

Elevated cerebrospinal fluid total tau in former professional athletes with multiple concussions

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Abstract

Objective

To identify CSF biomarkers that are related to decreased white matter (WM) integrity and poor cognitive performance in former professional athletes with a history of multiple concussions.

Methods

Concentrations of phosphorylated tau181, total tau (t-tau), and β -amyloid in the CSF were measured in 3 groups: 22 former professional athletes with multiple concussions (mean \pm SD age 55.9 ± 12.2 years), 5 healthy controls (age 57.4 ± 5.2 years), and 12 participants (age 60.0 ± 6.6 years) diagnosed with Alzheimer disease (AD). All participants in the former athletes group underwent diffusion tensor imaging to determine WM tract integrity and completed neuropsychological testing. We divided the former athletes group into those with normal (<300 pg/mL) and high (>300 pg/mL) CSF t-tau.

Results

CSF t-tau in the former athletes group was significantly higher than in the healthy control group (349.3 ± 182.6 vs 188.8 ± 39.9 pg/mL, $p = 0.003$) and significantly lower than in the patients with AD (349.3 ± 182.6 vs 857.0 ± 449.3 pg/mL, $p = 0.007$). Fractional anisotropy values across all the tracts were significantly lower in the high CSF t-tau group compared to the normal CSF t-tau group ($p = 0.036$). Participants in the high CSF t-tau group scored significantly lower on the Trail Making Test (TMT) Part B compared to the normal CSF t-tau group (t scores 45.6 ± 18.8 vs 62.3 ± 10.1 , $p = 0.017$).

Conclusion

Our findings indicate that former athletes with multiple concussions are at increased risk of elevated levels of CSF t-tau and that high CSF t-tau is associated with reduced WM integrity and worse scores on the TMT Part B.

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Glossary

$A\beta_{1-42}$ = β -amyloid₁₋₄₂; **AD** = Alzheimer disease; **ANOVA** = analysis of variance; **AxD** = axial diffusivity; **CC** = corpus callosum; **Cg** = cingulum; **CJD** = Creutzfeldt-Jakob disease; **CTE** = chronic traumatic encephalopathy; **DTI** = diffusion tensor imaging; **DWI** = diffusion-weighted imaging; **FA** = fractional anisotropy; **FOV** = field of view; **HC** = healthy control; **MD** = mean diffusivity; **p-tau** = phosphorylated tau181; **RD** = radial diffusivity; **SLF** = superior longitudinal fasciculus; **t-tau** = total tau; **TBI** = traumatic brain injury; **TMT** = Trail Making Test; **TR** = repetition time; **UHN** = University Health Network; **UNC** = uncinata fasciculus; **WM** = white matter.

Chronic traumatic encephalopathy (CTE) is a slowly progressive neurodegenerative disease that is characterized by a distinct pattern of hyperphosphorylated tau deposition in the brain.¹ CTE has been observed most often in professional contact sports athletes such as football or hockey players who have been subjected to repetitive concussive or subconcussive head impacts.² Patients with CTE may show a constellation of cognitive, psychiatric, and motor symptoms.³ However, CTE is still a postmortem diagnosis because in vivo diagnosis is currently impossible due to the strong overlap of symptoms with other neurodegenerative diseases (e.g., Alzheimer disease [AD], frontotemporal dementia, and Parkinson disease).⁴ In addition, studies have reported that CTE pathology is found in only a fraction of contact sports players who have a history of repetitive concussions and neurologic symptomatology.^{5,6} The potential for treating postconcussion degeneration such as CTE depends on being able to detect the in vivo pathology at an early stage to intervene before the disease progresses to an irreversible stage.

The biochemical composition of the CSF is thought to reflect changes that occur in the brain and thus might be an optimal source for potential biomarkers.^{7,8} CSF biomarkers, particularly β -amyloid₁₋₄₂ ($A\beta_{1-42}$), total tau (t-tau), and phosphorylated tau181 (p-tau), are commonly used in the diagnosis of AD.⁹ Various studies have suggested that CSF t-tau levels may reflect neuronal and axonal degeneration, while CSF $A\beta_{1-42}$ and p-tau levels reflect amyloid plaque load and neurofibrillary tangles in the brain, respectively.^{8,10-12} In a recent study, greater repetitive head impacts were reported to be associated with higher levels of CSF t-tau in former National Football League players.¹³

Diffusion tensor imaging (DTI) is widely used to investigate axonal integrity in contact sports athlete populations.¹⁴ The most frequently reported white matter (WM) abnormalities observed in sport-related concussions are in the corpus callosum (CC), uncinata fasciculus (UNC), superior longitudinal fasciculus (SLF), and cingulum (Cg).^{14,15}

The first aim of this study was to compare the CSF biomarkers $A\beta_{1-42}$, t-tau, and p-tau between former professional contact sports athletes with a history of repetitive concussions (former athletes group) and 2 groups: healthy controls (HC group) and participants diagnosed with AD (AD group). We hypothesized that former athletes would differ from the HC

and AD groups in CSF $A\beta_{1-42}$, t-tau, and p-tau. The second aim was to compare WM integrity and cognitive performance between former athletes with multiple concussions with high CSF t-tau and those with normal t-tau. We hypothesized that former athletes with multiple concussions with high CSF t-tau would have reduced WM integrity and perform worse on cognitive assessments compared to athletes with normal levels of CSF t-tau.

