Published Ahead of Print on June 16, 2023 as 10.1212/WNL.0000000000207389

OPEN

Neurology®

The most widely read and highly cited peer-reviewed neurology journal
The Official Journal of the American Academy of Neurology

AMERICAN ACADEMY OF NEUROLOGY®

Neurology Publish Ahead of Print
DOI: 10.1212/WNL.0000000000207389

Longitudinal Associations Between Blood Biomarkers and White-Matter MRI in Sport-Related Concussion: A Study of the NCAA-DoD CARE Consortium

Author(s):
Yu-Chien Wu, MD, PhD, DABMP[1]; Qiuting Wen, PhD[1]; Rhea Thukral, BS[1]; Ho-Ching Yang, PhD[1]; Jessica M. Gill, PhD, RN[2]; Sujuan Gao, PhD[3]; Kathleen A. Lane, MS[3]; Timothy B. Meier, PhD[4]; Larry D Riggen, MS[3]; Jaroslaw Harezlak, PhD[5]; Christopher C. Giza, MD[6]; Joshua Goldman, MD, FACSM[7]; Kevin M. Guskiewicz, PhD, ATC[8]; Jason P. Mihalik, PhD, CAT(C), ATC[8]; Stephen M. LaConte, PhD[9]; Stefan M. Duma, PhD[9]; Steven P Broglio, Ph.D.[10]; Andrew J. Saykin, PsyD[1]; Thomas Walker McAllister, MD[11]; Michael A McCrea, Ph.D[4]

Corresponding Author:
Yu-Chien Wu, yucwu@iu.edu

Affiliation Information for All Authors: 1. Department of Radiology and Imaging Sciences, Indiana University School of Medicine; 2. School of Nursing, Johns Hopkins; 3. Department of Biostatistics and Health Data Science, Indiana University School of Medicine; 4. Department of Neurosurgery, Medical College of Wisconsin; 5. Department of Epidemiology and Biostatistics, School of Public Health, Indiana University; 6. Department of Neurosurgery, David Geffen School of Medicine at University of California Los Angeles; 7. Family Medicine, Ronald Reagan UCLA Medical Center, UCLA Health - Santa Monica Medical Center; 8. Matthew Gfeller Center, Department of Exercise and Sport Science, University of North Carolina; 9. School of Biomedical Engineering and Sciences, Wake-Forest and Virginia Tech University; 10. Michigan Concussion Center, University of Michigan; 11. Department of Psychiatry, Indiana University School of Medicine.

Equal Author Contribution:
Drs. Yu-Chien Wu and Qiuting Wen are co-first authors.

Contributions:
Yu-Chien Wu: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data
Qiuting Wen: Analysis or interpretation of data
Rhea Thukral: Analysis or interpretation of data
Ho-Ching Yang: Analysis or interpretation of data
Jessica M. Gill: Major role in the acquisition of data; Analysis or interpretation of data
Sujuan Gao: Drafting/revision of the manuscript for content, including medical writing for content; Analysis or interpretation of data
Kathleen A. Lane: Drafting/revision of the manuscript for content, including medical writing for content; Analysis or interpretation of data
Timothy B. Meier: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Analysis or interpretation of data
Larry D Riggen: Major role in the acquisition of data; Analysis or interpretation of data
Jaroslaw Harezlak: Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data
Christopher C. Giza: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Joshua Goldman: Major role in the acquisition of data
Kevin M. Guskiewicz: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Jason P. Mihalik: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

Stephen M. LaConte: Major role in the acquisition of data
Stefan M. Duma: Major role in the acquisition of data
Steven P Broglio: Drafting/revision of the manuscript for content, including medical writing for content; Study concept or design
Andrew J. Saykin: Study concept or design
Thomas Walker McAllister: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design
Michael A McCrea: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design

Figure Count:4

Table Count:3

Search Terms:
[120] MRI, [264] Brain trauma, Sport-related concussion, blood biomarkers, diffusion tensor imaging

Acknowledgment:
The authors would like to thank Jody Harland, MS, Janetta Matesan, BA, Michael Menser (Indiana University School of Medicine); Ashley Rettmann, BS, Nicole L'Heureux, MBA (University of Michigan); Melissa Koschnitzke, MA (Medical College of Wisconsin); Michael Jarrett, MBA, Vibeke Brinck, MS and Bianca Byrne, BA (Quesgen); Melissa Baker, BS (Datalys Center for Sports Injury Research and Prevention); and the research and medical staff at each of the CARE participation sites. We thank Mr. Michael J. McGill (medical student at Indiana University School of Medicine) for organizing and summarizing literature reviews. We are grateful for the participation of the student athletes without whom this research would not be possible.

Study Funding:
This publication was made possible, in part, with support from the Grand Alliance Concussion Assessment, Research, and Education (CARE) Consortium, funded, in part by the National Collegiate Athletic Association (NCAA) and the Department of Defense (DOD). The U.S. Army Medical Research Acquisition Activity, 820 Chandler Street, Fort Detrick MD 21702-5014 is the awarding and administering acquisition office. This work was supported by the Office of the Assistant Secretary of Defense for Health Affairs, through the Combat Casualty Care Research

Program, endorsed by the Department of Defense, through the Joint Program Committee 6/ Combat Casualty Care Research Program – Psychological Health and Traumatic Brain Injury Program under Award No. W81XWH1420151. Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the Department of Defense. Other funding support includes National Institutes of Health grant R01 NS112303 to YCW and JH and R01 AG053993 to YCW.

Disclosures:
The authors report no relevant disclosures.

Preprint DOI:

Received Date:
2022-09-30

Accepted Date:
2023-03-22

Handling Editor Statement:
Submitted and externally peer reviewed. The handling editor was Associate Editor Rebecca Burch, MD.

Abstract

Objective

To study longitudinal associations between blood-based neural biomarkers (including total tau, neurofilament light (NfL), glial fibrillary acidic protein (GFAP), and ubiquitin C-terminal

hydrolase-L1 (UCH-L1)) and white-matter neuroimaging biomarkers in collegiate athletes with sport-related concussion (SRC) from 24-hours post-injury to 1 week after return-to-play.

Methods

We analyze clinical and imaging data of concussed collegiate athletes in the Concussion Assessment, Research and Education (CARE) Consortium. The CARE participants completed same-day clinical assessments, blood draws, and diffusion tensor imaging (DTI) at 3 time points: 24-48 hours post-injury, point of becoming asymptomatic, and 7 days following return-to-play. DTI probabilistic tractography was performed for each subject at each time point to render 27 subject-specific major white-matter tracts. The microstructural organization of these tracts was characterized by four DTI metrics. Mixed-effects models with random intercepts were applied to test whether white-matter microstructural abnormalities are associated with the blood-based biomarkers at the same time point. An interaction model was used to test if the association varies across time points. A lagged model was used to test if early blood-based biomarkers predict later microstructural changes.

Results

Data from 77 collegiate athletes were included in the following analyses. Among the 4 blood-based biomarkers, total tau had significant associations with the DTI metrics across the 3 time points. In particular, tau was positively associated with radial diffusivity (RD) in the right corticospinal tract (β=0.25, SE=0.07, $p_{FDR\text{-}adjusted}$=0.016) and superior thalamic radiation (β=0.21, SE=0.07, $p_{FDR\text{-}adjusted}$=0.042). NfL and GFAP had time-dependent associations with the DTI metrics. NfL showed significant associations only at the asymptomatic time point ($|\beta|$s>0.12,

SEs<0.09, $ps_{FDR\text{-}adjusted}$<0.05) and GFAP showed a significant association only at 7-days following return-to-play (βs>0.14, SEs<0.06, $ps_{FDR\text{-}adjusted}$<0.05). The p-values for the associations of early tau and later RD were not significant after multiple comparison adjustment, but were less than 0.1 in seven white-matter tracts.

Conclusions

This prospective study using data from the CARE Consortium demonstrated that in the early phase of SRC, white-matter microstructural integrity detected by DTI neuroimaging was associated with elevated levels of blood-based biomarkers of traumatic brain injury. Total tau in the blood showed the strongest association with white-matter microstructural changes.

