Investigating the use of plasma pTau181 in retired contact sports athletes.

Running title: Plasma pTau181 in retired athletes.

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Abstract

Background: Considering the wide range of outcomes following sport-related concussions, biomarkers are needed to detect underlying pathological changes. The objective was to analyze the use of plasma phosphorylated tau 181 (pTau181) as a non-invasive measure of underlying brain changes in a cohort of retired contact sports athletes at risk of neurodegeneration.

Methods: This study included 54 retired contact sport athletes and 27 healthy controls whose blood plasma was analyzed for pTau181. A portion (N=21) of retired athletes came for a 2-year follow-up visit. All participants had completed a neuropsychological battery and MRI imaging.

Results: Plasma pTau181 was significantly higher in retired athletes compared to healthy controls (8.94±5.08 pg/mL vs. 6.00±2.53 pg/mL; 95% CI 0.87-5.01; p=.02). When the retired athletes cohort was divided into high vs. normal pTau181 groups, the corpus callosum (CC) and entorhinal volumes were significantly lower in high pTau181 compared to older healthy controls (1.57±0.19 vs. 2.02±0.32, p=.002; and 2.07±0.35 vs. 2.82±0.51, p=.003, respectively). Lower white matter integrity was observed in the high pTau181 group in comparison to healthy controls (CC medial diffusivity: 0.96±0.04 $x10^{-3}$ mm^2/s vs. 0.90±0.03 $x10^{-3}$ mm^2/s, p=.003; CC axial diffusivity: 1.49±0.04 $x10^{-3}$ mm^2/s vs. 1.41±0.02 $x10^{-3}$ mm^2/s, p<.001, respectively).

Conclusions: Although high plasma pTau181 levels was associated with lower regional brain volumes and decreased white matter integrity, baseline pTau181 did not predict longitudinal changes in regional brain volumes or white matter integrity in retired contact sport athletes. pTau181 may be useful for identifying those with brain abnormalities related to repeated concussion but not for predicting progression.

Keywords: plasma, concussion, athletes, neurodegeneration, CTE, chronic traumatic encephalopathy

Introduction

Long-term effects of repeated concussions can involve delayed neurodegeneration including Alzheimer's disease (AD) and chronic traumatic encephalopathy (CTE)[1–3]. However, not all those who experience repeated concussions go on to develop long-term deleterious effects and some athletes do not report any neurological symptoms following concussions[3,4]. Considering the wide range of outcomes following repeated concussions, biomarkers are needed to detect underlying pathological changes.

Most blood biomarker research in athletes has been done in acute concussion, where neurofilament light chain (NfL) has been demonstrated as a sensitive biomarker of neuro-axonal injury.[5,6] NfL was able to discriminate professional hockey players with persistent post-concussion symptoms from those who were able to return to play shortly after sustaining injury[7]. Moreover, serum NfL levels were increased in boxers after a bout of boxing, and remained significantly increased following 3 months of rest[6]. In previously concussed retired professional athletes, we found that higher NfL levels years after concussions were associated with lower white matter (WM) integrity of the corpus callosum (CC) and fornix and higher NfL levels predicted a decrease in WM integrity of the CC over a 2-year span[8].

Plasma tau phosphorylated at threonine 181 (pTau181) has been identified as a blood biomarker for AD since its levels are significantly higher in AD patients compared to healthy controls, and it has a strong relationship with both amyloid and tau positron emission tomography (PET) positivity[9,10]. Plasma pTau181 detects amyloid PET-positive individuals regardless of clinical stage, and correlates with cortical deposition of the tau protein as measured by tau-PET[11–13]. Finally, plasma pTau181 differentiated AD from non-AD pathology in an autopsy confirmed cohort[14–16]. Elevated plasma pTau181 appears specific to AD and associated with brain amyloid and tau accumulation in AD-typical regions, while plasma NfL was increased in a number of neurodegenerative diseases[13,17–19].