Methods

Participants

A total of 39 participants participated in this study. For the former athletes group, 24 cognitively normal former professional contact sports players with a history of repetitive concussions were recruited, but 2 participants were excluded because of CSF biomarkers consistent with AD,^{16,17} so a total of 22 participants (all men, mean \pm SD age 55.9 ± 12.2 years) were used for analyses. These consisted of 1 snowboarder and 12 former Canadian Football League players (recruited through the Canadian Concussion Centre, University Health Network [UHN], Toronto, Ontario, Canada; Center 1) and 9 former professional hockey players (recruited through the Rotman Research Institute at Baycrest, Toronto, Ontario, Canada; Center 2). Concussion history (e.g., number of concussions, date of last concussion, date of first concussion, and number of years played) was based on participants' self-report of their concussions. Key exclusion criteria were the inability to recall the number of concussions, history of neurologic or psychiatric disorder or other illness affecting the brain, developmental disorder, or any lesions on routine MRI. The HC group consisted of 5 participants (2 men, 3 women, mean \pm SD age 57.4 ± 5.2 years). HCs were recruited from healthy volunteers in the community through advertising. Exclusion criteria for HC included a history of concussions, history of neurologic or psychiatric disorder, or other illness affecting the brain; developmental disorder; or any lesions on routine MRI or CT scans. The AD group included 12 participants with dementia (6 men, 6 women, mean \pm SD age 60.0 ± 6.6 years). Ten of the participants were diagnosed as having young-onset AD and 2 as having late-onset AD.

All participants underwent a lumbar puncture. For the purpose of this study, the neuroimaging and neuropsychological assessments were investigated only in the former athletes

group to compare participants with high CSF t-tau with those with normal t-tau values.

Standard protocol approvals and patient consents

The research ethics boards of the UHN and Rotman Research Institute approved the study. Consent was obtained from all participants before participating in the study.

CSF analysis

The lumbar punctures were performed by a neurologist at UHN (M.C.T.) according to Alzheimer's Disease Neuroimaging Initiative protocol.¹⁸ After CSF was collected in polypropylene tubes, it was transported to the adjacent laboratory within 30 minutes, divided into aliquots, and stored at -80°C until we used a sandwich ELISA method to measure concentrations of $\text{A}\beta_{1-42}$ [Innotest β -amyloid₍₁₋₄₂₎, Fujirebio, Malvern, PA], p-tau [Innotest phospho-tau (181p), Fujirebio], and t-tau (Innotest hTAU-Ag, Fujirebio) following the manufacturer's instructions.^{19,20} All analyses were done in 1 laboratory (Tanz Centre for Research in Neurodegenerative Diseases, Toronto, Ontario, Canada) by 1 operator (F.T.). All samples were measured in duplicates and repeated if the difference between individual optic density values was $>20\%$. All samples from the former athletes group were run on the same ELISA plate, while samples from the HC and AD groups were run on separate plates on 2 different dates. The same internal and external validation controls were used to normalize and ensure the quality and consistency across the plates. In addition to the ready-to-use calibrators and run validation controls, which were part of the assay kits, internal controls were included in each run. After calculating the mean absorbance for the calibrators, run validation controls, and unknown CSF samples, we used a sigmoidal 4-parameter curve fitting to determine the corresponding concentrations.

CSF biomarkers were considered consistent with AD diagnosis if p-tau was >68 pg/mL and the index of $\text{A}\beta_{1-42}$ to t-tau was >0.8 .^{16,17}