Glossary

AD = axial diffusivity; CARE = Concussion Assessment, Research and Education Consortium; CNS = central nervous system; DTI = diffusion tensor imaging; FA = fractional anisotropy; FSL = functional MRI of the brain software library; GFAP = glial fibrillary acidic protein; MD = mean diffusivity; MRI = magnetic resonance imaging; NfL = neurofilament light; RD = radial diffusivity; SRC = sport-related concussion; TBI = traumatic brain injury; TOI = tract-of-interest; UCH-L1 = ubiquitin C-terminal hydrolase-L1.

Introduction

Sport-related concussion (SRC) is a serious public health issue affecting 1.6 to 3.8 million high school and collegiate athletes.[1,2] Diffuse axonal injury is generally believed to be the initial neuropathology associated with mild traumatic brain injury (mTBI), including SRC.[3] As shown in animal models, closed head injury may initiate diffuse axonal injury that induces axonal pathologies and diffusion signal changes,[4,5] and repetitive brain injury may increase the burden of neocortical axonal injury.[5,6] Changes in the white matter following diffuse axonal injury may be detected by magnetic resonance imaging (MRI)-based methods, particularly diffusion tensor imaging (DTI). DTI measures the integrity of the white-matter microarchitecture, reflecting axonal organization, as well as supporting microstructures such as myelin, neuroglia, and substrates.[7] DTI has shown prognostic value in SRC,[4,5,8,9] and may serve as an objective imaging biomarker for white-matter abnormalities.

In response to brain injury, damaged axons and supporting cells (e.g., astrocytes) may release some metabolites into the circulation that can be detected in the serum or plasma of a peripheral blood sample. Changes in the concentration or levels of such biomarkers may serve as signs of specific biological processes in the central nervous system (CNS) in response to neurotrauma and may reflect the severity of neuronal and axonal damage.[10] For example, CNS blood-based **Tau** and neurofilament light (**NfL**) are axon-specific proteins, and ubiquitin C-terminal hydrolase L1 (**UCH-L1**) is a cytosolic neuronal protein that is highly and specifically expressed in neurons. In addition, glial fibrillary acidic protein (**GFAP**) is involved in the

structure and function of the cytoskeleton in astroglial cells. These blood-based biomarkers have demonstrated potential for clinical utility in the management of SRC.[11-14]

Few studies have investigated the relationship between the neuroimaging and fluid biomarkers in *chronic* SRC and shown significant associations.[15] While the above described MRI white-matter changes and proteomic biomarkers from peripheral blood have been characterized separately in acute injury settings,[8,11,12,16] their associations in the *acute post-injury* and recovery periods have not been well characterized. In addition, although tau, NfL, and UCH-L1 are most abundant in the cerebrum, they are also expressed in the peripheral nervous system. Thus, examining the strength of associations between their levels in the peripheral blood with neuroimaging is important in the context of sport-related brain injury.

Therefore, in this study, we examined (1) if white-matter microstructural integrity detected by DTI is associated with acute changes in blood-based neural biomarkers (i.e., tau, NfL, GFAP, and UCH-L1) in collegiate athletes sustaining SRC and (2) how the relationship between the white-matter microstructural integrity and the neural biomarkers varies across three time points; the acute time point (at 24–48 hours post-injury), the asymptomatic time point: and 7 days following return-to-play. We also evaluated (3) if early blood biomarkers can predict later white-matter microstructural integrity across this period of SRC. Similar to many groupwise analytical approaches, our analyses are based on the hypothesis that common vulnerabilities in white matter tracts may exist despite the heterogeneity in injury mechanisms in SRC.

Methods

Study cohorts

We analyzed previously acquired neuroimage and clinical data of concussed collegiate athletes recruited in a multi-site study of the natural history of concussion conducted through the NCAA-DoD Concussion Assessment, Research, and Education (CARE) Consortium. We downloaded all the available neuroimaging data acquired between the beginning of the CARE study in 2014 and the initiation of this analysis in July 2018. The inclusion and exclusion criteria have been previously described in the CARE publication by Broglio et al..[17] In brief, a large sample of student athletes were enrolled in the CARE studies. Consenting varsity athletes are assessed on a variety of baseline measures and followed over the duration of their college career. Number of previous concussions (self-report) and the age of the first concussion were recorded at the baseline screening, and participants were excluded from the follow-up visits if they had previous concussions within 6 months of the baseline assessments. When diagnosed with a concussion, they are assessed at five additional timepoints up to 6-months after injury. A subsample of the participants undergoes additional characterization with multi-modal MR imaging and fluid biomarkers. Concussions were diagnosed by the site research and medical staff based on the consensus guideline, which decided concussion as "a change in brain function following a force to the head, which may be accompanied by temporary loss of consciousness, but is identified in awake individuals with measures of neurologic and cognitive dysfunction".[18] This present study did not impose additional inclusion/exclusion criteria to the original CARE dataset other than the cutoff time when this analysis was initiated.

Standard Protocol Approvals, Registrations, and Patient Consents

This study did not actively recruit the participants. Nevertheless, in the CARE study (the source of the data), all participants provided written informed consent approved by the Medical College of Wisconsin Institutional Review Board (IRB) and the U.S. Department of Defense Human Research Protection Office (HRPO).[17]

Prospective longitudinal study design

All participants completed baseline clinical assessments when recruited into the study. After the concussion events, the concussed athletes received medical care and observations by the site physicians or medical staff. Clinical assessments, blood sample collection, and multimodal MRI scans were performed at multiple post-injury time points.[17,19] In this study, we examine the associations between DTI and the blood biomarkers at three time points: (1) 24-48 hours post-injury, (2) the point at which the concussed athletes became asymptomatic (cleared for return-to-play progression), and (3) 7 days following unrestricted return-to-play. This study design ensured that the MRI and blood biomarkers were collected at similar clinical recovery milestones across all the concussed athletes. All participants underwent MRI scans on the same day as blood collection. Serving as a reference for illustration, the blood biomarkers at baseline (preseason collection) and 6-hours post-injury were also included here. The decision on asymptomatic state and return-to-play was made by team physicians. When the concussed athletes became asymptomatic, they started a stepwise exercise progression protocol of five rehabilitation stages that had to be completed prior to unrestricted return to play.[20]

Blood sample collection and biomarker analysis

The collection of blood samples and biomarker analysis followed the CARE protocol described in a previous publication.[11] Briefly, blood samples were collected by venipuncture with a 10-ml purple-top EDTA tube prior to being centrifuged and aliquoted into cryovials. The cryovials were stored upright in a -80^{o} C freezer until analysis. The plasma biomarker levels were analyzed using single molecular array technology (SimoaTM, Quanterix Corp., Lexington, MA) with a multi-plex technology that simultaneously quantified total-tau, NfL, GFAP, and UCH-L1. Assays were batched to minimize variability, longitudinal samples from the same individual were run on the same plate, and each batch was run with the appropriate standards and controls to ensure reliability. For this study, we used all available plasma biomarker data regardless of their coefficient of variance (CV) values to preserve the data to the greatest extent. The average inter-plate CVs for total tau, NfL, GFAP, and UCH-L1 were 9.75% (SD=7.87), 5.96% (SD=4.65), 2.75% (SD=2.67), and 12.72% (SD=16.57), respectively. The percentage of biomarker data whose CV values exceeded 20% were 5.7% (total tau), 0% (NfL), 0.6% (GFAP), and 8.2% (UCH-L1).

Diffusion imaging protocol

The neuroimaging imaging acquisition protocol and longitudinal MRI quality assurance/control followed the original CARE design described in previous publications.[8,9,19] Briefly, for diffusion MRI, scans were performed on participants on Siemens MAGNETOM 3T scanners across three study sites, including the University of North Carolina (UNC), the University of California Los

Angeles (UCLA), and Virginia Tech (VT). Throughout the CARE study, a single 3T MRI scanner was utilized at each site. Both UNC and UCLA used Siemens Tim Trio scanners that were upgraded to Prisma in 2016; nevertheless, the MRI parameters were made identical prior to and following the upgrade. VT utilized a Siemens Tim Trio scanner for the duration of the study. A single-shot echo-planar imaging sequence with a twice-refocused spin echo was used. The diffusion-encoding scheme consisted of 30 directions at b-value of 1000 s/mm^2 and 8 b_0 (b-value= 0 s/mm^2). One of the b_0 volumes was acquired with a reversed phase-encoding direction. Other MRI parameters were echo time (TE) = 98 ms, repetition time (TR) = 7900 ms, field-of-view (FOV) = 243 mm, matrix size = 90 x 90, whole brain coverage of 60 slices with a slice thickness of 2.7 mm, and isotropic resolution of 2.7 mm.