Pathologically, the abnormal CTE tau shares some similarities with the AD tauopathy including being a combination of three and four microtubule binding domain repeats (3R and 4R) and being a paired helical filament [20]. Given the similarities between AD and CTE tau, the aim of this study was to investigate the use of plasma pTau181 as a non-invasive measure of underlying brain changes in a cohort of retired contact sports athletes at risk of neurodegeneration. The aims of this study would be accomplished by (1) comparing pTau181 between patients and controls; (2) comparing the high and normal pTau181 retired athlete groups for markers of brain degeneration and

neuropsychological performance; and (3) evaluating the predictive capabilities of pTau181 in a longitudinal cohort. We hypothesized that pTau181 could be a biomarker of *in vivo* CTE in retired contact sports athletes with elevated pTau181 without AD positive biomarkers. We also hypothesized that elevated pTau181 would be associated with MRI markers of neurodegeneration, such as atrophy and reduced WM integrity, and be associated with worse neuropsychological performance.

Methods

Participants

Data from a total of 81 participants was included in this retrospective study comprising athletes and controls. Athletes: fifty-four retired contact sports athletes with high risk of concussions were recruited through the Canadian Football League Alumni Association and the Canadian Concussion Centre concussion clinic. The inclusion criteria were participants under 85 years old who were retired contact sports athletes with high probability of multiple past concussions, fluent in English, and had blood plasma analyzed for pTau181, total tau (t-tau), and NfL. The relevant concussion history including number of concussions, years since last concussion, and total years of play were collected using self-report questionnaires. Exclusion criteria at the time of study visit were the diagnosis of a neurological or psychiatric disorder (i.e. epilepsy, stroke, major depression disorder, bipolar, schizophrenia), systemic illnesses affecting the brain, or pathology other than white matter hyperintensities (WMH) seen on brain MRI scans. A subset (21/57 (37%)) of the retired athletes returned for a 2-year follow up visit and were included in the longitudinal analysis. One included retired athlete had a neuropathological diagnosis of mixed CTE/AD/frontotemporal lobar degeneration (FTLD) TDP-43 type A six years post participation in this study. Controls: twenty-seven healthy controls recruited from the community through advertising underwent venipuncture for blood plasma and MRI. Exclusion criteria for the healthy controls were previous history of concussions, neurological or psychiatric disorder, other systemic illnesses affecting the brain, any significant lesions seen on brain MRI scans, or impaired performance on neuropsychological testing (ie. normed scores >1.5 standard deviations below the mean). This study was approved by the Research Ethics Board of the University Health Network and written consent was obtained from all participants.

Neuropsychological assessment

All participants completed neuropsychological assessments comprising the following domains: memory, executive functioning, and speed of processing/attention, as well as a neuropsychiatric battery. Memory assessments included

the Rey Auditory Verbal Learning Test (RAVLT)[21] and Rey Visual Design Learning Test (RVDLT)[22]. Attention and speed of processing measures included Trail Making Test (TMT) part A[23], Digit span forward[24], Stroop Color Naming and Word Reading Tests[25], and the Symbol Digit Modalities Test (SDMT)[22,26], both oral and written versions. Executive function assessments included the Digit span backwards[24], Wisconsin Card Sorting Test (WCST)[27] and TMT part B[28]. Neuropsychiatric assessment was completed using the Personality Assessment Inventory (PAI) sub-scales of aggression, depression, and anxiety.[29] The scores were standardized using established norms[23,24,26,29–31]. Higher normed scores on all cognitive measures represent better performance, while higher scores on the PAI normative scores represent poorer functioning. We operationalized neuropsychological impairment as more than 1.5 standard deviations below the normal range.

Blood analysis

Plasma from retired contact sport athletes and healthy controls was analyzed for pTau181 using an in-house assay on an HD-1 Single molecule array (Simoa) instrument (Quanterix, Billerica, MA), as previously described[10]. NfL and t-tau concentrations were also measured using Simoa and the commercial NF-light and Tau Advantage kits, respectively (Quanterix, Billerica, MA). Samples were diluted two-fold with assay diluent and analyzed as singlicates. Quality control samples (QCs) were analyzed in duplicates at the start and the end of each plate and used to assess analytical reproducibility. The intra-run and inter-run reproducibility were both < 20%. Plasma NfL analysis failed due to technical reasons in one retired athlete. The threshold for abnormally elevated plasma pTau181 levels among the retired athletes was based on published levels in cognitively unimpaired (CU) healthy adults[10,12]. For young adults (<60 y.o.) the threshold of >10.5 pg/mL for plasma pTau181 was considered high. For older adults (≥60 y.o.) two different cut-offs for pTau181were examined: (1) >13.3 pg/mL h; and (2) >15.4 pg/mL [10,12]. The threshold for abnormally high plasma NfL level was set at >25.7 pg/mL, based on previously published levels[32].

