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Longitudinal Associations Between Blood Biomarkers and White Matter MRI in Sport-Related Concussion

A Study of the NCAA-DoD CARE Consortium

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Abstract

Background and Objectives

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

Methods

We analyzed 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 postinjury, point of becoming asymptomatic, and 7 days after return to play. DTI probabilistic tractography was performed for each participant at each time point to render 27 participant-specific major white matter tracts. The microstructural organization of these tracts was characterized by 4 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 whether the association varies across time points. A lagged model was used to test whether 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, high tau level was associated with high radial diffusivity (RD) in the right corticospinal tract ($\beta = 0.25$, SE = 0.07, $p_{\text{FDR-adjusted}} = 0.016$) and superior thalamic radiation ($\beta = 0.21$, SE = 0.07, $p_{\text{FDR-adjusted}} = 0.042$). 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, $ps_{\text{FDR-adjusted}} < 0.05$) and GFAP showed a significant association only at 7 days after return to play (βs > 0.14, SEs <0.06, $ps_{\text{FDR-adjusted}} < 0.05$). 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 7 white matter tracts.

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Glossary

AD = axial diffusivity; **CARE** = Concussion Assessment, Research, and Education Consortium; **CoV** = coefficient of variance; **DoD** = Department of Defense; **DTI** = diffusion tensor imaging; **FA** = fractional anisotropy; **FDR** = false discovery rate; **GFAP** = glial fibrillary acidic protein; **MD** = mean diffusivity; **mTBI** = mild TBI; **NCAA** = National Collegiate Athletic Association; **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; **UCLA** = University of California Los Angeles; **UNC** = University of North Carolina; **VT** = Virginia Tech.

Discussion

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.

Sport-related concussion (SRC) is a serious public health issue affecting 1.6–3.8 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 after diffuse axonal injury may be detected by MRI-based methods, particularly diffusion tensor imaging (DTI). DTI measures the integrity of the white matter microarchitecture, reflecting axonal organization and 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 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 abovedescribed 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 postinjury 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) whether 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 3 time points; the acute time point (at 24–48 hours postinjury), the asymptomatic time point, and 7 days after return to play. We also evaluated (3) whether 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 multisite study of the natural history of concussion conducted through the National Collegiate Athletic Association (NCAA)-Department of Defense (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 were assessed on a variety of baseline measures and followed up over the duration of their college career. The 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 were assessed at 5 additional timepoints up to 6 months after injury. A subsample of the participants underwent additional characterization with multimodal MRI 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 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 data), all participants provided written informed consent approved by the Medical College of Wisconsin Institutional Review Board and the US Department of Defense Human Research Protection Office.[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 postinjury time points.[17,19] In this study, we examined the associations between DTI and the blood biomarkers at 3 time points: (1) 24–48 hours postinjury, (2) the point at which the concussed athletes became asymptomatic (cleared for return-to-play progression), and (3) 7 days after 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 postinjury were also included in this study. 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 5 rehabilitation stages that had to be completed before 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] In brief, blood samples were collected by venipuncture with a 10-mL purple-top EDTA tube before being centrifuged and aliquoted into cryovials. The cryovials were stored upright in a −80°C freezer until analysis. The plasma biomarker levels were analyzed using single molecular array technology (Simoa; Quanterix Corp., Lexington, MA) with a multiplex 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 (CoV) values to preserve the data to the greatest extent. The average interplate CoVs 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 CoV values exceeded 20% were 5.7% (total tau), 0% (NfL), 0.6% (GFAP), and 8.2% (UCH-L1).

Diffusion Imaging Protocol

The neuroimaging acquisition protocol and longitudinal MRI quality assurance/control followed the original CARE design described in previous publications.[8,9,19] In brief, for diffusion MRI, scans were performed on participants on Siemens MAGNETOM 3T scanners across 3 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 used 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 before and after the upgrade. VT used 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 a b value of 1,000 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 = 98 milliseconds, repetition time = 7,900 milliseconds, field-of-view = 243 mm, matrix size = 90 × 90, whole brain coverage of 60 slices with a slice thickness of 2.7 mm, and isotropic resolution of 2.7 mm.

Image Preprocessing

For diffusion-weighted images, we used the same preprocessing 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, links.lww.com/WNL/C807). Maps of the DTI metrics were transformed to the standard Montreal Neurological Institute space using Advanced Neuroimaging Tools nonlinear 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 further.