Image pre-processing

For diffusion-weighted images, we used the same pre-processing pipelines described in previous studies.[8,9] DTI metrics include fractional anisotropy (FA, the coherence of microstructure water diffusion), mean diffusivity (MD, the magnitude of overall water diffusion), radial diffusivity (RD, perpendicular to the principal water diffusion direction), and axial diffusivity (AD, along the principal water diffusion direction) (**eTable 1**). Maps of the DTI metrics were transformed to the standard MNI space using Advanced Neuroimaging Tools (ANTs) non-linear registration.[21] Moreover, the directionality of the underlying microstructural organization in white matter, the major eigenvector (V1) of the diffusion tensor, was extracted from each voxel for probability tractography described below.

Probability tractography for subject-specific tracts of interest

Similar to our previous publication,[22] a within-voxel multi-fiber tract orientation structure was modeled using BEDPOSTx followed by probabilistic tractography (with crossing fiber modeling) using PROBTRACKx[23] and AutoPtx plugin for FSL).[24] Tract-specific measures of diffusion metrics (i.e., FA, MD, RD, AD) were derived for the following 27 tracts-of-interest (including bilateral tracts) covering most of the brain major white matter tracts: middle cerebellar peduncle (mcp); medial lemniscus (ml); uncinate fasciculus (unc); cingulate gyrus and parahippocampal portions of the cingulum bundle (cgc, cgh); forceps major and minor (fma, fmi); corticospinal tract (cst); acoustic radiation (ar); anterior, superior, and posterior thalamic radiation (atr, str, ptr); and superior, inferior longitudinal, and inferior fronto-occipital fasciculus (slf, ilf, ifo) (**eFigure 1**).

Means of the DTI metrics in the subject-specific tracts of interest (TOI) were computed for each subject at each time point to study: (1) if microstructural organization of TOIs associates with axonal biomarkers (total-tau and NfL), neuroglial biomarker (GFAP), or neuron biomarker (UCH-L1) in the blood; (2) changes in such associations over time; and (3) if blood-based biomarkers can predict later white-matter changes in this acute to subacute phases of SRC.

Statistical analyses

The statistical analyses were conducted using SAS software version 9.4. The blood biomarkers were logarithmically transformed to adjust for the right skewness in the distributions, and

values of the DTI metrics in TOIs were standardized to z-scores using all the data points (i.e., 173, **eTable 2**). To adjust for correlations among longitudinal measures from the same individual, mixed effects models were used to analyze the data. The mixed effects models provide unbiased estimates under the missing at random assumption.[25] Mixed effects models with random intercepts for each subject were used to test for differences in blood biomarkers between time points. If the overall test was significant, post-hoc pairwise tests were performed with Tukey adjustments for multiple comparisons.

Similarly, mixed effects models with random intercepts were used to study the associations of the blood biomarkers (as dependent variables) with each of the DTI metrics. The covariates included time, age, sex, and site. In the initial assessment, for each blood biomarkers, the percentage of significant findings for the 4 DTI metrics in the 27 white-matter tracts were reported. Benjamini & Hochberg false discovery rate (FDR)[26] was used for adjusting p-values for multiple comparisons in post-hoc analyses.

To study time-varying effects on the associations, DTI-time interaction was added as an independent variable in the mixed effects model. If the time interaction was significant, post-hoc analyses were carried out to test the associations at individual time points. p-Values were adjusted to account for multiple comparisons in the post-hoc analyses by controlling the FDR.

To determine whether blood biomarkers can be used to predict latent microstructural changes observed at a later time point, mixed effects models were used with DTI metrics as dependent variables and blood biomarkers measured from an earlier time point as independent variables adjusting for time, age, sex, and site. In the initial assessment, for each blood biomarker, total numbers of significant findings for the 4 DTI metrics in the 27 white-matter tracts were reported. To identify those white-matter tracts in which a blood biomarker can significantly predict later diffusion metrics, p-values were adjusted for multiple comparisons by controlling the FDR. P-values <0.05 were considered statistically significant unless otherwise stated. Nevertheless, results with adjusted p values < 0.05 and adjusted p values < 0.1 were reported.

Data availability

The MRI data and clinical data were collected through the CARE project funded by the NCAA-DoD Grand Alliance. Deidentified data are available following the existing data sharing plans outlined in the CARE consortium (https://redcap.uits.iu.edu/surveys/?s=ngUQpwiuHG). The CARE neuroimaging data and clinical data are also available on the Federal Interagency Traumatic Brain Injury Research (FITBIR, https://fitbir.nih.gov/content/access-data) platform since March 2019.

Results

A total of 77 collegiate athletes who sustained SRC, completed the assessment protocol, and completed MRI scans in Siemens 3T scanners by July 2018 were included in this study. As subjects could have multiple concussions, only data from the first concussion was used for the analysis. The characteristics of the participating athletes are listed in **Table 1**. The 77 concussed athletes were participants in college football (n=45), soccer (n=24), and lacrosse (n=8). The overall post-injury time span was approximately 1 month, ranging from the acute time point at 24–48 hours post-injury (2.09 ± 1.50), to asymptomatic (12.62 ± 28.78) and 7 days following return-to-play (29.66 ± 36.52 days). The asymptomatic and 7 days following return-to-play varied among the participants due to their natural history of recovery. Note that while the clinical recovery time may be different for individual athletes, the MRI and blood biomarkers were collected at similar clinical recovery milestones (i.e., asymptomatic time point and 7 days after return-to-play). The position for individual sports, previous concussion history, and self-report medical history and previous concussion are listed in **Table 1**. Similar to many longitudinal studies, not all the baseline subjects received blood draws and MRIs at every follow-up time point despite our best efforts. **eTable 2** lists the numbers of subjects who had usable diffusion MRI or available blood biomarker data at each follow-up time point. Overall, there were 173 usable DTI data, 157 tau, 160 NfL, 160 GFAP, and 122 UCH-L1 biomarker data.

Longitudinal changes in blood biomarkers

Mean levels of the blood biomarkers at each time point are reported in **Table 1** and **Figure 1**. During 24-hour post-SRC to 7 days following return-to-play, NfL and GFAP did not change significantly over the three time points (white zone in **Figure 1**), while tau and UCH-L1 exhibited significant longitudinal changes. Plasma tau increased significantly from 24–48 hours post-injury to asymptomatic time point ($p = 0.001$) and from 24–48 hours post-injury to 7 days post return-to-play ($p < 0.001$). Plasma UCH-L1 significantly increased from asymptomatic time point to 7 days post return-to-play ($p = 0.003$). Blood biomarker levels at baseline (preseason collection) and 6 hours post-injury are illustrated in **Figure 1** (gray zone) for reference purposes as neuroimaging data were not available for these 2 time points for the following association analyses.

Associations between the blood biomarkers and DTI metrics

Plasma tau had the most significant associations with DTI. The significance rate was 18.5% for the 4 DTI metrics in the 27 white-matter tracts (20 significant associations divided by 4*27, **Table 2**). GFAP had a 10.2% significance rate, while NfL and UCH-L1 had a 1.9% and 0% significance rate, respectively. The direction of the associations of the significant tau and GFAP was negative with FA and positive with diffusivities (i.e., MD, AD, and RD). The beta coefficient (i.e., slope) of the associations for tau ranged between 0.13 and 0.25 ln(pg/ml) in absolute values per unit change of standardized DTI measures (**Figure 2A**). For GFAP, the beta

coefficient ranged between 0.08 and 0.12 ln(pg/ml) per unit change of standardized DTI measures (**eTable 3**).

After FDR adjustment, RD demonstrated significant associations with tau in the right corticospinal tract (beta coefficient = 0.25, $p_{FDR\text{-}corrected} < 0.05$, **Figure 3, A** and **B**) and superior thalamic radiation (beta coefficient = 0.21, $p_{FDR\text{-}corrected} < 0.05$, **Figure 3, A** and **C**). The blood tau levels were higher with elevated RD in these 2 white-matter tracts. MD also demonstrated significant associations with tau in the same tracts with weaker significance ($0.05 < p_{FDR\text{-}corrected} < 0.1$, **Figure 4**). Similar to RD, the blood tau levels were higher with higher MD in the right corticospinal tract and superior thalamic radiation (**Figure 4, B** and **C**).

