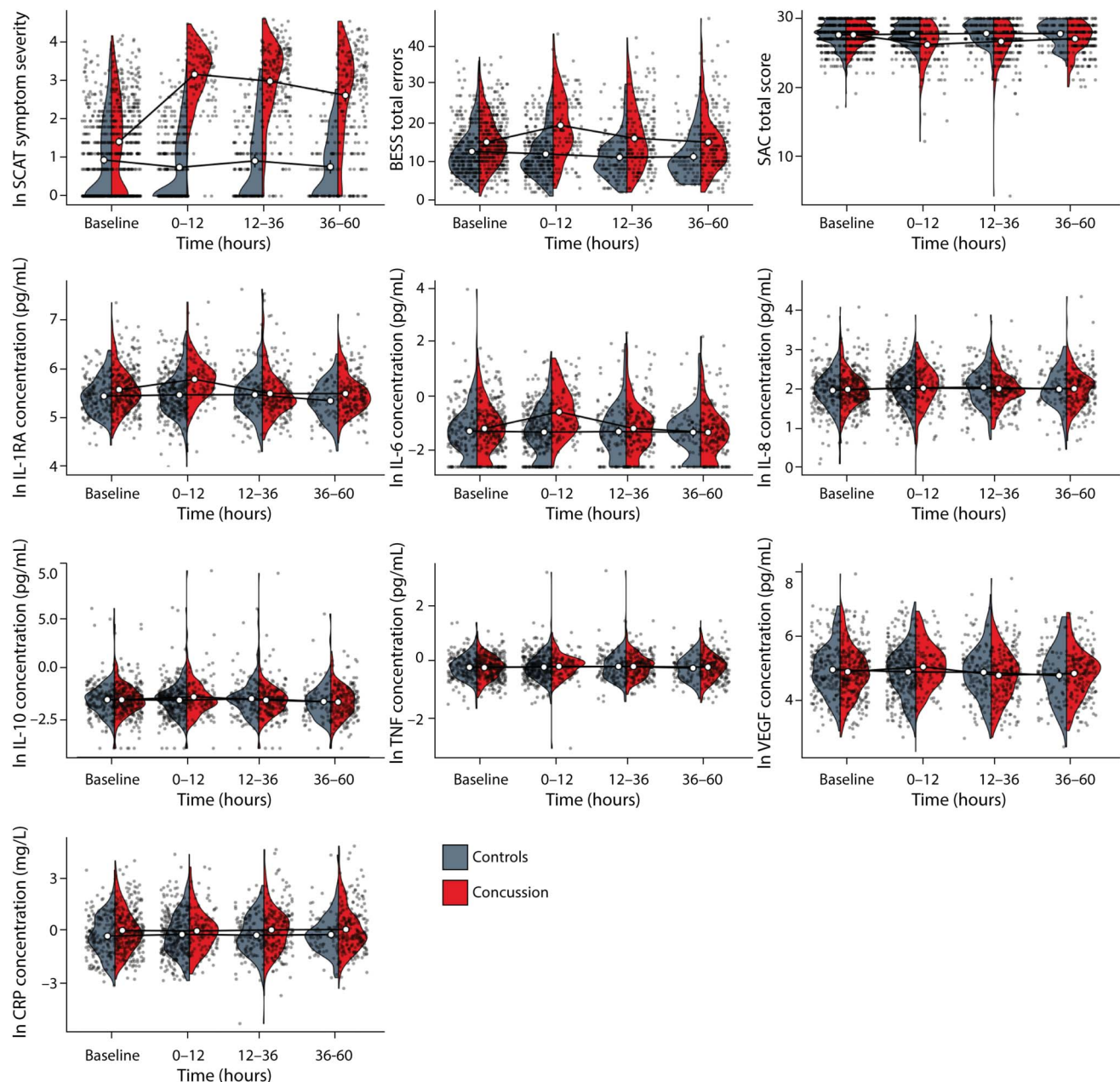
### Association of Concussion With Inflammatory Markers: Whole Sample