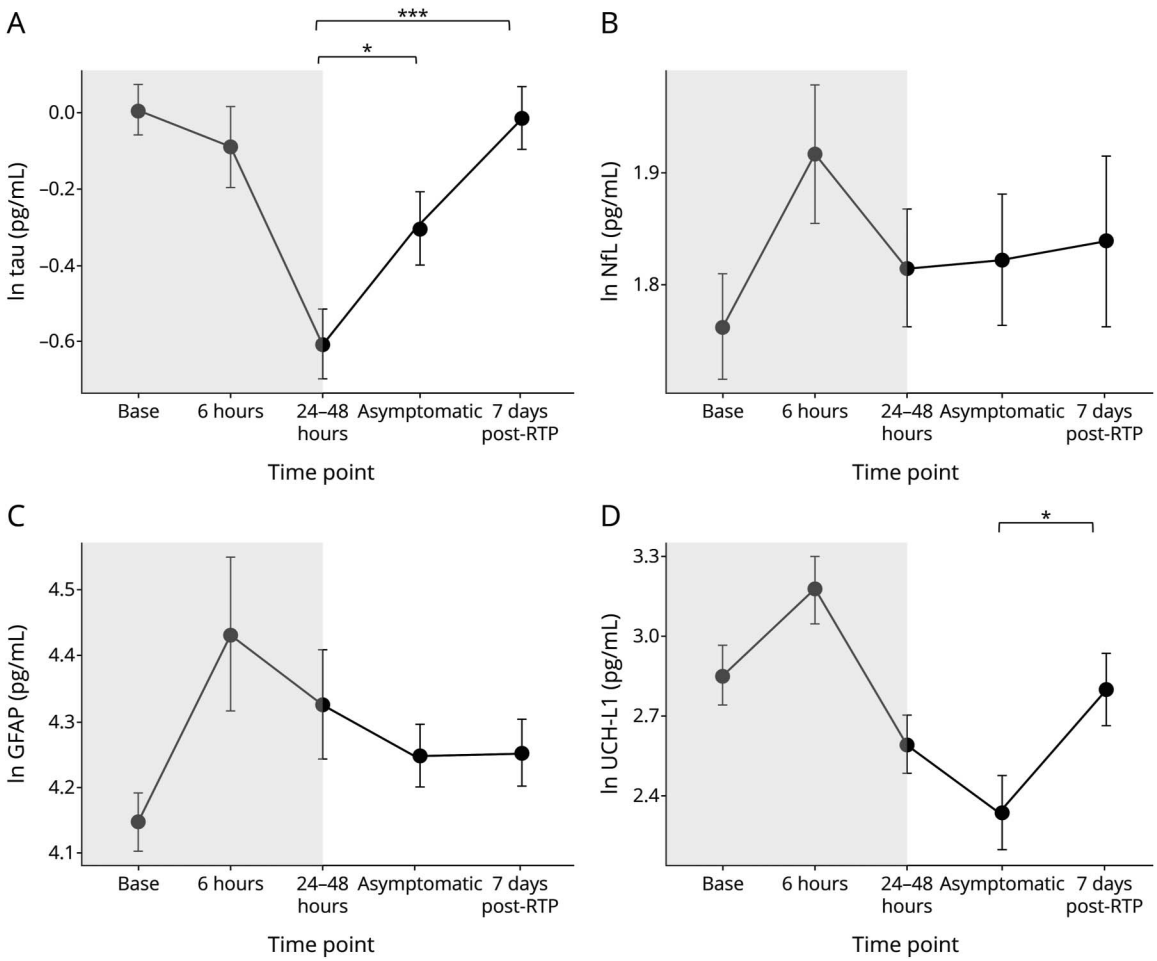
Table 1 Demographics and Characteristics of the Concussed-Athlete Participants

Demographics	Mean (SD); n = 77
Age (y)	18.82 (0.87)
Sex (male:female)	64:13
Race (White:AfricanAmerican:multiple:Hawaiian:unknown)	38:29:7:2:1
BMI (kg/m^2)	27.16 (5.76)
Education (y)	13.50 (0.75)
WTAR standard score	106.23 (14.00)
Time until asymptomatic (d)	9.72 (6.27)
Time until 7 d after unrestricted return to play (d)	25.82 (14.10)
Sport types (n) (football, soccer, and 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 (y) (n = 35)[c]	16.37 (1.9)
Premorbid risk factors (n)[d]	22
ADD/ADHD, headache, depression, diabetes, hearing problems, learning disorder, memory disorder, sleep disorder, balance disorder, bipolar disorder, seizure disorder, psychiatric disorder, and 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

	Time point			Overall[f]	Tukey pairwise adjusted		
Blood biomarkers[e] **ln(pg/mL)**	**24–48 h**[g]	**Asymp**[h]	**7d post RTP**[i]	***p* Value**	***p* Value**[j]	***p* Value**[k]	***p* Value**[l]
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**