Longitudinal changes in the associations between blood biomarkers and DTI metrics

Among the 4 blood biomarkers, NfL had the highest number of time-dependent associations with DTI described by a significant DTI-time interaction term in the mixed effect models. NfL had 12.03% significance rate for the DTI-time interaction among the 27 white-matter tracts (13 significant interactions divided by 4*27, **Table 2** and **eTable 4**). GFAP, UCH-L1, and tau had a 3.70%, 2.78%, and 0% significance rate, respectively. In the post-hoc association analyses at individual time points, the significant associations ($p_{FDR\text{-}adjusted} < 0.05$) between NfL and the DTI metrics occurred only at asymptomatic point. At this time point, the direction of the NfL associations was positive with FA and AD and negative with MD and RD (**Table 3**). The beta coefficient of the associations ranged between 0.12 and 0.21 ln(pg/ml) in absolute values per

unit change of standardized DTI measures. In contrast, for GFAP, only 7 days post return-to-play had significant GFAP-DTI associations ($p_{\text{FDR-adjusted}} < 0.05$, **Table 3**). Unlike NfL, GFAP positively associated with diffusivities (MD & AD) with beta coefficients ranged between 0.14 and 0.21 ln(pg/ml) per unit change of standardized DTI measures.

Associations between early blood biomarkers and later DTI metrics

Using the lagged mixed effects model, early tau levels in the blood were significantly associated with later DTI metrics with a 15.7% significance rate among the 27 white-matter tracts (17 significant associations divided by 4*27, **Table 2** and **eTable 5**). The significance associations rates for NfL, GFAP, and UCH-L1 were 3.7%, 3.7%, and 0%, respectively. Similar to the concurrent associations, early tau levels in the blood were negatively associated with FA and positively associated with diffusivities, including MD, AD, and RD (**Figure 2B**). The beta coefficient of the early-tau-later-DTI associations ranged between 0.23 and 0.32 $[\ln(\text{pg/ml})]^{-1}$ in absolute values. After adjusting for multiple comparisons by controlling for the FDR, the associations did not reach significance in any particular tract. Though, positive trends ($0.05 < p_{\text{FDR-adjusted}} < 0.10$) were observed in 7 white matter tracts, including the bilateral acoustic radiation, right anterior thalamic radiation, middle cerebellar peduncle, left medial lemniscus, left posterior thalamic radiation, and right uncinate fasciculus (**eFigure 2**).

Discussion

In our previous studies using the CARE data, we detected group differences in the DTI metrics between the concussed football players and contact-sport controls.[9] In addition, the acute changes in DTI metrics were associated with the severity of initial symptoms after SRC, including psychological distress, cognition, and recovery time.[8,9] On the other hand, we have also demonstrated that acute changes in the blood biomarkers were associated with loss of consciousness or post-traumatic amnesia.[11] In the present study, we combined these two objective measures and investigated whether the changes in neuronal blood biomarkers can be explained by microstructural changes in brain white matter detected by DTI. This prospective study demonstrated that in the interval spanning 1-day post-SRC to 1 week after return-to-play, white-matter microstructural integrity detected by DTI was associated with CNS-related metabolites in the blood. The associations showed temporal variations during this period of SRC. In addition, the early CNS blood biomarkers showed promises in predicting later white-matter microstructural composition.

The longitudinal trajectories of this subset of blood biomarker data are consistent with a larger study of the CARE consortium primarily focusing on the relationship between blood biomarkers and clinical outcome measures.[11] The longitudinal changes in the blood biomarkers showed acute responses to SRC with peak changes at 6-hours post-injury in NfL, GFAP, and UCH-L1. Tau appeared to have a slightly delayed response, bottoming out at 24–48 hours post-injury.

During the 24–48 hours post-injury time point to the 7-days following return-to-play time point, the tau level in the blood continued to evolve and return toward the baseline level while other blood biomarkers were relatively stable.

This evolution of tau in the blood may reflect longitudinal changes of axons during the initial response and recovery phase following SRC. This hypothesis was supported by the same-time association analyses, in which tau was the most sensitive blood biomarker reflecting brain microstructural integrity detected by the DTI metrics. Further support for this hypothesis was provided by the prediction analyses, where only the early tau level was significantly associated with the later brain microstructure integrity within this time period of SRC. Furthermore, the significant prediction results suggest the clinical and prognostic utility of the tau blood biomarker.

In these analyses, higher tau levels were significantly associated with higher radial diffusivity and mean diffusivity, as well as, to a minor extent, lower fractional anisotropy. Overall, the directions of change in DTI-metrics are consistent with the consequences of axonal degradation with increased organizational dispersion and increased water diffusion freedom perpendicular to the axons. The underlying pathophysiological explanation for increased radial diffusivity could be axonal beading, reduced axonal packing density, and/or compromised myelin sheaths.[27,28] This observation complements our previous findings of significant group differences in the DTI mean and radial diffusivities between concussed and control athletes, as

well as persistent elevation of these diffusivities in the white matter of concussed athletes.[8] Our results in humans are supported by a rat model of mTBI with closed-head injury, where decreased FA was observed in the corpus callosum 21 days post injury.[5] In another closed-head-injury rat model, decreased FA and increased MD and RD were observed longitudinally from 1 day post-injury to 30 days post-injury. These changes in DTI metrics were associated with myelin compactness detected by immunohistochemistry analysis.[4]

This finding of degraded white-matter microstructural integrity coinciding with higher tau may be the underlying mechanisms of previous observations, where higher tau levels associate with longer time needed for return-to-play in hockey players,[29] and collegiate athletes.[30,31] Furthermore, our previous publication provides direct evidence connecting poor white-matter integrity and longer recovery time.[8] Nevertheless, interestingly, the longitudinal evolution of the plasma tau level after SRC followed a paradoxical direction. Namely, unlike the other three biomarkers, the plasma tau level decreased right after the concussion. Similar paradoxical trajectory was also observed in previous SRC studies in collegiate athletes.[11,31,32] One possible explanation for this paradoxical direction is that, in addition to the CNS, the origin of total tau in the peripheral blood also includes the peripheral nervous system as well as peripheral tissues (e.g., liver, kidney, and heart).[33,34] The increase in the total tau level in the blood between the asymptomatic and return-to-play time points might be due to the reintroduction of exercise (i.e., the rehabilitation program) and its impact on the peripheral tissues. Nevertheless, it remains puzzling that the total tau level increased between 24-48 hours post-injury and the asymptomatic time point when prescribed rest was recommended. While more studies are

needed, the potential explanation may relate to the tau species' releasing process through the blood-brain barrier, phosphorylation state, and subsequent metabolism.

Few published studies have focused on the relationship of tau and DTI white-matter imaging in SRC. Our results of this early period of SRC may fill in the temporal gap of a previous study in TBI (including mild, moderate, and severe), in which serum tau was found to be weakly associated with the DTI metrics, namely FA (negative associations), ranging from 3 to 17 months after injury.[15] Similarly, in another study of preseason football players, tau was positively associated with DTI mean diffusivity and negatively associated with the neurite density index derived from diffusion compartment modeling.[35] Such results, albeit with a modest sample (n=17), may support the potential explanation of the underlying pathology of low axonal packing in high diffusivity in cases of accumulated head impacts.