There was a significant time point-by-group interaction for IL-1RA and IL-6 ( $ps < 0.0001$ ; Figure 2; eTable 6, links.lww.com/WNL/D290). For IL-1RA, athletes and cadets with

concussion had elevated IL-1RA at the 0- to 12-hour time point relative to all other time points including baseline, MD = 0.242 (0.038),  $t(1,084) = 6.366$ ,  $p < 0.0001$ ; the 12- to 36-hour time point, MD = 0.322 (0.041),  $t(1,149) = 7.856$ ,  $p < 0.0001$ ; and the 36- to 60-hour time point, MD = 0.317 (0.040),  $t(1,095) = 7.864$ ,  $p < 0.0001$ . IL-1RA levels were lower relative to baseline in athletes and cadets with concussion at the 12- to 36-hour time point, MD = 0.081 (0.030),  $t(1,012) = 2.693$ ,  $p = 0.036$ . There were no significant differences between time points in controls following Tukey adjustment for multiple comparisons. Finally, athletes and cadets with concussion had elevated IL-1RA relative to controls at the 0- to 12-hour time point, MD = 0.283 (0.042),  $t(1,520) = 6.674$ ,  $p < 0.0001$  and at baseline, MD = 0.117 (0.036),  $t(1,461) = 3.205$ ,  $p = 0.0014$ .

For IL-6, athletes and cadets with concussion had elevated levels at the 0- to 12-hour time point relative to all other

**Figure 2** Clinical and Blood Biomarker Data



Shown are violin plots with individual data points and group means and 95% CIs for clinical measures and blood-based biomarkers across visit. BESS = Balance Error Scoring System; CRP = C-reactive protein; IL = interleukin; RA = receptor antagonist; SAC = Standardized Assessment of Concussion; SCAT = Sport Concussion Assessment Tool symptom severity; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor.

time points, including baseline, MD = 0.656 (0.078),  $t(946) = 8.443$ ,  $p < 0.0001$ ; the 12- to 36-hour time point, MD = 0.609 (0.086),  $t(1,022) = 7.113$ ,  $p < 0.0001$ ; and the 36- to 60-hour time point, MD = 0.818 (0.084),  $t(969) = 9.759$ ,  $p < 0.0001$ . In addition, IL-6 levels in this group at the 36- to 60-hour time point were lower than at baseline, MD = 0.163 (0.062),  $t(860) = 2.646$ ,  $p = 0.041$  and at the 12- to 36-hour time point, MD = 0.209 (0.075),  $t(1,080) = 2.787$ ,  $p = 0.028$ . There were no significant differences between time points in controls. Finally, relative to controls, athletes, and cadets with concussion had elevated

IL-6 levels at the 0- to 12-hour time point, MD = 0.701 (0.091),  $t(1,411) = 7.699$ ,  $p < 0.0001$ .

There were no significant main effects of group or group-by-time point interactions for secondary inflammatory markers with correction for multiple comparisons ( $ps > 0.01$ ; eTables 6 and 7, [links.lww.com/WNL/D290](https://links.lww.com/WNL/D290)).

### Subanalysis: Split by Injury Population

In the athlete subset, there was a group-by-time point interaction for IL-1RA ( $p < 0.0001$ ; Figure 3; eTable 6, [links.lww.com/WNL/D290](https://links.lww.com/WNL/D290)).