Bold entries denote *p* values lower than 0.05.
Abbreviations: ADD/ADHD = attention-deficit/hyperactivity disorder; BMI = body mass index; GFAP = glial fibrillary acidic protein; NfL = neurofilament light; UCH-L1 = ubiquitin C-terminal hydrolase-L1; WTAR = Wechsler Test of Adult Reading.
[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 before the baseline assessments.
[c] We have 36 concussed participants who had previous concussion history. One of them did not report the age of the first concussion.
[d] Participants might report multiple previous medical history.
[e] Logarithmically transformed for statistical tests.
[f] Mixed-effects models with random intercepts for each participant to test whether there is any difference in blood biomarkers between time points.
[g] 24–48 hours postinjury.
[h] Asymp denotes the point at which the concussed athletes were asymptomatic (cleared for return-to-play progression).
[i] 7d post-RT denotes 7 days after unrestricted return to play.
[j] *p* Values for post hoc comparisons between 24 and 48 hours postinjury and asymptomatic time point.
[k] *p* Values for post hoc comparisons between 24 and 48 hours postinjury and 7 days after return to play.
[l] *p* Values for post hoc comparisons between asymptomatic and 7 days after return to play.

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; 6 hours = 6 hours postinjury; 24–48 hours = 24–48 hours postinjury; Asymptomatic = the point at which the concussed athletes became asymptomatic; and 7 days post-RTP = 7 days after 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.

Probability Tractography for Subject-Specific Tracts of Interest

Similar to our previous publication,[22] a within-voxel multifiber tract orientation structure was modeled using BEDPOSTx followed by probabilistic tractography (with crossing fiber modeling) using PROBTRACKx[23] and AutoPtx plugin for functional MRI of the brain software library).[24] Tract-specific measures of diffusion metrics (i.e., FA, MD, RD, and 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, and ptr); and superior, inferior longitudinal, and inferior fronto-occipital fasciculus (slf, ilf, and ifo) (eFigure 1, links.lww.com/WNL/C812).

Mean values of the DTI metrics in the subject-specific tracts of interest (TOI) were computed for each subject at each time point to study: (1) whether microstructural organization of TOI 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) whether blood-based biomarkers can predict later white matter changes in these acute to subacute phases of SRC.

Statistical Analyses

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, links.lww.com/WNL/C808). To adjust for correlations among longitudinal measures from the same individual, mixed-effects models were used to

Table 2 Significant Association Rate of the Blood Biomarkers With DTI in the White Matter Tracts

	Total tau	NfL	GFAP	UCH-L1
Same-time associations (%)[a]	18.5	1.9	10.2	0
Time interactions (%)[b]	0	12.0	3.7	2.8
Predictions (%)[c]	15.7	3.7	3.7	0

Abbreviations: DTI = diffusion tensor imaging; GFAP = glial fibrillary acidic protein; NfL = neurofilament light; UCH-L1 = ubiquitin C-terminal hydrolase-L1.
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.
[a] 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 (links.lww.com/WNL/C809).
[b] Rate for significant DTI-time interaction (indicating time-dependent associations) in the overall associations. Also see eTable 4 (links.lww.com/WNL/C810).
[c] The associations between early blood biomarkers and later DTI metrics after adjusting for covariates (time, age, sex, and site). Also see eTable 5 (links.lww.com/WNL/C811).

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 biomarker, the percentage of significant findings for the 4 DTI metrics in the 27 white matter tracts were reported. Benjamini and 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 conducted 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

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 (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.nih.gov/content/access-data) platform since March 2019.