A cutoff for normal CSF t-tau

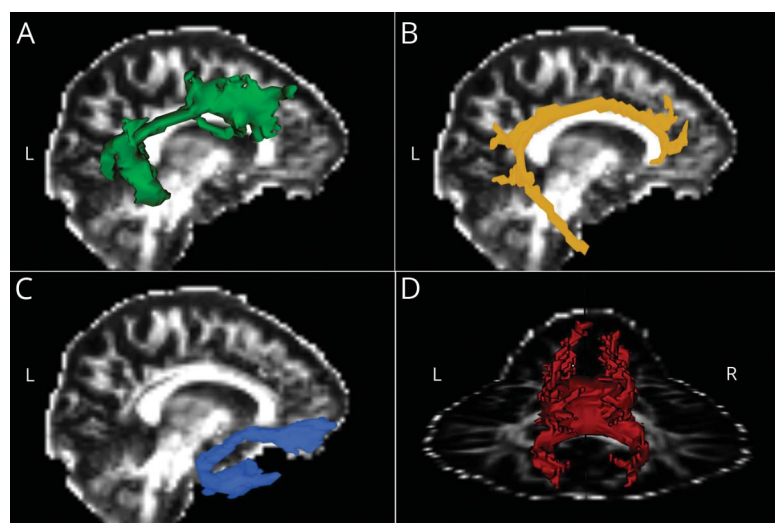
We determined the optimal cutoff for normal CSF t-tau as 300 pg/mL on the basis of a review of the literature that used the same assay method as ours.^{21,22} We divided the former athletes group into those with normal (<300 pg/mL) and those with high (>300 pg/mL) CSF t-tau. This cutoff value was consistent with the bimodally distributed levels of CSF t-tau in the former athletes group.

Neuroimaging acquisition, DTI processing, and WM tractography

All participants in the former athletes group underwent MRI at 2 centers: 13 were recruited and scanned at Center 1 and 9 were recruited and scanned at Center 2.

The imaging protocol at Center 1 included a 3T MRI system (Signa HDx; GE Healthcare, Milwaukee, WI) with a standard 8-channel head coil used to obtain structural and diffusion-weighted imaging (DWI). A high-resolution T1-weighted whole-brain scan was acquired using an inversion recovery fast spoiled gradient echo sequence with the following parameters: 180 axial slices with 1-mm thickness; 3-millisecond echo time; 7.8-millisecond repetition time (TR); 450-millisecond inversion time; 15° flip angle; 25.6-cm field of view (FOV); 256×256 matrix size; and $1 \times 1 \times 1$ -mm³ voxel size. At least 1 DWI scan was obtained with diffusion gradients applied across 60 spatial directions ($b = 1,000$ s/mm²) and 10 non-diffusion-weighted (B_0) scans. The DWI had the following parameters: 2.4-mm-thick axial slices, TR of 17,000 milliseconds, FOV of 23 cm, and 2.4×2.4 -mm² in-plane resolution.

Figure 1 Tractography of white matter tracts for a single patient



(A) Superior longitudinal fasciculus, (B) cingulum bundle, (C) uncinate fasciculus, and (D) corpus callosum.

The imaging protocol at Center 2 called for a 3T MRI system (Siemens Magnetom Trio Tim, Numaris/4Syngo MR B13; Siemens, Germany) with a standard 12-channel head coil used to obtain structural imaging and DWI. A high-resolution T1-weighted whole-brain scan was acquired with a magnetization-prepared rapid gradient echo sequence with the following parameters: 160 axial slices with 1-mm thickness; echo time of 2.63 milliseconds; TR of 2,000 milliseconds; FOV of 25.6-cm; and $1 \times 1 \times 1\text{-mm}^3$ voxel size. Two DWI scans were obtained with diffusion gradients applied across total of 60 spatial directions ($b = 1,000 \text{ s/mm}^2$) and 8 non-diffusion-weighted (B_0) scans. The DWI had the following parameters: 2.2-mm-thick axial slices, TR of 7,900 milliseconds, FOV of 24.2 cm, and $2.2 \times 2.2\text{-mm}^2$ in-plane resolution.

The DTI analysis was performed with the FMRIB Software Library tools (fmrib.ox.ac.uk/fsl/fdt/index.html). The DTI processing, region of interest definition, and fiber tracking were performed as previously reported.²³ Briefly, axial diffusivity (AxD), radial diffusivity (RD), mean diffusivity (MD), and fractional anisotropy (FA) were measured for the following WM tracts: right and left SLF, right and left UNC, right and left Cg, and CC (figure 1). Before the DTI analyses, the participant's fluid-attenuated inversion recovery images were reviewed by a neurologist (M.C.T.) to confirm the presence of no to minimal amount of WM hyperintensity that could affect the tractography results.

Neuropsychological assessment

As part of ongoing studies, the athletes completed comprehensive neuropsychological assessments. The Trail Making Test (TMT) Parts A and B^{24,25} and the Symbol Digit Modalities Test written format^{25,26} were analyzed in the present study because these are widely used standardized tests sensitive to distributed dysfunction caused by traumatic brain injury (TBI),²⁷ and the tests were common to the batteries administered at both centers. To account for variability of age and education in our sample, these scores were standardized

with the use of established norms.^{26,28} Higher scores on these assessments represent better performance.