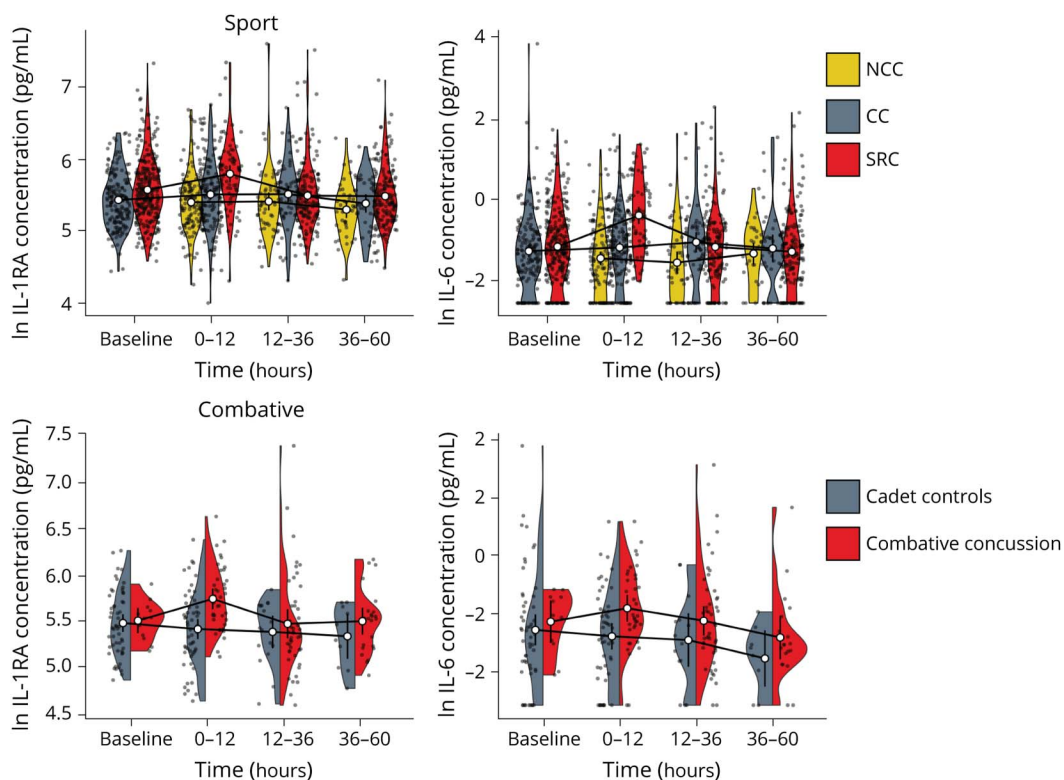
IL-1RA was significantly elevated in athletes with concussion at the 0- to 12-hour time point relative to baseline, MD = 0.249 (0.045),  $t(884) = 5.488$ ,  $p < 0.0001$ ; the 12- to 36-hour time point, MD = 0.333 (0.050),  $t(951) = 6.634$ ,  $p < 0.0001$ ; and the 36- to 60-hour time point, MD = 0.333 (0.048),  $t(909) = 6.878$ ,  $p < 0.0001$ . IL-1RA levels at the 36- to 60-hour time point were lower than those at baseline, MD = -0.084 (0.031),  $t(834) = -2.665$ ,  $p = 0.039$ . There were no significant differences between time points in controls. Additional pairwise comparisons showed that athletes with concussion had elevated IL-1RA at the 0- to 12-hour time point relative to both contact controls, MD = 0.304 (0.055),  $t(1,333) = 5.571$ ,  $p < 0.0001$  and noncontact controls, MD = 0.269 (0.058),  $t(1,333) = 4.626$ ,  $p < 0.0001$ . Athletes with concussion also had elevated IL-1RA levels at baseline compared with contact controls, MD = 0.134 (0.040),  $t(1,110) = 3.395$ ,  $p = 0.002$ .

There was also a group-by-time point interaction for IL-6 in the athlete subsample ( $p < 0.0001$ ; eTable 6, [links.lww.com/WNL/D290](https://links.lww.com/WNL/D290)). This effect was driven by an elevation of IL-6 in athletes with concussion at the 0- to 12-hour time point relative to baseline, MD = 0.766 (0.089),  $t(752) = 8.609$ ,  $p < 0.0001$ ; the 12- to 36-hour time point, MD = 0.749 (0.102),  $t(821) = 7.377$ ,  $p < 0.0001$ ; and the 36- to 60-hour time point, MD = 0.937 (0.096),  $t(779) = 9.740$ ,  $p < 0.0001$ . IL-6 levels at

the 36- to 60-hour time point were also lower than those at baseline, MD = -0.171 (0.065),  $t(758) = -2.643$ ,  $p = 0.042$ . Controls did not differ over time points. Relative to controls, athletes with concussion also had elevated IL-6 at the 0- to 12-hour time point relative to contact controls, MD = 0.748 (0.115),  $t(1,245) = 6.514$ ,  $p < 0.0001$  and noncontact controls, MD = 0.935 (0.125),  $t(1,227) = 7.508$ ,  $p < 0.0001$ .