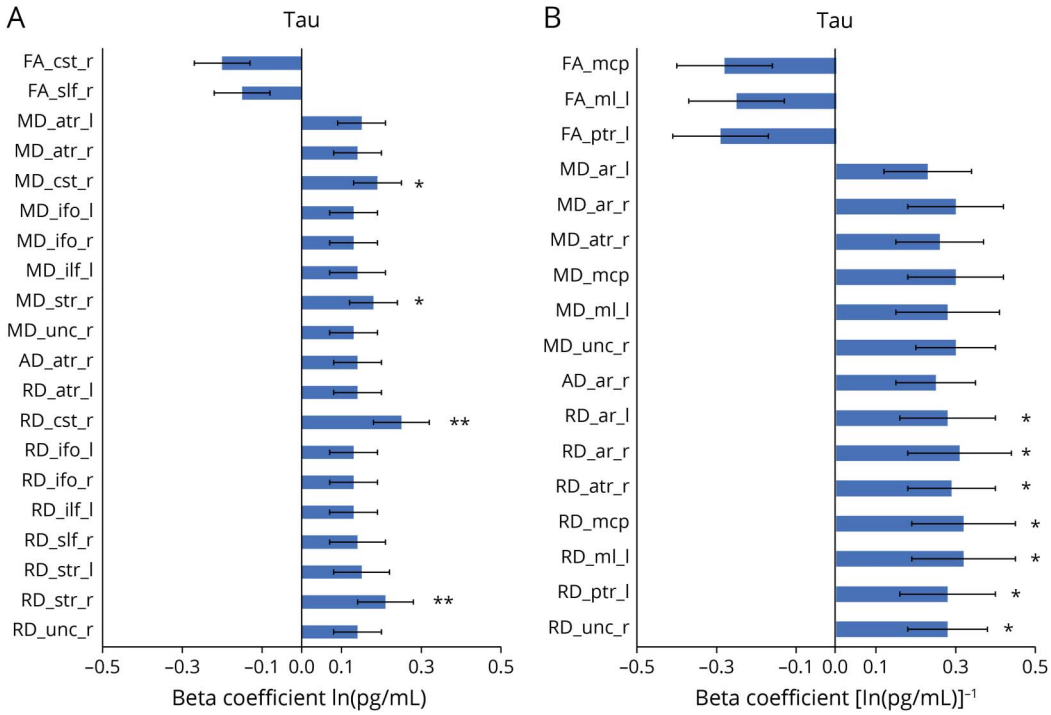
Results

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. Because participants 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 postinjury time span was approximately 1 month, ranging from the acute time point at 24–48 hours postinjury (2.09 ± 1.50 days) to asymptomatic (12.62 ± 28.78 days) and 7 days after return to play (29.66 ± 36.52 days). The asymptomatic and 7 days after 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 participants received blood draws and MRIs at every follow-up time point despite our best efforts. eTable 2 (links.lww.com/WNL/C808) lists the numbers of participants who had useable diffusion MRI or available blood biomarker data at each follow-up time point. Overall, there were 173 useable DTI data, 157 tau, 160 NfL, 160 GFAP, and 122 UCH-L1 biomarker data.

Longitudinal Changes in Blood Biomarkers

The mean levels of the blood biomarkers at each time point are summarized in Table 1 and presented in Figure 1. During 24-hour post-SRC to 7 days after return to play, NfL and GFAP did not change significantly over the 3 time points (white zone in Figure 1), while tau and UCH-L1 exhibited significant longitudinal changes. Plasma tau increased significantly from 24–48 hours postinjury to asymptomatic time point ($p = 0.001$) and from 24–48 hours postinjury 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

Figure 2 β 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 diffusion tensor imaging (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 β coefficients of the associations from mixed-effects models with random intercepts after adjusting for time, sex, and site at uncorrected $p < 0.05$. Error bars denote standard error of the β 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 (links.lww.com/WNL/C812). "_l" denotes left hemisphere and "_r" denotes right hemisphere.

levels at baseline (preseason collection) and 6 hours postinjury are illustrated in Figure 1 (gray zone) for reference purposes because neuroimaging data were not available for these 2 time points for the following association analyses.