Statistical analysis

Statistical analyses were conducted with SPSS software (version 24; SPSS Inc, Chicago, IL). A 1-way analysis of variance (ANOVA) was used to compare age between the groups (i.e., former athletes, HC, and AD). We performed multiple ANOVAs with age as a covariate to compare CSF biomarkers between the former athletes group and the 2 other groups. Dunnett-T3 post hoc²⁹ analysis was performed to compare the former athletes with each of the 2 other groups. An omnibus mixed-design ANOVA was used for each DTI measure (i.e., FA, MD, AxD, and RD) across all the tracts (i.e., right/left SLF, right/left UNC, right/left Cg, and CC), which were entered as a 7-level factor to test significance of the differences observed in the WM integrity between the former athlete groups with normal and high CSF t-tau. In all of the analyses, the variable of age was entered as a covariate. The Pearson correlation coefficient was used for correlations. Before we ran the above-mentioned statistical analyses, we used a Kolmogorov-Smirnov test, and we confirmed the normal distribution of each variable. Statistical significance level was conservatively set at $p < 0.05$.

Data availability

All data can be shared at the request of other investigators.

Results

Results for CSF biomarkers are summarized in table 1 and plotted in figure 2. Age was not significantly different between the groups ($F = 0.645$, $p = 0.530$, ANOVA) (table 1). There were no significant correlations between age and CSF t-tau in the AD ($r = -0.27$, $p = 0.40$) and HC ($r = -0.1$, $p = 0.87$) groups, whereas a significant correlation was found in the former athletes group ($r = 0.580$, $p = 0.005$). After ANOVA was performed with CSF t-tau as the dependent

Table 1 CSF biomarkers in 3 groups

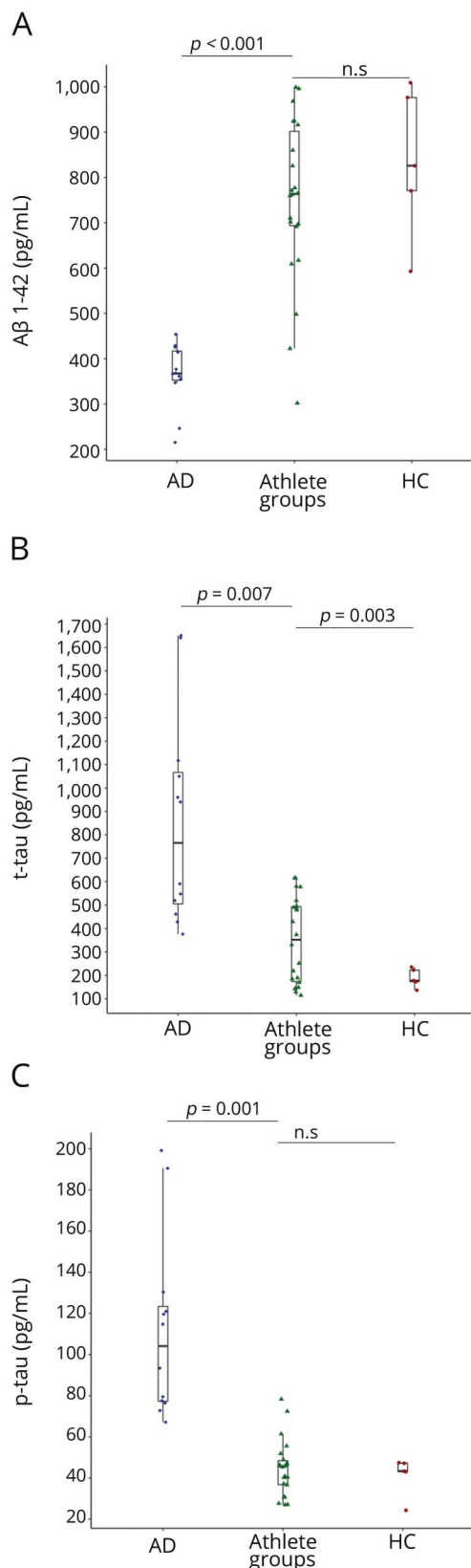
	Former athletes group	HC group	AD group	ANOVA p Value	Post hoc p values ^a	
					Former athletes vs HC	Former athletes vs AD
No.	22	5	12			
Age, y	55.9 ± 12.2	57.4 ± 5.2	60.0 ± 6.6	0.530	0.665	0.496
Education, y	14.95 ± 2.2	14.8 ± 1.6	14.42 ± 2.7	0.810	0.997	0.909
A β ₁₋₄₂ , pg/mL	750.0 ± 182.1	835.0 ± 168.2	363.3 ± 70.9	<0.001 ^b	0.694	<0.001 ^b
p-tau, pg/mL	44.8 ± 13.6	41.1 ± 9.6	111.9 ± 44.4	<0.001 ^b	0.858	0.001 ^b
t-tau, pg/mL	349.3 ± 182.6	188.8 ± 39.9	857.0 ± 449.3	<0.001 ^b	0.003 ^b	0.007 ^b

Abbreviations: A β ₁₋₄₂ = β -amyloid₁₋₄₂; AD = Alzheimer disease; ANOVA = analysis of variance; HC = healthy controls; p-tau = phosphorylated tau181; t-tau = total tau.