During this period of 24-hour post-SRC to 1 week following return-to-play, our results showed stable NfL levels as well as insignificant associations with the DTI metrics, except at the asymptomatic state. Similarly, GFAP was relatively stable in this phase and did not associate with the DTI metrics, except at 7 days following return-to-play. It is possible that these 2 blood biomarkers are more sensitive to chronic white-matter changes as reported in the aforementioned TBI study, in which both NfL and GFAP became significantly associated with DTI at 3 to 17 months post-injury.[14,15] Interestingly, the direction of associations between NfL and the DTI metrics were opposite between this SRC study and the previous TBI study. Unlike the

previous study, in this study, high NfL levels were associated with high FA and AD, but with low MD and RD, suggesting higher packing density or cellularity. This discrepancy may arise from a different phase of recovery (subacute vs. chronic) or different brain-injury mechanisms, suggested by previous preclinical studies.[36,37]

There are some limitations in the present study. Although plasma total tau showed differences between preseason and postconcussion in ice hockey players[13] and group differences in collegiate contact sport players,[11] the total tau in the blood may not directly reflect the level of CNS damage owing to unknown blood-brain-barrier penetration. Studies showed poor correlations between plasma total tau and cerebrospinal fluid (CSF) total tau in individuals with Alzheimer's disease[38] and with persistent postconcussive symptoms for more than 3 months after repetitive concussions.[39] Phosphorylated tau might be a better blood biomarker with higher CNS specificity.[40] On the other hand, plasma NfL and GFAP demonstrated significant correlations with their CSF counterparts in TBI[14,39,41] and in Alzheimer disease.[42] In the most recent study of moderate-severe TBI, cerebral microdialysis of brain extracellular fluid seems to correlate well with NfL, tau and UCH-L1.[43] Despite being cost-effective with minimally invasive, blood biomarkers do not provide anatomical specificity, which can be followed up by detailed neuroimaging examination.

Among many diffusion MRI approaches, DTI has the advantages of simplicity and efficiency in image acquisition and mathematical model computation. However, unlike sophisticated

diffusion compartment modeling approaches (such as neurite orientation dispersion and density imaging[44] or kurtosis-based white-matter tract integrity imaging[45]), DTI metrics provide only summarized descriptions of tissue organization with ambiguities in pathophysiological specificities. The present study does not include direct comparisons with controls or correlations with clinical assessments, which have been described and published on the same cohorts.[8,11] Previous concussion history as well as the age of first concussion might play a significant role in the brain recovery, which will be included in our future studies.

Despite the limitations, the CNS blood biomarkers and DTI neuroimaging (a product sequence in most MRI scanners for research, though not yet included in standard clinical imaging protocols) may provide convenient and objective measures of SRC. We have demonstrated that these two objective measures are associated. Specifically, elevated blood tau levels appeared to be associated with higher radial and mean diffusivity and lower fractional anisotropy. Linking blood biomarkers to neuroimaging and the CNS specificity of plasma total tau remain active research endeavors; our findings may contribute insights for future studies.

Figure legends

Figure 1. Longitudinal changes in blood biomarkers

(A). The total tau level in the blood across study time points. (B). The neurofilament light (NfL) in the blood across time. (C). The glial fibrillary acidic protein (GFAP) level in the blood across time. (D). The ubiquitin C-terminal hydrolase-L1 (UCH-L1) level in the blood across time. All the blood biomarker levels were natural-logarithm transformed to adjust for skewness. Time-point abbreviations: Base = baseline collection at preseason; 6h = 6-hours post-injury; 24-48h = 24-48 hours post-injury; Asymp = the point at which the concussed athletes became asymptomatic; and 7d post RTP = seven days following unrestricted return-to-play. Asterisk * indicates significant differences at $p < 0.05$ between time points using post-hoc pairwise tests with Tukey adjustments for multiple comparisons. Asterisk *** indicates significant differences at $p < 0.001$ between time points.

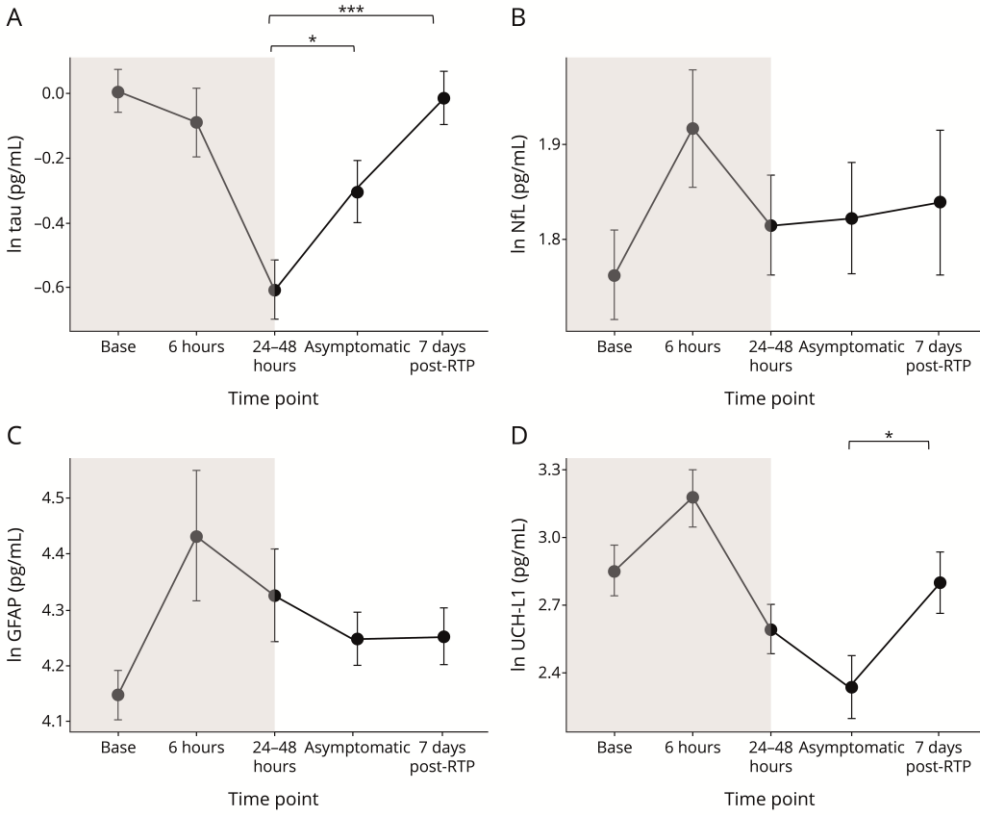


Figure 2. Beta coefficients of associations between tau and the DTI metrics in white-matter tracts

(A). Same-time associations. (B). Association between early tau and the later DTI metrics. The total tau levels in the blood were natural-logarithm transformed to adjust for skewness, and values of the DTI metrics in white-matter tracts were standardized. Blue bars denote beta coefficients of the associations from mixed effects models with random intercepts after adjusting for time, sex, and site at uncorrected $p < 0.05$. Errorbars denote standard error of the beta coefficients. Asterisk * indicates $0.05 < p_{FDR\text{-}adjusted} < 0.1$ and asterisk ** indicates $p_{FDR\text{-}adjusted} < 0.05$. DTI metrics: FA = fractional anisotropy; MD = mean diffusivity; AD = axial diffusivity; and RD = radial diffusivity. Abbreviations for white-matter tracts are listed in the **Methods** section, subsection ***Probability tractography for subject-specific tracts of interest*** and **eFigure 1**. '_l' denotes left hemisphere and '_r' denotes right hemisphere.