Associations Between the Blood Biomarkers and DTI Metrics

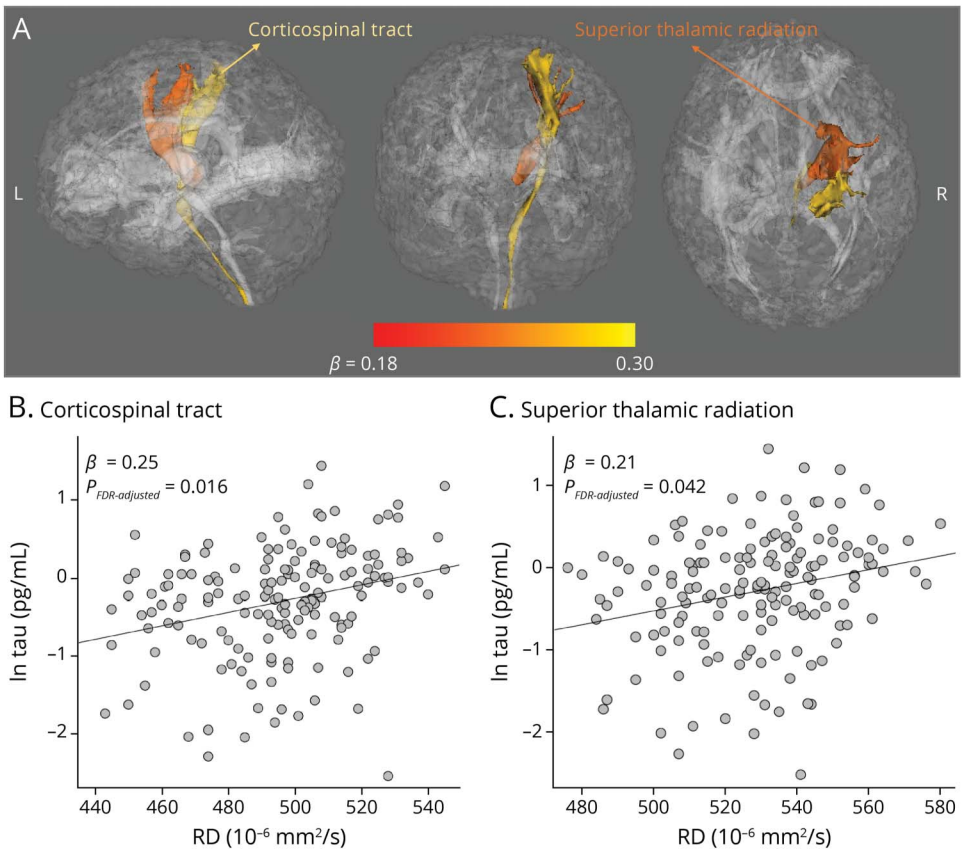
Plasma tau had the most significant associations with DTI. The significance rate was 18.5% for the 4 DTI metrics in the 27 white matter tracts (20 significant associations divided by 4 × 27, Table 2). GFAP had a 10.2% significance rate, while NfL and UCH-L1 had a 1.9% and 0% significance rate, respectively. 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 β 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 β coefficient ranged between 0.08 and 0.12 ln(pg/mL) per unit change of standardized DTI measures (eTable 3, links.lww.com/WNL/C809).

After FDR adjustment, RD demonstrated significant associations with tau in the right corticospinal tract (β coefficient = 0.25, $p_{FDR\text{-}corrected} < 0.05$, Figure 3, A and B) and superior thalamic radiation (β coefficient = 0.21, $p_{FDR\text{-}corrected} < 0.05$, Figure 3, A and C). The plasma 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 plasma 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, links.lww.com/WNL/C810). 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 β coefficient of the associations ranged between 0.12 and 0.21

Figure 3 White Matter Tracts With Significant Tau-RD Associations



(A) The right corticospinal tract had a β coefficient = 0.25 (yellow), and the right superior thalamic radiation had a β coefficient = 0.21 (orange). Both had false discovery rate–adjusted $p < 0.05$. (B) Post hoc scatter plot of tau and diffusion tensor imaging (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.

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

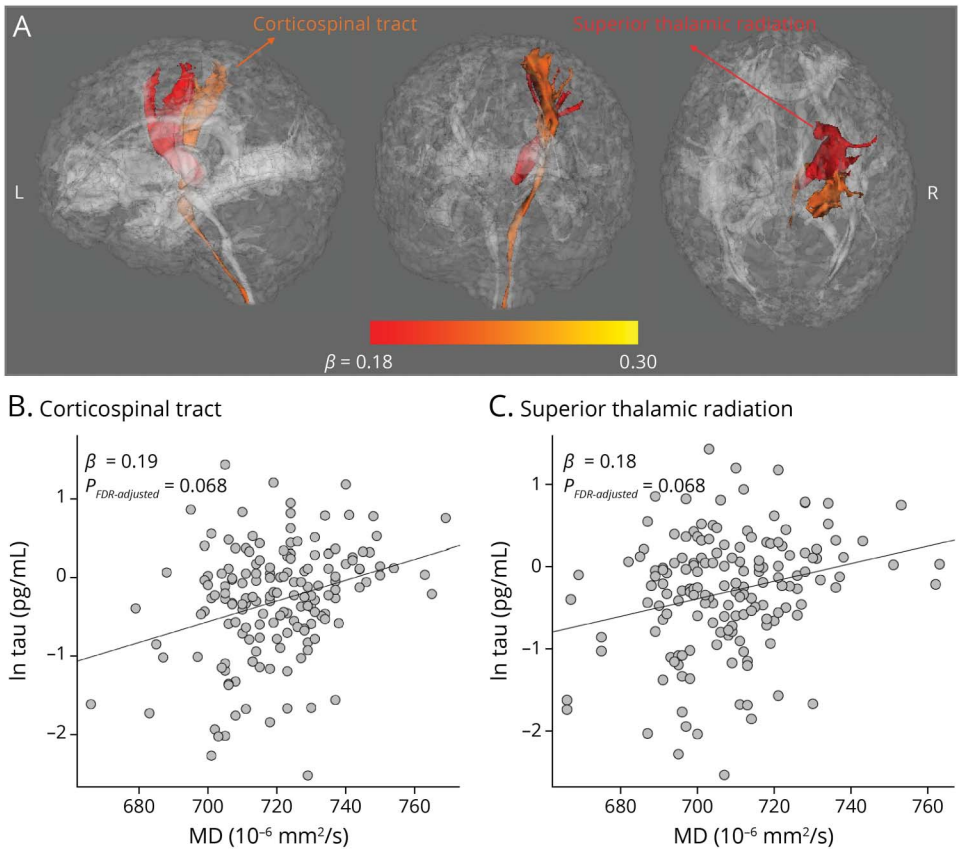
Associations Between Early Blood Biomarkers and Later DTI Metrics