^a Dunnett T3 post hoc analysis.

^b Significant at the level of <0.05.

Figure 2 Comparison of the CSF biomarkers between former athletes and HC and AD groups



(A) CSF β -amyloid₁₋₄₂ (A β ₁₋₄₂), (B) CSF total tau (t-tau), and (C) CSF phosphorylated tau181 (p-tau). AD = Alzheimer disease; HC = healthy controls; n.s. = not significant.

variable and age and group (i.e., former athletes, HC, and AD) as the independent variables, this analysis demonstrated a significant effect by group ($F = 16.2$, $p = 0.001$) but not by age ($F = 1.4$, $p = 0.288$). The interaction effect of group by age showed only a trend ($F = 2.93$, $p = 0.051$). Furthermore, post hoc analyses showed that CSF t-tau in the former athletes group was significantly higher than in the HC group (349.3 ± 182.6 vs 188.8 ± 39.94 pg/mL, $p = 0.003$) and significantly lower than in the AD group (349.3 ± 182.6 vs 857.0 ± 449.3 pg/mL, $p = 0.007$). Levels of CSF A β ₁₋₄₂ and p-tau were significantly different between the 3 groups ($F = 28.62$, $p < 0.0001$; and $F = 26.54$, $p < 0.0001$, respectively). However, post hoc analyses demonstrated no statistically significant differences in the levels of CSF A β ₁₋₄₂ and p-tau between the former athletes group and HC group (750.0 ± 182.1 vs 835.0 ± 168.2 pg/mL, $p = 0.694$; and 44.8 ± 13.6 vs 41.1 ± 9.6 pg/mL, $p = 0.858$, respectively), whereas the AD group had significantly higher levels of CSF p-tau compared to both the former athletes and HC groups (111.9 ± 44.4 vs 44.8 ± 13.6 pg/mL, $p = 0.001$; and 111.9 ± 44.4 vs 41.1 ± 9.6 , $p < 0.001$, respectively). Patients with AD also showed significantly lower levels of CSF A β ₁₋₄₂ compared to both the former athletes and HC groups (363.3 ± 70.9 vs 750.0 ± 182.1 pg/mL, $p < 0.001$; and 363.3 ± 70.9 vs 835.0 ± 168.2 pg/mL, $p = 0.006$, respectively) (figure 2).

CSF analysis in former athletes with multiple concussions

On the basis of the cutoff for CSF t-tau established for this study (i.e., 300 pg/mL), 12 of the 22 participants (54.5%) showed a high t-tau concentration in their CSF. Table 2 summarizes the demographics, levels of CSF t-tau, p-tau, and A β ₁₋₄₂, and performance on the neuropsychological assessments between former athletes with multiple concussions and high CSF t-tau and those with normal CSF t-tau. Participants in the high CSF t-tau group were significantly older than the normal CSF t-tau group (age 62.6 ± 7.6 vs 47.8 ± 12.0 years, $p = 0.004$). The differences in CSF p-tau and A β ₁₋₄₂ between the former athletes with multiple concussions with high CSF t-tau and normal CSF t-tau were not statistically significant ($p = 0.33$ and $p = 0.52$, respectively).

Neuropsychological comparison in former athletes with multiple concussions

No statistically significant differences were seen in the TMT Part A (t scores 59.7 ± 10.9 vs 53.7 ± 9.02 , $p = 0.18$) and the Symbol Digit Modalities Test (z scores 0.669 ± 0.64 vs 0.258 ± 1.6 , $p = 0.43$) between the normal and high CSF t-tau groups. However, participants in the high CSF t-tau group scored significantly lower on the TMT Part B compared to the participants in the normal CSF t-tau group (t scores 45.6 ± 18.8 vs 62.3 ± 10.1 , $p = 0.017$).