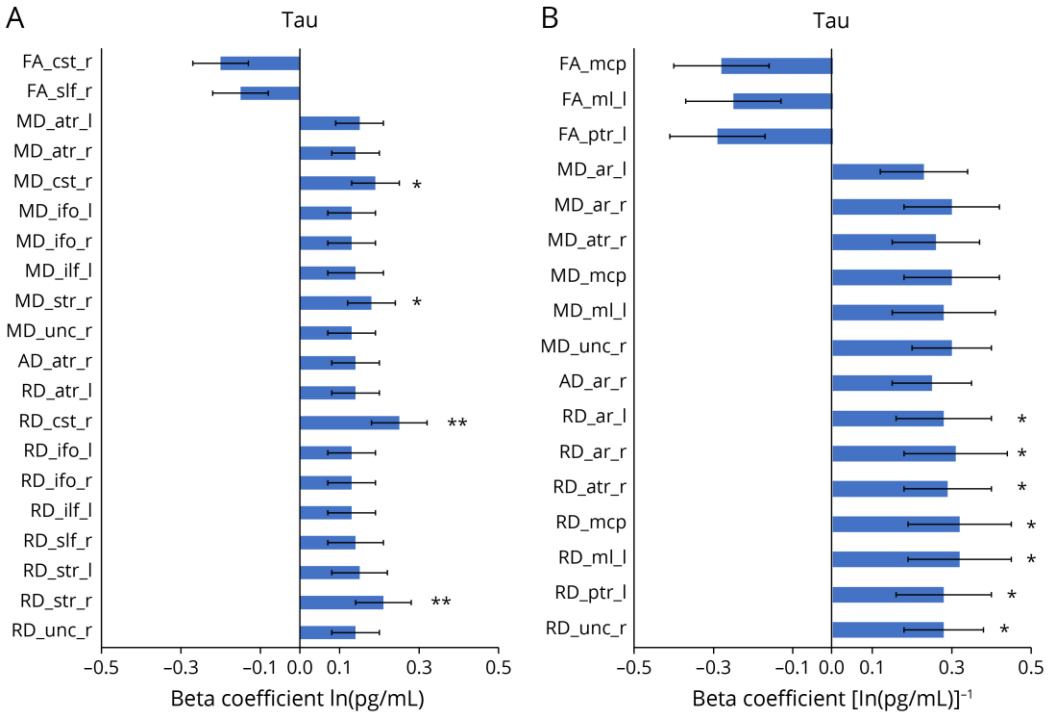


Figure 3. White-matter tracts with significant tau-RD associations

(A) The right corticospinal tract had a beta coefficient = 0.25 (yellow), and the right superior thalamic radiation had a beta coefficient = 0.21 (orange). Both had FDR adjusted $p < 0.05$. (B) Post-hoc scatter plot of tau and DTI derived radial diffusivity (RD) in the right corticospinal tract. Each gray dot indicates a data point of a concussed athlete. (C) Post-hoc scatter plot of tau and RD in the right superior thalamic radiation.

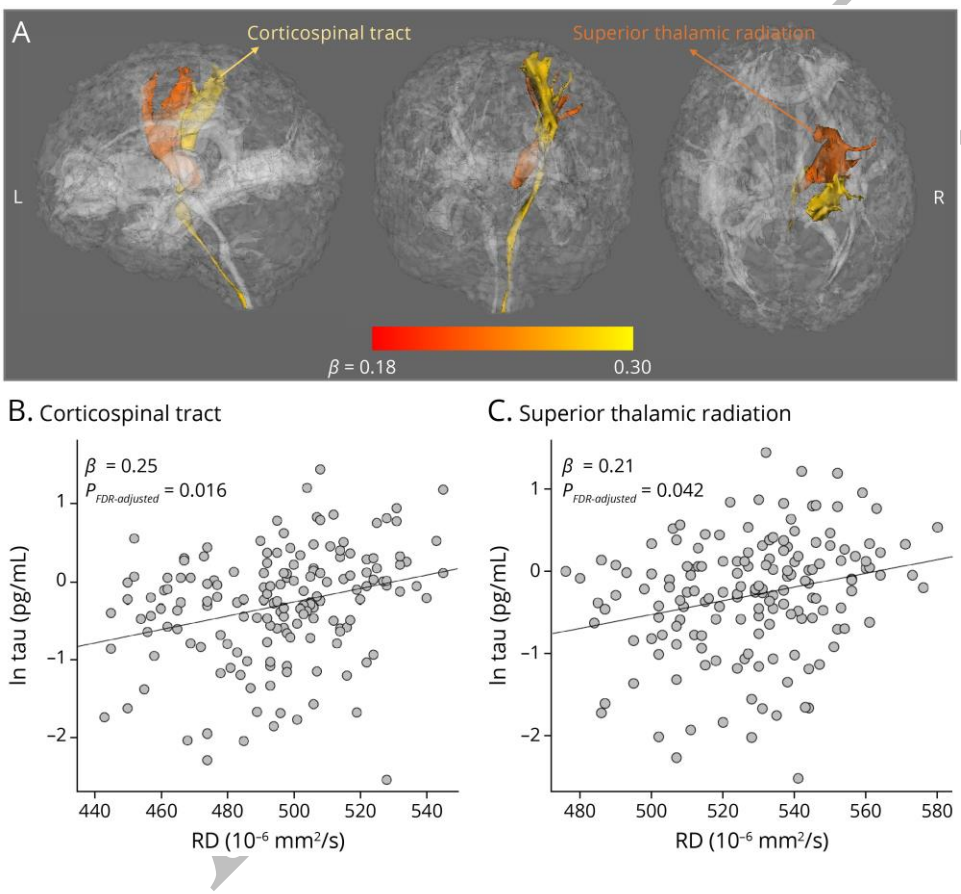
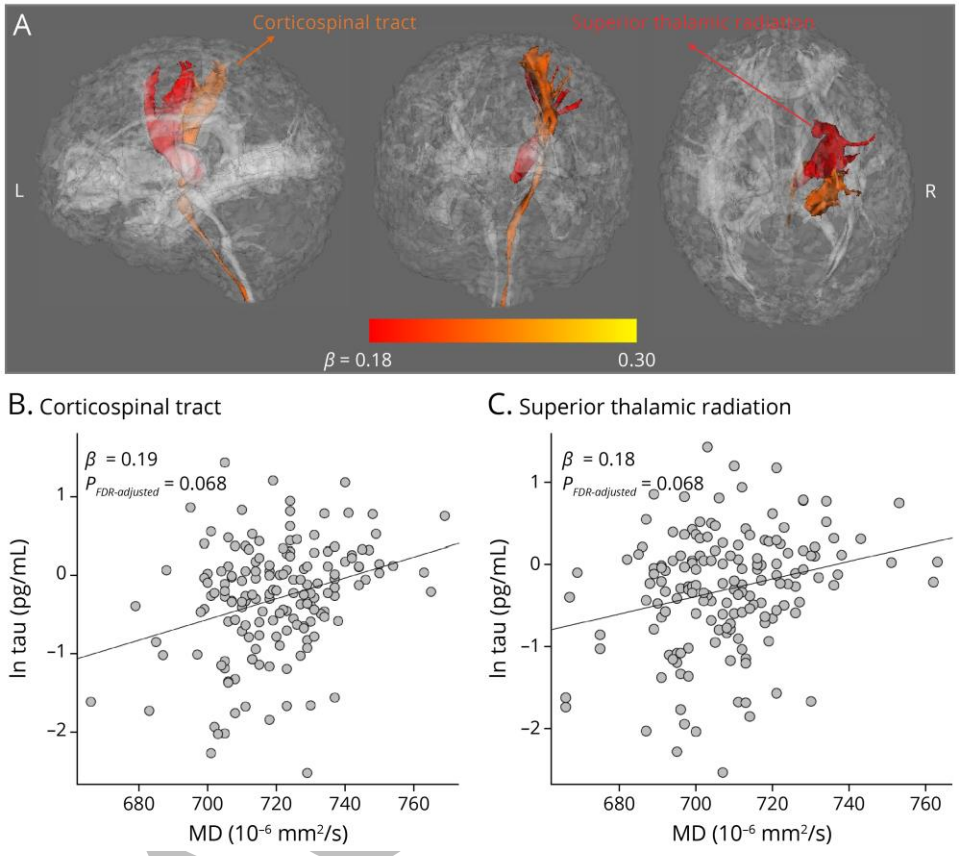


Figure 4. White-matter tracts with significant tau-MD associations

(A) The right corticospinal tract had a beta coefficient = 0.19 (orange), and the right superior thalamic radiation had a beta coefficient = 0.18 (red). Both had FDR adjusted p < 0.1. (B) Post-hoc scatter plot of tau and DTI derived mean diffusivity (MD) in the right corticospinal tract. Each gray dot indicates a data point of a concussed athlete. (C) Post-hoc scatter plot of tau and MD in the right superior thalamic radiation.