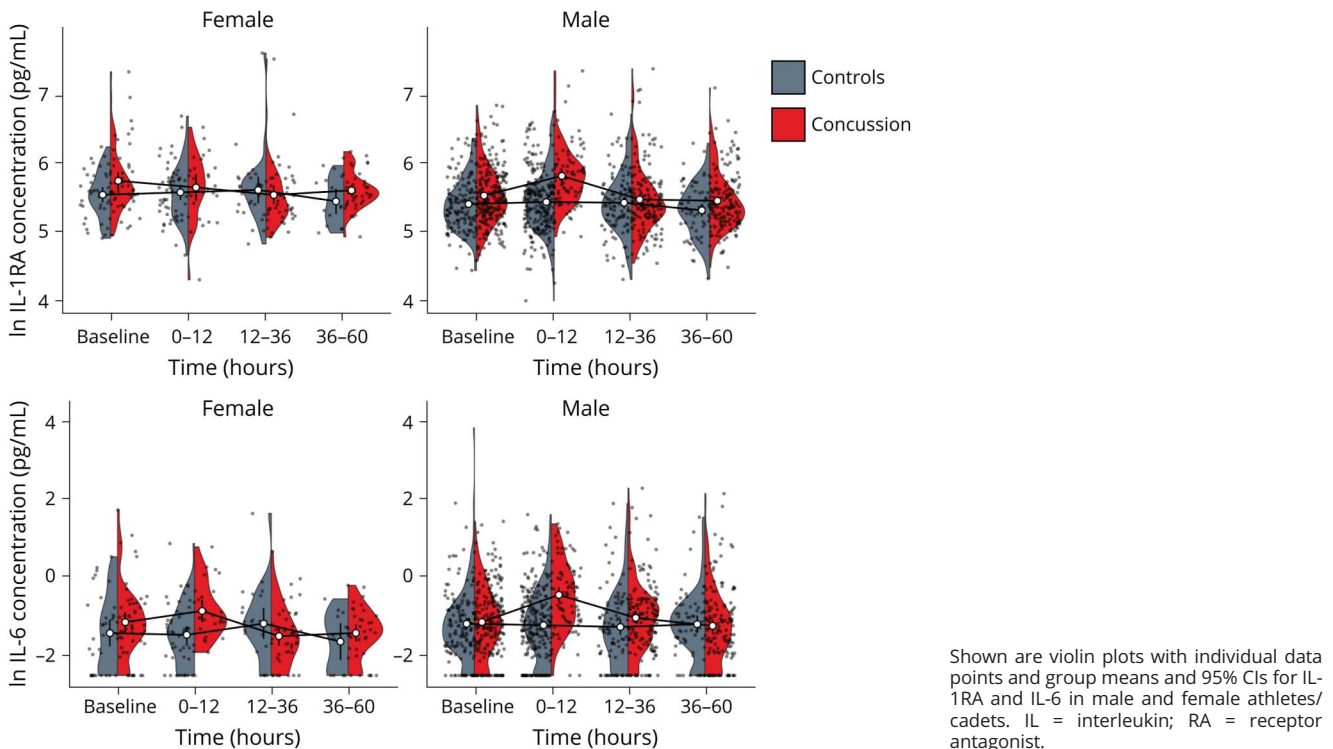
In cadets, there was a significant group-by-time point interaction for IL-1RA ( $p = 0.009$ ; Figure 3; eTable 6, [links.lww.com/WNL/D290](https://links.lww.com/WNL/D290)). IL-1RA was elevated in cadets with concussion at the 0- to 12-hour time point relative to the 12- to 36-hour time point, MD = 0.301 (0.069),  $t(199) = 4.367$ ,  $p = 0.0001$  and relative to the 36- to 60-hour time point, MD = 0.240 (0.080),  $t(178) = 3.012$ ,  $p = 0.016$ . IL-1RA levels in cadets with concussion were also elevated relative to controls at the 0- to 12-hour time point, MD = 0.312 (0.081),  $t(209) = 3.869$ ,  $p = 0.0001$ . There was a main effect of group on IL-6 levels in cadets, with higher levels in cadets with concussion relative to controls, MD = 0.416 (0.150),  $t(173) = 2.777$ ,  $p = 0.006$ . Pairwise comparisons showed that this effect was driven by higher IL-6 in cadets with concussion at the 0- to 12-hour time point, MD = 0.583 (0.178),  $t(218) = 3.280$ ,  $p = 0.0012$ ; groups did not significantly differ at any other time point ( $ps > 0.05$ ; eTables 9 and 10). Neither IL-1RA nor IL-6 levels changed across time points in cadet controls (eTables 9 and 10).

**Figure 3** IL-1RA and IL-6 Levels Split by Concussion Population



Shown are violin plots with individual data points and group means and 95% CIs for IL-1RA and IL-6 in athletes (top row) and cadets (bottom row). CC = contact control; IL = interleukin; NCC = noncontact control; RA = receptor antagonist; SRC = sport-related concussion.