DTI metrics in former athletes with multiple concussions

Omnibus mixed-design ANOVA (controlled for age) showed that the FA value across all the tracts was significantly lower in

Table 2 Comparison of normal and high CSF t-tau groups in former athletes with multiple concussions

	Normal CSF t-tau group	High CSF t-tau group	p Value
No.	10	12	
Recruited at Canadian Concussion Centre/Rotman Research Institute, n	6/4	7/5	1.00
Age, y	47.8 ± 12.0	62.6 ± 7.6	0.004 ^a
Education, y	15.2 ± 1.9	14.6 ± 2.4	0.52
Reported concussions, n	6.3 ± 4.1	4.0 ± 2.0	0.13
Years played	11.8 ± 5.0	10.8 ± 6.3	0.70
Age at the time of first concussion, y	18.2 ± 4.1	18.2 ± 2.9	0.99
Age at which participation in contact sport started, y	8.8 ± 2.9 ^b	11.4 ± 2.6 ^c	0.06
Interval from last reported concussion, y	19.6 ± 11.6 (minimum 2, maximum 39)	28.4 ± 16.7 (minimum 2, maximum 41)	0.18
CSF biomarkers			
t-Tau, pg/mL	169.3 ± 42.5	499.3 ± 90.4	<0.001 ^a
p-Tau, pg/mL	40.8 ± 11.0	48.1 ± 15.0	0.33
Aβ ₁₋₄₂ , pg/mL	761.5 ± 172.6	740.5 ± 196.8	0.52
Neuropsychological assessments			
TMT Part A t score	59.7 ± 10.9	53.7 ± 9.0	0.18
TMT Part B t score	62.3 ± 10.1	45.6 ± 18.8	0.017 ^a
SDMT written format z score	0.669 ± 0.64	0.258 ± 1.6	0.43

Abbreviation: Aβ₁₋₄₂ = β-amyloid₁₋₄₂; p-tau = phosphorylated tau₁₈₁; SDMT = Symbol Digit Modalities Test; t-tau = total tau; TMT = Trail Making Test.

^a Significant at the level of <0.05.

^b This information was missing for 1 participant in this group because the participant was lost to follow-up.

^c This information was missing for 1 participant in this group because the participant died.

the high CSF t-tau group compared to the normal CSF t-tau group (between-participant effect, $F = 5.1$, $p = 0.036$). Furthermore, both MD and RD values were significantly higher in the high CSF t-tau group (between-participant effect, $F = 5.0$, $p = 0.038$; and $F = 4.71$, $p = 0.043$, respectively), whereas no statistically significant difference was seen in AxD of the tracts (between-participant effect, $F = 3.2$, $p = 0.1$). FA, MD, AxD, and RD values for each tract separately can be found in table 3.

No significant interaction effect (group × factor [all the tracts]) was observed in FA ($p = 0.36$), MD ($p = 0.81$), AxD ($p = 0.49$), or RD ($p = 0.73$) values.

Discussion

The results of this study showed a higher t-tau concentration in the CSF of former athletes with a history of multiple concussions compared to HCs. The elevated t-tau level was, however, significantly lower than in participants with AD. Among the former athletes with a history of multiple concussions, those with high concentrations of CSF t-tau performed worse on the TMT Part B and showed reduced integrity in some WM tracts.

A high concentration of CSF t-tau has been reported in a number of neurodegenerative diseases in addition to AD. Previous studies have established high levels of CSF t-tau and low levels of CSF Aβ₁₋₄₂ as a characteristic profile of AD.^{16,17} Similarly, patients with Creutzfeldt-Jakob disease (CJD) present with high levels of CSF t-tau. However, in patients with CJD, the increase in the concentration of CSF t-tau is usually multifold and beyond the range seen in AD.³⁰ In addition to AD and CJD, there has been a report of high CSF t-tau in some patients with frontotemporal dementia syndromes or Lewy body disease,³¹ although not consistently.³¹

Although CSF t-tau, p-tau, and Aβ₁₋₄₂ have been widely studied in AD,^{16,17} a smaller body of literature has focused on CSF proteins in TBI.¹¹ CSF t-tau has been reported as higher in patients with severe TBI compared to control groups.³² In addition, studies investigating mild TBI in boxers reported an immediate increase in CSF t-tau after a bout, which normalized within 8 to 12 weeks if they did not participate in any further matches.³³ These reports have studied changes in the acute phase,^{34,35} so there is a paucity of data on remote TBI in general, with even less information on dealing with concussion or mild TBI.³³ A recent study reported that higher levels of CSF t-tau in former National Football League players were

Table 3 Comparing DTI measures in former athletes with multiple concussions between the normal and high CSF t-tau groups