http://links.lww.com/WNL/C807

http://links.lww.com/WNL/C808

http://links.lww.com/WNL/C809

http://links.lww.com/WNL/C810

http://links.lww.com/WNL/C811

http://links.lww.com/WNL/C812

References

1. Langlois J A, Rutland-Brown W, and Wald M M. The epidemiology and impact of traumatic brain injury: A brief overview. J Head Trauma Rehabil 2006;21:375-378.
2. Daneshvar D H, Nowinski C J, McKee A C, and Cantu R C. The epidemiology of sport-related concussion. Clin Sports Med 2011;30:1-17, vii.
3. Laskowski R A, Creed J A, and Raghupathi R, Pathophysiology of mild tbi: Implications for altered signaling pathways, in Brain neurotrauma: Molecular, neuropsychological, and rehabilitation aspects, Kobeissy F H, Editor: Boca Raton (FL); 2015.
4. Tu T W, Williams R A, Lescher J D, Jikaria N, Turtzo L C, and Frank J A. Radiological-pathological correlation of diffusion tensor and magnetization transfer imaging in a closed head traumatic brain injury model. Ann Neurol 2016;79:907-920.
5. Kao Y J, Lui Y W, Lu C F, Chen H L, Hsieh B Y, and Chen C Y. Behavioral and structural effects of single and repeat closed-head injury. AJNR Am J Neuroradiol 2019;40:601-608.
6. Ogino Y, Vascak M, and Povlishock J T. Intensity specific repetitive mild traumatic brain injury evokes an exacerbated burden of neocortical axonal injury. J Neuropathol Exp Neurol 2018;77:782-792.
7. Basser P J, and Pierpaoli C. Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor mri. J Magn Reson B 1996;111:209-219.
8. Wu Y C, Harezlak J, Elsaid N M H, Lin Z, Wen Q, Mustafi S M, et al. Longitudinal white-matter abnormalities in sports-related concussion: A diffusion mri study. Neurology 2020;95:e781-e792.
9. Mustafi S M, Harezlak J, Koch K M, Nencka A S, Meier T B, Giza C C, et al. Acute white-matter abnormalities in sports-related concussion: A diffusion tensor imaging study from the ncaa-dod care consortium. Journal of Neurotrauma 2018;35:2653-2664.
10. Zetterberg H, Smith D H, and Blennow K. Biomarkers of mild traumatic brain injury in cerebrospinal fluid and blood. Nat Rev Neurol 2013;9:201-210.

11. McCrea M, Broglio S P, McAllister T W, Gill J, Giza C C, Huber D L, et al. Association of blood biomarkers with acute sport-related concussion in collegiate athletes: Findings from the ncaa and department of defense care consortium. JAMA Netw Open 2020;3:e1919771.
12. Meier T B, Huber D L, Bohorquez-Montoya L, Nitta M E, Savitz J, Teague T K, et al. A prospective study of acute blood-based biomarkers for sport-related concussion. Ann Neurol 2020;87:907-920.
13. Shahim P, Tegner Y, Wilson D H, Randall J, Skillback T, Pazooki D, et al. Blood biomarkers for brain injury in concussed professional ice hockey players. JAMA Neurol 2014;71:684-692.
14. Shahim P, Politis A, van der Merwe A, Moore B, Chou Y Y, Pham D L, et al. Neurofilament light as a biomarker in traumatic brain injury. Neurology 2020;95:e610-e622.
15. Shahim P, Politis A, van der Merwe A, Moore B, Ekanayake V, Lippa S M, et al. Time course and diagnostic utility of nfl, tau, gfap, and uch-l1 in subacute and chronic tbi. Neurology 2020;95:e623-e636.
16. Wu Y C, Mustafi S M, Harezlak J, Kodiweera C, Flashman L A, and McAllister T W. Hybrid diffusion imaging in mild traumatic brain injury. J Neurotrauma 2018;35:2377-2390.
17. Broglio S P, McCrea M, McAllister T, Harezlak J, Katz B, Hack D, et al. A national study on the effects of concussion in collegiate athletes and us military service academy members: The ncaa-dod concussion assessment, research and education (care) consortium structure and methods. Sports Med 2017;47:1437-1451.
18. Carney N, Ghajar J, Jagoda A, Bedrick S, Davis-O'Reilly C, du Coudray H, et al. Concussion guidelines step 1: Systematic review of prevalent indicators. Neurosurgery 2014;75 Suppl 1:S3-15.
19. Nencka A S, Meier T B, Wang Y, Muftuler L T, Wu Y C, Saykin A J, et al. Stability of mri metrics in the advanced research core of the ncaa-dod concussion assessment, research and education (care) consortium. Brain Imaging Behav 2018;12:1121-1140.
20. McCrory P, Meeuwisse W, Dvorak J, Aubry M, Bailes J, Broglio S, et al. Consensus statement on concussion in sport-the 5(th) international conference on concussion in sport held in berlin, october 2016. Br J Sports Med 2017;51:838-847.

21. Avants B B, Tustison N J, Song G, Cook P A, Klein A, and Gee J C. A reproducible evaluation of ants similarity metric performance in brain image registration. Neuroimage 2011;54:2033-2044.
22. Wen Q, Mustafi S M, Li J, Risacher S L, Tallman E, Brown S A, et al. White matter alterations in early-stage alzheimer's disease: A tract-specific study. Alzheimers Dement (Amst) 2019;11:576-587.
23. Behrens T E, Berg H J, Jbabdi S, Rushworth M F, and Woolrich M W. Probabilistic diffusion tractography with multiple fibre orientations: What can we gain? Neuroimage 2007;34:144-155.
24. de Groot M, Vernooij M W, Klein S, Ikram M A, Vos F M, Smith S M, et al. Improving alignment in tract-based spatial statistics: Evaluation and optimization of image registration. Neuroimage 2013;76:400-411.
25. Ibrahim J G, and Molenberghs G. Missing data methods in longitudinal studies: A review. Test (Madr) 2009;18:1-43.
26. Benjamini Y, and Hochberg Y. Controlling the false discovery rate: A practical and powerful approach to multiple testing. Journal of the Royal Statistical Society. Series B (Methodological) 1995;57:289–300.
27. Klawiter E C, Schmidt R E, Trinkaus K, Liang H F, Budde M D, Naismith R T, et al. Radial diffusivity predicts demyelination in ex vivo multiple sclerosis spinal cords. Neuroimage 2011;55:1454-1460.
28. Janve V A, Zu Z, Yao S Y, Li K, Zhang F L, Wilson K J, et al. The radial diffusivity and magnetization transfer pool size ratio are sensitive markers for demyelination in a rat model of type iii multiple sclerosis (ms) lesions. Neuroimage 2013;74:298-305.
29. Shahim P, Linemann T, Inekci D, Karsdal M A, Blennow K, Tegner Y, et al. Serum tau fragments predict return to play in concussed professional ice hockey players. J Neurotrauma 2016;33:1995-1999.
30. Pattinson C L, Meier T B, Guedes V A, Lai C, Devoto C, Haight T, et al. Plasma biomarker concentrations associated with return to sport following sport-related concussion in

collegiate athletes-a concussion assessment, research, and education (care) consortium study. JAMA Netw Open 2020;3:e2013191.

31. Gill J, Merchant-Borna K, Jeromin A, Livingston W, and Bazarian J. Acute plasma tau relates to prolonged return to play after concussion. Neurology 2017;88:595-602.
32. Meier T B, Bergamino M, Bellgowan P S, Teague T K, Ling J M, Jeromin A, et al. Longitudinal assessment of white matter abnormalities following sports-related concussion. Hum Brain Mapp 2016;37:833-845.
33. Dugger B N, Whiteside C M, Maarouf C L, Walker D G, Beach T G, Sue L I, et al. The presence of select tau species in human peripheral tissues and their relation to alzheimer's disease. J Alzheimers Dis 2016;54:1249.
34. Fischer I, and Baas P W. Resurrecting the mysteries of big tau. Trends Neurosci 2020;43:493-504.
35. Kawata K, Steinfeldt J A, Huibregtse M E, Nowak M K, Macy J T, Kercher K, et al. Association between proteomic blood biomarkers and dti/noddi metrics in adolescent football players: A pilot study. Front Neurol 2020;11:581781.
36. Mac Donald C L, Dikranian K, Bayly P, Holtzman D, and Brody D. Diffusion tensor imaging reliably detects experimental traumatic axonal injury and indicates approximate time of injury. J Neurosci 2007;27:11869-11876.
37. Budde M D, Janes L, Gold E, Turtzo L C, and Frank J A. The contribution of gliosis to diffusion tensor anisotropy and tractography following traumatic brain injury: Validation in the rat using fourier analysis of stained tissue sections. Brain : a journal of neurology 2011;134:2248-2260.
38. Mattsson N, Zetterberg H, Janelidze S, Insel P S, Andreasson U, Stomrud E, et al. Plasma tau in alzheimer disease. Neurology 2016;87:1827-1835.
39. Shahim P, Zetterberg H, Simren J, Ashton N J, Norato G, Scholl M, et al. Association of plasma biomarker levels with their csf concentration and the number and severity of concussions in professional athletes. Neurology 2022;99:e347-e354.