Structural MRI and DTI acquisition

All structural and diffusion tensor imaging (DTI) scans were acquired using a 3T MRI scanner (GE Signa DHx, Milwaukee, WI, USA) with an 8-channel head coil. The T1-weighted structural MRI scans were acquired using inversion recovery spoiled gradient echo (IR-SPGR) in the sagittal plain using the following parameters: TE=2.8ms, TR=7ms, flip angle = 11°; 176 slices, slice thickness=1.2mm, 256x256 matrix, and FOV=26 cm. At least one diffusion weighted imaging (DWI) scan was obtained with the diffusion gradient applied across 60 spatial directions

(b=1000 s/mm^2) and 10 non-diffusion weighted B_0 scans. The DWI scans were acquired using the following parameters: 2.4 mm thick axial slices, TR=17000ms, FOV=23cm, and 2.4x2.4 mm in-plane resolution.

Volumetric MRI analysis

Regional brain volumes were derived by applying FreeSurfer v.6 software (http://surfer.nmr.mgh.harvard.edu/) to the T1-weighted structural 3D MRI images. Structural MRI scans were preprocessed using the standard "recon-all" FreeSurfer pipeline, previously described.[33] Volumes of interest included CC, hippocampus, superior frontal, thalamus, inferior parietal, precuneus, and entorhinal. To control for individual differences in head size, each regional volume of interest was divided by the intracranial volume (ICV) and presented as volume-to-ICV ratio x10^{-3}.

WM tractography

The DTI analysis was completed using the FMRIB Software library (FSL) tools (http://www.fmrib.ox.ac.uk/fsl/fdt/index.html). Multiple DTI metrics including fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AxD), and radial diffusivity (RD) were extracted for the following tracts of interest: (a) right and left superior longitudinal fasciculus (SLF), (b) right and left uncinate (UNC), (c) right and left cingulum (Cg), (d) CC, and (e) fornix. The processing steps of the DTI data, region of interest (ROI) definition and fibre tracking steps for SLF, UNC, Cg and CC were previously described elsewhere[34]. The ROI for the fornix was placed on the coronal slice at the point where the posterior pillars of the fornix join together to form the body of fornix[35]. All subjects' images were reviewed by a neurologist for presence of WMH prior to DTI analysis. All subjects had no to minimal amount of WMH. MD, AxD, and RD values are presented as mm^2/s x10^{-3}.

PET acquisition and PET processing

A subset (N=23; 43%) of the 54 retired athletes who had blood analysis also underwent PET imaging with 5mCi of [F-18]AV1451 ([F-18]T807; Flortaucipir, AVID Radiopharmaceuticals) tau specific tracer. All retired athletes were scanned using a Biograph HiRez XVI PET/CT scanner (Siemens Molecular Imaging, Knoxville, TN, USA). The detailed acquisition parameters and analysis pipeline is described elsewhere[35]. Following a 45-minute uptake time, emission PET data were acquired in list mode for 75 mins. ROI analysis of the PET data was completed using in-house ROMI software[36] and Statistical Parametric Mapping version 8 (SPM8; https://www.fil.ion.ucl.ac.uk/spm/software/spm8/). The PET images were corrected for head motion and partial volume effect[37]. ROI included whole cortical grey matter. AD-typical pattern of tracer retention was characterized

either as increased activity in the posterolateral temporal, occipital, parietal, or precuneus regions; or as increased activity in the frontal regions accompanied by increased activity in the posterolateral temporal, occipital, parietal, or precuneus regions, as previously described elsewhere[38,39]. Standardized uptake value ratios (SUVRs) were calculated for full cortical grey matter using cerebellar grey matter as a reference region and averaged for the 50-80 min post tracer injection timeframe.

Statistical analysis

Statistical analysis was completed using IBM SPSS Statistics v.27 (IBM Corp., Armonk, NY, USA). Data were visualized using R studio and R v4.0.2 (https://www.r-project.org/). Firstly, the retired athletes cohort was compared to healthy controls using blood markers and scores on neuropsychological/neuropsychiatric assessments. Then, retired athletes were divided into those with normal plasma pTau181 levels and high pTau181 levels based on published values for CU healthy adults[10,12]. To explore the predictive ability of plasma pTau181, the neuroimaging markers related to the plasma markers in the cross-sectional analysis were examined in the subset of retired athletes with a 2-year follow up visit. To ensure that AD pathology was not influencing the results, the sub-cohort of retired contact sports athletes who had [18F]-AV1451 PET-tau scan not consistent for AD-like pattern of tracer signal[38] were compared to healthy controls. All matched comparisons were completed using ANCOVA with age as a covariate for continuous variables and Fisher's exact test for categorical variables. Pearson partial correlations adjusted for age were implemented to explore the associations between blood markers and neuroimaging outcomes. Results were controlled for multiple comparisons using Bonferroni method.