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, links.lww.com/WNL/C811). 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 β 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. However, 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, links.lww.com/WNL/C812).

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 contrary, we have also demonstrated that acute changes in the blood biomarkers were associated with loss of consciousness or posttraumatic amnesia.[11] In this study, we combined these 2 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

Figure 4 White Matter Tracts With Significant Tau-MD Associations



(A) The right corticospinal tract had a β coefficient = 0.19 (orange), and the right superior thalamic radiation had a β coefficient = 0.18 (red). Both had false discovery rate–adjusted $p < 0.1$. (B) Post hoc scatter plot of tau and diffusion tensor imaging (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.

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 postinjury in NfL, GFAP, and UCH-L1. Tau seemed to have a slightly delayed response, bottoming out at 24–48 hours postinjury. During the 24–48 hours postinjury time point to the 7 days after 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 after 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 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 and 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 pathophysiologic 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 and 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 postinjury.[5] In another closed head injury rat model, decreased FA and increased MD and RD were observed longitudinally from 1 day postinjury to 30 days postinjury. These changes in DTI metrics were associated with myelin compactness detected by immunohistochemistry analysis.[4]

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	β 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: DTI = diffusion tensor imaging; FDR = false discovery rate; fmi = forceps minor; GFAP = glial fibrillary acidic protein; ifo = inferior fronto-occipital fasciculus; ilf = inferior longitudinal faciculus; mcp = middle cerebellar peduncle; ml = medial lemniscus; NfL = neurofilament light; ptr = medial lemniscus; _l = left hemisphere; _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 7 days after unrestricted return to play.

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 3 biomarkers, the plasma tau level decreased right after 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 and 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 postinjury 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 after return to play, our results showed stable NfL levels and 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 after 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 3–17 months postinjury.[14,15] Of interest, 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 this study. Although plasma total tau showed differences between preseason and post-concussion 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 CSF total tau in individuals with Alzheimer 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 contrary, 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-to-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 pathophysiologic specificities. This 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 and 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 2 objective measures are associated. Specifically, elevated plasma tau levels seemed 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.