40. Barthelemy N R, Horie K, Sato C, and Bateman R J. Blood plasma phosphorylated-tau isoforms track cns change in alzheimer's disease. J Exp Med 2020;217.

41. Vos P E, Jacobs B, Andriessen T M, Lamers K J, Borm G F, Beems T, et al. Gfap and s100b are biomarkers of traumatic brain injury: An observational cohort study. Neurology 2010;75:1786-1793.

42. Blennow K. A review of fluid biomarkers for alzheimer's disease: Moving from csf to blood. Neurol Ther 2017;6:15-24.

43. Graham N S N, Zimmerman K A, Moro F, Heslegrave A, Maillard S A, Bernini A, et al. Axonal marker neurofilament light predicts long-term outcomes and progressive neurodegeneration after traumatic brain injury. Sci Transl Med 2021;13:eabg9922.

44. Zhang H, Schneider T, Wheeler-Kingshott C A, and Alexander D C. Noddi: Practical in vivo neurite orientation dispersion and density imaging of the human brain. Neuroimage 2012;61:1000-1016.

45. Fieremans E, Jensen J H, and Helpern J A. White matter characterization with diffusional kurtosis imaging. Neuroimage 2011;58:177-188.

46. Jones D K, and Oxford University Press., Diffusion mri theory, methods, and applications. 2011, Oxford University Press,: New York ; Oxford. p. 1 online resource (xvi, 767 p.

47. Kim J H, Budde M D, Liang H F, Klein R S, Russell J H, Cross A H, et al. Detecting axon damage in spinal cord from a mouse model of multiple sclerosis. Neurobiol Dis 2006;21:626-632.

48. Song S K, Yoshino J, Le T Q, Lin S J, Sun S W, Cross A H, et al. Demyelination increases radial diffusivity in corpus callosum of mouse brain. Neuroimage 2005;26:132-140.

Table 1. Demographics and characteristics of the concussed-athlete participants

Demographics	Mean (SD); n = 77
Age (years)	18.82 (0.87)
Sex (M:F)	64:13
Race (White:AfricanAmerican:Multiple:Hawaiian:Unknown)	(38:29:7:2:1)
BMI (kg/m^2)	27.16 (5.76)
Education (years)	13.50 (0.75)
WTAR standard score	106.23 (14.00)
Time until asymptomatic (days)	9.72 (6.27)
Time until seven days following unrestricted return-to-play (days)	25.82 (14.10)
Sport types (n) (football, soccer, lacrosse)	(45, 24, 8)
Position[a]	
Football (QB:C:CB:DL:WR:LB:Off:RB:S:ST)	(1:1:4:9:4:10:6:4:5:1)
Soccer (DB:FA:G:MF)	(5:7:5:7)
Lacrosse (DB:FA:G:MF)	(4:2:0:2)
Concussion history[b]	
No. of participants with previous concussion (0:1:2:3)	(41:27:7:2)
No. of football players with previous concussion (0:1:2:3)	(24:16:4:1)
No. of soccer players with previous concussion (0:1:2:3)	(12:9:2:1)
No. of lacrosse players with previous concussion (0:1:2:3)	(5:2:1:0)
Age at the first concussion (n = 35)* (years)	16.37 (1.9)
Premorbid risk factors (n)[c]	22
(ADD/ADHD, headache, depression, diabetes, hearing problems, learning disorder, memory disorder, sleep disorder, balance disorder, bipolar disorder, seizure disorder, psychiatric disorder, moderate/severe traumatic brain injury)	(13, 7, 3, 2, 2, 2, 2, 2, 1, 1, 1, 1, 1)
Loss of consciousness (no:yes)	73:4

Blood biomarkers[d] ln(pg/ml)	**Time point**			**Overall**[e]	**Tukey pairwise adjusted**		
	24-48 h[f]	Asymp[g]	7d post RTP[h]	*p* value	*p* value[i]	*p* value[j]	*p* value[k]
Tau (SD)	-0.60 (0.70)	-0.24 (0.73)	-0.01 (0.57)	**< 0.001**	**0.001**	**< 0.001**	0.200
NfL (SD)	1.81 (0.45)	1.81 (0.52)	1.85 (0.51)	0.200	--	--	--
GFAP (SD)	4.35 (0.70)	4.23 (0.40)	4.26 (0.34)	0.059	--	--	--
UCH-L1 (SD)	2.65 (0.76)	2.33 (0.98)	2.83 (0.80)	**0.004**	0.090	0.360	**0.003**

* We have 36 concussed participants who had previous concussion history. One of them did not report the age of the first concussion.

[a] Position abbreviations: QB = quarterback; C = center; CB = connerback; DL = defensive line; :WR = wide receiver; LB = linebacker; LS = long snapper; Off = tight end + off guard + off tackle; RB = running back; S = safety; ST = special team (FG offense + punt return); DB = Defensive Back; FA = Forward Attack; G = Goalkeeper; MF = Midfielder.

[b] Participants were excluded if previous concussions happened within 6 months prior to the baseline assessments.

[c] Participants might report multiple previous medical history.

[d] Logarithmically transformed for statistical tests.

[e] Mixed effects models with random intercepts for each subject to test if there is any difference in blood biomarkers between time points.

[f] 24-48-hours post-injury.

[g] Asymp denotes the point at which the concussed athletes were asymptomatic (cleared for return-to-play progression);

[h] 7d post RT denotes seven days following unrestricted return-to-play.

[i] *p*-Values for post-hoc comparisons between 24-48-hours post-injury and asymptomatic time point;

[j] *p*-Values for post-hoc comparisons between 24-48-hours post-injury and seven days following return-to-play;

[k] *p*-Values for post-hoc comparisons between asymptomatic and seven days following return-to-play.

Table 2. The significant association rate of the blood biomarkers with DTI in the white-matter tracts.

	total tau	NfL	GFAP	UCH-L1
Same-time associations[1]	18.5%	1.9%	10.2%	0%
Time interactions[2]	0%	12.0%	3.7%	2.8%
Predictions[3]	15.7%	3.7%	3.7%	0%

The rate was calculated by counting the significant associations (uncorrected $p < 0.05$) among the 4 DTI metrics in the 27 whole-brain white-matter tracts.

[1] The overall associations between the blood biomarkers with the DTI metrics at the same time point after adjusting for covariates (time, age, sex, and site). Also see eTable 3.

[2] Rate for significant DTI-time interaction (indicating time-dependent associations) in the overall associations. Also see eTable 4.

[3] The associations between early blood biomarkers and later DTI metrics after adjusting for covariates (time, age, sex, and site). Also see eTable 5.

Table 3. Significant associations between the blood biomarkers and DTI metrics at individual time points when time interaction is significant

DTI_Tract_Side	Time point of significant association	Beta coefficient	Standard Error	$P_{FDR\text{-}adjusted}$ value
		NfL		
FA_ifo_l	Asymp[a]	0.15	0.05	0.022
FA_ilf_r	Asymp	0.13	0.05	0.047
FA_mcp	Asymp	0.14	0.05	0.026
MD_ml_r	Asymp	-0.21	0.09	0.039
AD_ifo_l	Asymp	0.13	0.05	0.041
RD_mcp	Asymp	-0.12	0.04	0.020
		GFAP		
MD_fmi	7d post RTP[b]	0.14	0.06	0.022
MD_ifo_r	7d post RTP	0.15	0.05	0.008
MD_ptr_l	7d post RTP	0.17	0.05	0.004
AD_ilf_l	7d post RTP	0.21	0.06	0.001

Abbreviations: **fmi** = forceps minor; **ifo** = inferior fronto-occipital fasciculus; **ilf** = inferior longitudinal faciculus; **mcp** = middle cerebellar peduncle; **ml** = medial lemniscus; **ptr** = medial lemniscus; _l denotes left hemisphere; and _r denotes right hemisphere.

[a] Asymp denotes the point at which the concussed athletes were asymptomatic (cleared for starting return-to-play progression);
[b] 7d post RT denotes seven days following unrestricted return-to-play.f

Longitudinal Associations Between Blood Biomarkers and White-Matter MRI in Sport-Related Concussion: A Study of the NCAA-DoD CARE Consortium

Yu-Chien Wu, Qiuting Wen, Rhea Thukral, et al.

Neurology published online June 16, 2023
DOI 10.1212/WNL.0000000000207389