**Figure 4** IL-1RA and IL-6 Levels Split by Sex



Finally, for secondary markers, there was a main effect of group for CRP in the athlete subsample ( $p = 0.005$ ; eTable 6, [links.lww.com/WNL/D290](https://links.lww.com/WNL/D290)), with athletes with concussion having higher CRP than noncontact controls, MD = 0.357 (0.124),  $t(910) = 2.868$ ,  $p = 0.012$ . There were no other significant main effects of group or group-by-time point interactions for secondary inflammatory markers in cadets or in athletes when analyzed separately, accounting for multiple comparisons ( $ps > 0.01$ ; eFigure 2 and 3; eTable 6).

### Subanalysis: Split by Sex

In male participants, there was a group-by-visit interaction for IL-1RA and IL-6 ( $ps < 0.0001$ ; Figure 4; eTable 6). For IL-1RA, male participants with concussion had significantly higher levels at 0- to 12-hour time point relative to baseline, MD = 0.334 (0.042),  $t(856) = 7.866$ ,  $p < 0.0001$ ; the 12- to 36-hour time point, MD = 0.386 (0.046),  $t(914) = 8.330$ ,  $p < 0.0001$ ; and the 36- to 60-hour time point, MD = 0.381 (0.045),  $t(869) = 8.411$ ,  $p < 0.0001$ . In male participants who were controls, IL-1RA levels were higher at the 0- to 12-hour time point relative to baseline, MD = 0.087 (0.033),  $t(724) = 2.619$ ,  $p = 0.045$ . IL-1RA levels were higher in male participants with concussion relative to controls at the 0- to 12-hour time point, MD = 0.356 (0.047),  $t(1,214) = 7.531$ ,  $p < 0.0001$  and at baseline, MD = 0.109 (0.040),  $t(1,171) = 2.709$ ,  $p = 0.007$ .

For IL-6, male participants with concussion had significantly higher levels at the 0- to 12-hour time point relative to baseline, MD = 0.730 (0.091),  $t(759) = 8.065$ ,  $p < 0.0001$ ; the

12- to 36-hour time point, MD = 0.619 (0.101),  $t(827) = 6.152$ ,  $p < 0.0001$ ; and the 36- to 60-hour time point, MD = 0.839 (0.098),  $t(780) = 8.590$ ,  $p < 0.0001$ . Finally, IL-6 levels were elevated in male participants with concussion relative to controls at the 0- to 12-hour time point, MD = 0.734 (0.105),  $t(1,135) = 6.991$ ,  $p < 0.0001$ .

In female participants, there was no main effect of group or group-by-visit interaction of IL-1RA, in contrast to results in the overall sample ( $ps > 0.05$ ; Figure 4; eTable 6, [links.lww.com/WNL/D290](https://links.lww.com/WNL/D290)). There was a significant interaction for IL-6 ( $p = 0.003$ ; eTable 6). IL-6 was significantly elevated in female participants with concussion at the 0- to 12-hour time point, relative to the 12- to 36-hour time point, MD = 0.544 (0.146),  $t(174) = 3.72$ ,  $p = 0.0015$  and the 36- to 60-hour time point, MD = 0.791 (0.149),  $t(170) = 5.319$ ,  $p < 0.0001$  while baseline levels were higher than those at the 36- to 60-hour visit, MD = 0.438 (0.115),  $t(151) = 3.819$ ,  $p = 0.0011$ . IL-6 levels were also elevated in female participants with concussion compared with controls at the 0- to 12-hour time point, MD = 0.600 (0.177),  $t(258) = 3.393$ ,  $p = 0.0008$ .

There were no significant main effects of group or group-by-time point interactions for any secondary inflammatory marker in either male or female participants when analyzed separately after accounting for multiple comparisons ( $ps > 0.01$ ; eFigure 4; eTables 11 and 12, [links.lww.com/WNL/D290](https://links.lww.com/WNL/D290)).

**Table 2** AUCs for Data at 0- to 12-Hour Visit

	AUC	95% CI
IL-1RA	0.708	0.654–0.763
IL-6	0.720	0.666–0.774
Brain injury	0.730	0.662–0.798
Brain injury + inflammatory	0.775	0.713–0.838
SAC/BESS	0.811	0.758–0.863
SAC/BESS + inflammatory	0.868	0.826–0.911
SAC/BESS/SCAT	0.970	0.953–0.988
SAC/BESS/SCAT + inflammatory	0.977	0.963–0.991

Abbreviations: AUC = area under the receiver operating characteristic curve; BESS = Balance Error Scoring System; IL = interleukin; RA = receptor antagonist; SAC = Standardized Assessment of Concussion; SCAT = Sport Concussion Assessment Tool symptom severity.