	Right hemisphere				Left hemisphere			
	FA	MD, mm ² ·s ⁻¹ ·10 ⁻³	AxD, mm ² ·s ⁻¹ ·10 ⁻³	RD, mm ² ·s ⁻¹ ·10 ⁻³	FA	MD, mm ² ·s ⁻¹ ·10 ⁻³	AxD, mm ² ·s ⁻¹ ·10 ⁻³	RD, mm ² ·s ⁻¹ ·10 ⁻³
SLF								
Normal CSF t-tau group	0.406 ± 0.02	0.792 ± 0.03	1.14 ± 0.03	0.616 ± 0.04	0.412 ± 0.02	0.780 ± 0.03	1.13 ± 0.03	0.604 ± 0.03
High CSF t-tau group	0.391 ± 0.02	0.837 ± 0.04	1.21 ± 0.08	0.650 ± 0.03	0.401 ± 0.03	0.822 ± 0.04	1.18 ± 0.09	0.644 ± 0.04
<i>p</i> Value	0.344	0.016 ^a	0.02 ^a	0.115	0.202	0.049 ^a	0.153	0.101
UNC								
Normal CSF t-tau group	0.390 ± 0.03	0.853 ± 0.05	1.23 ± 0.05	0.663 ± 0.04	0.378 ± 0.04	0.835 ± 0.05	1.19 ± 0.04	0.657 ± 0.06
High CSF t-tau group	0.350 ± 0.03	0.896 ± 0.08	1.26 ± 0.09	0.712 ± 0.05	0.339 ± 0.04	0.885 ± 0.08	1.21 ± 0.06	0.724 ± 0.06
<i>p</i> Value	0.029 ^a	0.140	0.344	0.148	0.046 ^a	0.221	0.893	0.128
Cg								
Normal CSF t-tau group	0.374 ± 0.04	0.822 ± 0.04	1.17 ± 0.05	0.655 ± 0.01	0.392 ± 0.01	0.837 ± 0.05	1.21 ± 0.06	0.652 ± 0.05
High CSF t-tau group	0.339 ± 0.04	0.866 ± 0.03	1.20 ± 0.04	0.699 ± 0.01	0.371 ± 0.01	0.852 ± 0.05	1.20 ± 0.07	0.676 ± 0.04
<i>p</i> Value	0.223	0.036 ^a	0.074	0.052	0.063	0.621	0.868	0.367
CC								
Normal CSF t-tau group	0.501 ± 0.03	0.900 ± 0.06					1.44 ± 0.08	0.629 ± 0.06
High CSF t-tau group	0.489 ± 0.03	0.956 ± 0.07					1.52 ± 0.11	0.673 ± 0.06
<i>p</i> Value	0.256	0.546					0.672	0.494

Abbreviations: AxD = axial diffusivity; Cg = cingulum fasciculus; CC = corpus callosum; DTI = diffusion tensor imaging; FA = fractional anisotropy; MD = mean diffusivity; RD = radial diffusivity; SLF = superior longitudinal fasciculus; t-tau = total tau; UNC = uncinata fasciculus.

^a Significant at the level of $p \leq 0.05$ (controlled for age).

associated with more repetitive head impacts.¹³ Although our study showed that the levels of CSF t-tau were higher in the former athletes with repetitive head impacts than in the HC group with no history of concussion, we did not find any difference in the number of reported concussions and number of years played between the normal and high CSF t-tau groups. This may be due to the different population included in the 2 studies (i.e., National Football League vs Canadian Football League/hockey players).

High CSF t-tau is thought to reflect ongoing axonal injury or neurodegeneration.³⁶ This is in keeping with the observation that in AD the levels of CSF t-tau increase over time.³⁷ Some studies have suggested that the CSF p-tau may be a better CSF biomarker for the tangle pathology that is seen in the brain of patients with AD and CTE.^{8,11} This has been shown to be true in AD because CSF p-tau correlates with neurofibrillary changes in the brain of patients with this disease.³⁸ However, in CTE, the pathologic changes are distinct from AD, and although neurofibrillary tangles are seen, they are at the depths of sulci, around small vessels, and in the superficial cortical layers.^{3,4} In the current study, we did not observe elevated concentrations of CSF p-tau in the former athletes group. Although the main reason for this discrepancy is unknown, in addition to the distinct pattern of tau distribution within the brain, it might be due to the aggregation of different isoforms (e.g., different phosphorylation sites) of p-tau in various tauopathies that cannot be equally measured by all assays.⁷