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Disclosure

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Continued

Appendix *(continued)*

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References

1. Langlois JA, Rutland-Brown W, Wald MM. The epidemiology and impact of traumatic brain injury: a brief overview. *J Head Trauma Rehabil*. 2006;21(5):375-378. doi:10.1097/00001199-200609000-00001
2. Daneshvar DH, Nowinski CJ, McKee AC, Cantu RC. The epidemiology of sport-related concussion. *Clin Sports Med*. 2011;30(1):1-17, vii. doi:10.1016/j.csm.2010.08.006
3. Laskowski RA, Creed JA, Raghupathi R. Pathophysiology of mild TBI: implications for altered signaling pathways. In: Kobeissy FH, editor. *Brain Neurotrauma: Molecular, Neuropsychological, and Rehabilitation Aspects*. CRC Press/Taylor & Francis; 2015. p 35 - 42.
4. Tu TW, Williams RA, Lescher JD, Jikaria N, Turtzo LC, Frank JA. Radiological-pathological correlation of diffusion tensor and magnetization transfer imaging in a closed head traumatic brain injury model. *Ann Neurol*. 2016;79(6):907-920. doi:10.1002/ana.24641
5. Kao YJ, Lui YW, Lu CF, Chen HL, Hsieh BY, Chen CY. Behavioral and structural effects of single and repeat closed-head injury. *AJNR Am J Neuroradiol*. 2019;40(4):601-608. doi:10.3174/ajnr.a6014
6. Ogino Y, Vascak M, Povlishock JT. Intensity specific repetitive mild traumatic brain injury evokes an exacerbated burden of neocortical axonal injury. *J Neuropathol Exp Neurol*. 2018;77(9):782-792. doi:10.1093/jnen/nly054
7. Basser PJ, Pierpaoli C. Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI. *J Magn Reson B*. 1996;111(3):209-219. doi:10.1006/jmrb.1996.0086
8. Wu YC, Harezlak J, Elsaid NMH, et al. Longitudinal white-matter abnormalities in sports-related concussion: a diffusion MRI study. *Neurology*. 2020;95(7):e781-e792. doi:10.1212/wnl.0000000000009930
9. Mustafi SM, Harezlak J, Koch KM, et al. Acute white-matter abnormalities in sports-related concussion: a diffusion tensor imaging study from the NCAA-DoD care consortium. *J Neurotrauma*. 2018;35(22):2653-2664. doi:10.1089/neu.2017.5158
10. Zetterberg H, Smith DH, Blennow K. Biomarkers of mild traumatic brain injury in cerebrospinal fluid and blood. *Nat Rev Neurol*. 2013;9(4):201-210. doi:10.1038/nrneurol.2013.9
11. McCrea M, Broglio SP, McAllister TW, 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(1):e1919771. doi:10.1001/jamanetworkopen.2019.19771
12. Meier TB, Huber DL, Bohorquez-Montoya L, et al. A prospective study of acute blood-based biomarkers for sport-related concussion. *Ann Neurol*. 2020;87(6):907-920. doi:10.1002/ana.25725
13. Shahim P, Tegner Y, Wilson DH, et al. Blood biomarkers for brain injury in concussed professional ice hockey players. *JAMA Neurol*. 2014;71(6):684-692. doi:10.1001/jamaneurol.2014.367
14. Shahim P, Politis A, van der Merwe A, et al. Neurofilament light as a biomarker in traumatic brain injury. *Neurology*. 2020;95(6):e610-e622. doi:10.1212/wnl.0000000000009983
15. Shahim P, Politis A, van der Merwe A, et al. Time course and diagnostic utility of NfL, tau, GFAP, and UCH-l1 in subacute and chronic TBI. *Neurology*. 2020;95(6):e623-e636. doi:10.1212/wnl.0000000000009985
16. Wu YC, Mustafi SM, Harezlak J, Kodiweera C, Flashman LA, McAllister TW. Hybrid diffusion imaging in mild traumatic brain injury. *J Neurotrauma*. 2018;35(20):2377-2390. doi:10.1089/neu.2017.5566
17. Broglio SP, McCrea M, McAllister T, 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(7):1437-1451. doi:10.1007/s40279-017-0707-1
18. Carney N, Ghajar J, Jagoda A, et al. Concussion guidelines step 1: systematic review of prevalent indicators. *Neurosurgery*. 2014;75(suppl 1):S3-S15. doi:10.1227/neu.0000000000000433
19. Nencka AS, Meier TB, Wang Y, 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(4):1121-1140. doi:10.1007/s11682-017-9775-y
20. McCrory P, Meeuwisse W, Dvořák J, 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(11):838-847. doi:10.1136/bjsports-2017-097699
21. Avants BB, Tustison NJ, Song G, Cook PA, Klein A, Gee JC. A reproducible evaluation of ANTs similarity metric performance in brain image registration. *Neuroimage*. 2011;54(3):2033-2044. doi:10.1016/j.neuroimage.2010.09.025
22. Wen Q, Mustafi SM, Li J, et al. White matter alterations in early-stage Alzheimer's disease: a tract-specific study. *Alzheimers Dement (Amst)*. 2019;11(1):576-587. doi:10.1016/j.dadm.2019.06.003
23. Behrens TE, Berg HJ, Jbabdi S, Rushworth MF, Woolrich MW. Probabilistic diffusion tractography with multiple fibre orientations: what can we gain? *Neuroimage*. 2007;34(1):144-155. doi:10.1016/j.neuroimage.2006.09.018