### Added Discriminative Value of IL-6 and IL-1RA

The AUCs for IL-6 and IL-1RA at the 0- to 12-hour time point were 0.72 and 0.71, respectively (see Table 2 for AUCs and 95% CIs). Logistic regression analyses showed that the inclusion of IL-6 and IL-1RA significantly improved the discrimination of participants with concussion from controls relative to traditional brain injury markers (GFAP, UCH-L1, NfL, tau) at the 0- to 12-hour time point,  $\chi^2(2) = 17.855$ ,  $p = 0.0001$  and also improved the AUC from 0.73 to 0.78, although this was not statistically significant, Delong test  $Z = 1.854$ ,  $p = 0.06$ . Similarly, the inclusion of IL-6 and IL-1RA also improved the discrimination of participants with concussion from controls relative to objective clinical markers (BESS and SAC) at the 0- to 12-hour time point,  $\chi^2(2) = 40.661$ ,  $p < 0.0001$ , improving the AUC from 0.81 to 0.87, Delong test  $Z = 3.391$ ,  $p = 0.0007$ . Finally, the inclusion of IL-6 and IL-1RA significantly improved the discrimination of participants with concussion from controls relative to objective markers and symptom severity scores,  $\chi^2(2) = 13.456$ ,  $p = 0.001$ , although there was no significant improvement in AUC (0.97–0.98, Delong test  $Z = 1.446$ ,  $p = 0.15$ ).

## Discussion

A central and systemic inflammatory response is a known consequence of mild traumatic brain injury,<sup>17</sup> although the sensitivity of peripheral inflammatory markers to concussion is understudied compared with other putative brain injury markers. Here, we replicate and extend previous evidence of elevated IL-6 and IL-1RA in the early-acute window after concussion<sup>19,20</sup> in an independent cohort of athletes and cadets. Although both IL-6 and IL-1RA were elevated regardless of cause of injury (i.e., sport vs combative training injuries), analyses split by sex showed that elevations in IL-1RA after concussion are only present in male participants. Finally,

logistic regression analyses showed that the inclusion of peripheral inflammatory markers improved the classification of participations with concussion from controls relative to traditional brain injury markers and relative to clinical markers that are currently used in the clinical management of concussion, although AUCs were only significantly improved based on the Delong test relative to objective clinical markers alone. These results demonstrate the importance of considering sex as a biological factor when investigating blood-based biomarkers of concussion and provide further evidence for the potential additive clinical value of peripheral inflammatory markers for the identification of concussion.

These results add to the growing literature demonstrating the sensitivity of IL-6 to mTBI/concussion. For example, elevated serum or plasma levels of IL-6 have been reported in football players approximately within 6 hours postinjury, within 8 hours after concussion in military personnel, in emergency department patients with CT-negative mTBI within 24 hours of injury, and in military personnel after moderate blast exposure.<sup>9,10,18-20</sup> Consistent with these previous reports, our results show that this elevation occurs rapidly (0–12 hours) and subsequently returns to baseline levels, at a group level, by 12–24 hours postinjury. Moreover, this effect was observed across both athlete and cadet populations, as well as in both male and female participants, suggesting that an acute systemic IL-6 response is a characteristic response of concussion, although the MD was qualitatively greater in male relative to female participants.

It is well-documented that brain injury also effects IL-1 activity based on preclinical models and results from patients with moderate-to-severe TBI.<sup>32</sup> IL-1RA is an acute-phase protein that competitively inhibits IL-1 activity<sup>33</sup>; thus, the observation of elevated serum levels of IL-1RA conceivably provides indirect evidence for elevated IL-1 activity after concussion. In contrast to IL-6, split analyses by sex showed that IL-1RA was only elevated in the early acute phase (0–12 hours) in male athletes/cadets; IL-1RA levels in female participants were not associated with concussion. There are well-established sex differences in immune function, partially mediated by sex hormones, which vary by immune cell-type and age.<sup>25</sup> For example, after puberty, female individuals have a more robust inflammatory response to infection and vaccination compared with male individuals,<sup>34,35</sup> whereas autoimmune diseases are more common in female than male individuals.<sup>36</sup> Similarly, sex differences in IL-1 activity have been reported, including differences in microglial IL-1 $\beta$  messenger RNA expression in injured cortex after experimental TBI in mice,<sup>37</sup> differences in IL-1 $\beta$  expression in the brain due to stress,<sup>38</sup> in the secretion of IL-1 $\alpha$ , IL-1 $\beta$ , and IL-1RA from mononuclear cells,<sup>39</sup> and in IL-1RA gene polymorphisms.<sup>40</sup> Although there is growing appreciation that sex moderates the effects of brain injury,<sup>26,27</sup> female participants are still underrepresented in concussion research,<sup>41</sup> including the current project (~20% female participants). Additional studies are needed to characterize potential sex differences in IL-1 activity after concussion and the potential role of sex hormones, menstrual cycle, and other sex-specific factors.