In comparing former athletes with high and normal CSF t-tau, our study revealed that former athletes with high levels of CSF t-tau were significantly older than those with normal levels. The relationship between tau and age is still unclear. Although there was a significant correlation between age and CSF t-tau in the former athletes group, this was not observed in the AD or HC group. Although a correlation between age and CSF t-tau was reported in 1 study,³⁹ 2 large CSF studies, one with 150 patients with AD and 100 HCs¹⁹ and another with 407 patients with AD and 65 elderly HCs,⁴⁰ found no correlation between age and CSF t-tau. In addition, the increase in CSF t-tau seen in the former athletes in our study cannot be explained solely by the effects of aging because the mean age of former athletes in the high CSF t-tau group (i.e., 62.6 ± 7.6 years) is similar to that of participants in the HC group (i.e., 57.4 ± 5.2 years), who have lower levels of CSF t-tau (tables 1 and 2). In addition, as mentioned, the normal cutoff (i.e., 300 pg/mL) that was used to divide the athletes into normal and high CSF t-tau groups was chosen on the basis of studies that had included HCs similar in age to the former athletes in our study.^{21,22} These findings support the hypothesis proposed by some studies that multiple concussions or subconcussive head impacts can accelerate age-associated brain changes⁴¹ that consequently can be associated with elevated tau.³⁹ Similarly, it has recently been shown that multiple concussions may accelerate the aging process and lead to greater brain regional atrophy than what might be expected for age.⁴²

Although there was only a trend toward significance, our findings (table 2) showed that the normal CSF t-tau group started playing contact sports at younger ages compared to the high CSF t-tau group. These results may seem inconsistent with the study that suggested that participation in tackle football in younger ages is associated with greater cognitive impairment later in life.⁴³ However, that study compared cognitive functions between athletes who started playing tackle football before 12 years of age and those who started later. In our study, the mean age at which participation in contact sports started was <12 years in both the normal and high CSF t-tau groups. Other factors such as genetic vulnerability may play a more important role in the long-term outcome of multiple concussions.⁴⁴

The relationship between CSF levels of t-tau and WM integrity has previously been studied in both participants with AD and those without AD.⁴⁵ In a study of healthy adults with risk factors for AD, the authors reported a relationship between CSF t-tau and WM integrity, particularly in the temporal lobe.⁴⁶ Another study of participants with mild cognitive impairment reported that those with high CSF t-tau showed a greater FA reduction in the Cg and SLF relative to controls over time; however, this was not seen in participants with mild cognitive impairment with normal levels of CSF t-tau.⁴⁷ These findings suggest that ongoing neurodegeneration that can be associated with increased levels of CSF t-tau may be associated with reduced integrity of the WM. Our results are consistent with these findings because the participants with high levels of CSF t-tau showed a reduced integrity in the WM tracts.

In the current study, we also found that former athletes with high levels of CSF t-tau performed worse on the TMT Part B. The TMT is a sensitive test for cognitive impairment⁴⁸; in particular, TMT Part B is a popular test for assessing executive function.⁴⁹ Previously, it has been reported that cognitive function can be altered in former athletes with multiple concussions even decades after their last concussion.⁵⁰ Therefore, our findings suggest that increased levels of CSF t-tau may be indicative of neurodegeneration.

While the results of this study are promising for providing a better understanding of the pathophysiology of chronic brain trauma and suggest that CSF t-tau may be a biomarker of possible neurodegeneration, potential limitations should be noted. The small sample size, particularly in the HC group (i.e., 5 participants), and the lack of female participants in the former athletes group make the generalizability of our results unknown. Therefore, more studies with a larger sample size including both men and women are required to confirm and extend the results of this study. In addition, although our results showed differences in WM integrity between the high and normal CSF t-tau groups, comparison to HCs is required to investigate whether WM integrity in the high CSF t-tau is abnormal. Another potential limitation was that 2 different MRI scanners were used because the former athletes were

scanned at 2 centers. However, it should be noted that both the normal and high CSF t-tau groups included a similar number of former athletes scanned at each center (table 2). This enabled us to compare the 2 groups (i.e., normal and high CSF t-tau groups) and to be confident that differences were not due to differences in scanners. Finally, we could not find any differences in the levels of p-tau between the former athletes group and the HC group. However, we specifically measured only one of the p-tau isoforms (p-tau181) that is commonly being used in AD.⁸ It may be worthwhile to also measure and investigate other isoforms of this protein in the CSF.

Overall, our findings indicate that former athletes with multiple concussions are at increased risk of elevated levels of CSF t-tau and that high CSF t-tau is associated with reduced WM integrity and worse scores on the TMT Part B even after controlling for the effects of age. Furthermore, we found an association between age and CSF t-tau only in the former athletes group, not in the HC or AD group. Taken together, these findings suggest that multiple concussive or subconcussive events may trigger neurodegeneration to a greater degree than expected on the basis of age alone. However, on the basis of solely these findings and before investigation of the brain pathology of the participants with elevated CSF t-tau, no conclusion can be made as to whether these participants have CTE. We are engaged in longitudinal studies to track neurologic and neuropsychological function, CSF biomarkers, and structural brain changes over time to further assess the delayed effects of multiple concussions on the brain.

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Appendix (continued)

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