24. de Groot M, Vernooij MW, Klein S, et al. Improving alignment in tract-based spatial statistics: evaluation and optimization of image registration. *Neuroimage*. 2013;76:400-411. doi:10.1016/j.neuroimage.2013.03.015
25. Ibrahim JG, Molenberghs G. Missing data methods in longitudinal studies: a review. *Test (Madr)*. 2009;18(1):1-43. doi:10.1007/s11749-009-0138-x
26. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Series B Stat Methodol*. 1995;57(1):289-300. doi:10.1111/j.2517-6161.1995.tb02031.x
27. Klawiter EC, Schmidt RE, Trinkaus K, et al. Radial diffusivity predicts demyelination in ex vivo multiple sclerosis spinal cords. *Neuroimage*. 2011;55(4):1454-1460. doi:10.1016/j.neuroimage.2011.01.007
28. Janve VA, Zu Z, Yao SY, 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. doi:10.1016/j.neuroimage.2013.02.034
29. Shahim P, Linemann T, Inekci D, et al. Serum tau fragments predict return to play in concussed professional ice hockey players. *J Neurotrauma*. 2016;33(22):1995-1999. doi:10.1089/neu.2014.3741
30. Pattinson CL, Meier TB, Guedes VA, 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(8):e2013191. doi:10.1001/jamanetworkopen.2020.13191
31. Gill J, Merchant-Borna K, Jeromin A, Livingston W, Bazarian J. Acute plasma tau relates to prolonged return to play after concussion. *Neurology*. 2017;88(6):595-602. doi:10.1212/wnl.0000000000003587
32. Meier TB, Bergamino M, Bellgowan PS, et al. Longitudinal assessment of white matter abnormalities following sports-related concussion. *Hum Brain Mapp*. 2016; 37(2):833-845. doi:10.1002/hbm.23072
33. Dugger BN, Whiteside CM, Maarouf CL, et al. The presence of select tau species in human peripheral tissues and their relation to Alzheimer's disease. *J Alzheimers Dis*. 2016;54(3):1249. doi:10.3233/jad-169007
34. Fischer I, Baas PW. Resurrecting the mysteries of big tau. *Trends Neurosci*. 2020; 43(7):493-504. doi:10.1016/j.tins.2020.04.007
35. Kawata K, Steinfeldt JA, Huibregtse ME, et al. Association between proteomic blood biomarkers and DTI/NODDI metrics in adolescent football players: a pilot study. *Front Neurol*. 2020;11:581781. doi:10.3389/fneur.2020.581781
36. Mac Donald CL, Dikranian K, Bayly P, Holtzman D, Brody D. Diffusion tensor imaging reliably detects experimental traumatic axonal injury and indicates approximate time of injury. *J Neurosci*. 2007;27(44):11869-11876. doi:10.1523/jneurosci.3647-07.2007
37. Budde MD, Janes L, Gold E, Turtzo LC, Frank JA. 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*. 2011;134(8): 2248-2260. doi:10.1093/brain/awr161
38. Mattsson N, Zetterberg H, Janelidze S, et al. Plasma tau in Alzheimer disease. *Neurology*. 2016;87(17):1827-1835. doi:10.1212/wnl.0000000000003246
39. Shahim P, Zetterberg H, Simren J, et al. Association of plasma biomarker levels with their CSF concentration and the number and severity of concussions in professional athletes. *Neurology*. 2022;99(4):e347-e354. doi:10.1212/wnl.0000000000200615
40. Barthelemy NR, Horie K, Sato C, Bateman RJ. Blood plasma phosphorylated-tau isoforms track CNS change in Alzheimer's disease. *J Exp Med*. 2020;217(11): e20200861. doi:10.1084/jem.20200861
41. Vos PE, Jacobs B, Andriessen TM, et al. GFAP and S100B are biomarkers of traumatic brain injury: an observational cohort study. *Neurology*. 2010;75(20):1786-1793. doi: 10.1212/wnl.0b013e3181fd62d2
42. Blennow K. A review of fluid biomarkers for Alzheimer's disease: moving from CSF to blood. *Neurol Ther*. 2017;6(suppl 1):15-24. doi:10.1007/s40120-017-0073-9
43. Graham NSN, Zimmerman KA, Moro F, et al. Axonal marker neurofilament light predicts long-term outcomes and progressive neurodegeneration after traumatic brain injury. *Sci Transl Med*. 2021;13(613):eabg9922. doi:10.1126/scitranslmed.abg9922
44. Zhang H, Schneider T, Wheeler-Kingshott CA, Alexander DC. NODDI: practical in vivo neurite orientation dispersion and density imaging of the human brain. *Neuroimage*. 2012;61(4):1000-1016. doi:10.1016/j.neuroimage.2012.03.072
45. Fieremans E, Jensen JH, Helpern JA. White matter characterization with diffusional kurtosis imaging. *Neuroimage*. 2011;58(1):177-188. doi:10.1016/j.neuroimage.2011.06.006
46. Jones DK. *Diffusion MRI Theory, Methods, and Applications*. Oxford University Press; 2011:1 online resource (xvi, 767 p).
47. Kim JH, Budde MD, Liang HF, et al. Detecting axon damage in spinal cord from a mouse model of multiple sclerosis. *Neurobiol Dis*. 2006;21(3):626-632. doi:10.1016/j.nbd.2005.09.009
48. Song SK, Yoshino J, Le TQ, et al. Demyelination increases radial diffusivity in corpus callosum of mouse brain. *Neuroimage*. 2005;26(1):132-140. doi:10.1016/j.neuroimage.2005.01.028

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