Current results suggest that IL-6 and IL-1RA levels early after concussion may have clinical utility in identifying individuals with concussion from uninjured participants because both markers alone showed fair discrimination (AUCs >0.70). Peripheral inflammatory markers are sensitive to multiple nonconcussion-related factors, including those relevant to athletes and cadets.<sup>17,21</sup> For example, exercise can result in an acute elevation of inflammatory markers, including IL-6 and IL-1RA,<sup>42-45</sup> while peripheral injuries can also elevate inflammatory markers in blood.<sup>46</sup> Furthermore, we are unable to determine the cellular origin of IL-1RA or IL-6 (e.g., peripheral vs brain origin). Accordingly, the current results should be interpreted with some caution, and it is unlikely that these markers have utility as blood-based biomarkers for concussion in isolation. Nevertheless, although the improvement in AUC was not significant ( $p = 0.06$ ), likelihood ratio tests showed that IL-6 and IL-1RA provided additional diagnostic utility above and beyond blood-based biomarkers thought to be more specific to brain injury (GFAP, UCH-L1, tau, and NfL). These markers should be carefully considered for future inclusion in biomarker panels for mTBI and concussion.

Furthermore, our results also suggest that IL-6 and IL-1RA could potentially be used to supplement current clinical decision-making tools. Although blood biomarkers will never replace objective clinical testing, they could provide additional information in scenarios where there is concern about performance validity or where balance testing or comprehensive neurocognitive testing are not typically conducted (e.g., in an emergency department setting). The fact that the addition of IL-6 and IL-1RA did not statistically improve the AUC relative to symptom reporting is not surprising given the AUC for symptom alone (0.96). The results from the logistic regression analysis did show, however, that IL-6 and IL-1RA did improve the model fit above and beyond clinical symptoms. Thus, these markers could potentially help lower the reliance on subjective symptom report, such as in cases where there is concern about either malingering or symptom underreporting by athletes or cadets based on motivational factors.

Data for current analyses were collected as part of a prospective study of cadets and athletes with concussion recruited from a geographically diverse set of data collection sites. Both male and female cadets and athletes were included, and athletes from multiple sports were enrolled, which should improve the generalizability of the findings. There are limitations that should be considered. First, female participants were underrepresented compared with male participants, and follow-up studies in female athletes and cadets with concussion are merited. Second, limiting self-report of biological sex to a binary variable (male/female) may have led to exclusion or misclassification of some individuals. Third, the age range of included participants was narrow, and it is uncertain whether observed results are generalizable to younger or older individuals with concussion. Fourth, inflammatory markers

and brain injury markers were measured on different platforms. The MSD platform was used for inflammatory markers to maintain consistency with our previous work.<sup>19,20</sup> MSD V-PLEX assays use high-sensitivity multiplexed technology with wide dynamic range and bridged assays to maximize reliability across lots.<sup>47,48</sup> Nevertheless, it is possible that alternative technology (i.e., Simoa) could be more sensitive to some of the measured inflammatory markers. Finally, the panel of biomarkers focused on relatively common markers of inflammation (as well as VEGF) based on previous literature, and it is possible that other inflammatory markers not investigated may also be sensitive to concussion.

These data show the discriminative ability of serum levels of IL-6 (all participants) and IL-1RA (male participants only) acutely after concussion in athletes and cadets and demonstrate that these markers should be considered for future biomarker arrays aimed at supplementing clinical decision-making regarding concussion diagnosis. Furthermore, IL-1RA results highlight sex differences in the immune response to concussion which should be considered in future biomarker work.

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