Results

Participants

A total of 81 participants were included in this study [age (52.04±12.89 years); sex (79 males, 2 females)]. Retired contact sports athletes and control clinical characteristics are displayed in Table 1. One participant had a neuropathological diagnosis of mixed CTE/AD/FTLD-TDP six years post participation in this study; at the time of the study visit this participant had low plasma pTau181 level of 6.23 pg/mL and elevated NfL of 25.87 pg/mL.

Comparison between retired contact sport athletes and healthy controls

Retired athletes when compared to healthy controls did not differ on demographics or neuropsychological assessments but had significantly higher PAI depression (51.30±13.17 vs. 44.31±9.43; 95% CI 0.99-12.12; adjusted p=.03) and PAI aggression scores (50.15±10.22 vs. 43.42±5.91; 95% CI 2.84-11.18; adjusted p<.003). Plasma pTau181 was significantly higher in retired athletes in comparison to healthy controls (8.94±5.08 pg/mL vs. 6.00±2.53 pg/mL, respectively; 95% CI 0.87-5.01; adjusted p=.02; see Figure 1A) while there was no difference in t-tau or NfL. Three retired athletes had considerably higher plasma pTau181 levels (outliers >2 standard deviations above the mean) with the concentrations of 34.64, 18.96, and 20.83 pg/mL so were excluded, but pTau181 levels remained significantly higher in the retired athletes in comparison to healthy controls (8.25±3.38 pg/mL vs. 6.00±2.53 pg/mL, respectively; 95% CI 0.78-3.73; adjusted p=.009).

Relationship between blood plasma markers

No significant correlations were found between plasma markers of pTau181, t-tau, and NfL after controlling for age, in the retired contact sports athletes (N=54) or healthy controls (N=27).

Relationship between regional brain volumes and blood plasma markers

In the retired contact sports athletes, a significant relationship was found between blood plasma NfL levels and CC (N=54; r=-0.384, p=.005) and hippocampal volumes (N=54; r=-0.381, p=.005), controlled for age and ICV (Figure 3A). No significant relationships between other plasma markers (i.e., pTau181 and t-tau) and regional brain volumes of CC, hippocampi, superior frontal, thalamus, inferior parietal, precuneus, and entorhinal areas, controlled for age and ICV volume, were found in retired contact sports athletes. No significant relationships were found between any plasma biomarkers and measures of regional brain volumes in 27 healthy controls.

Relationship between white matter integrity and blood plasma markers

Fifty-three retired contact sport athletes had a DTI scan available for analysis. There was a significant negative relationship between plasma pTau181 levels and fornix FA values (r=-0.393, p=.004), controlled for age (Figure 2B). We also found a significant relationship between plasma NfL levels and all measures of white matter integrity of the fornix: FA (r=-0.455, p<.001), MD (r=0.462, p<.001), AxD (r=0.444, p<.001), and RD (r=0.468, p<.001), controlled for age. In contrast, there were no significant relationships between plasma t-tau levels and measures of white matter integrity in retired athletes, controlled for age. Finally, there were no significant relationships between any plasma markers and measures of white matter integrity among healthy controls (N=27), controlled for age.

Separating the cohort of retired athletes based on plasma pTau181

The full cohort of retired athletes was separated into normal and high plasma pTau181 groups based on published levels for CU healthy adults[10,12]. When using the thresholds of 10.5 pg/mL for young athletes (<60 y.o.) and 13.3 pg/mL for older athletes, the cohort was divided into 45/54 (83%) athletes with normal pTau181 levels, and 9/54 (17%) athletes with high pTau181 levels. Aside from the normal pTau181 group being significantly younger than the high pTau181 group (51.32±12.86 years vs. 63.78±9.48 years; 95% CI -21.54 to -3.37; p=.01), there were no significant differences in demographics, neuropsychological or behavioral scores between these 2 groups (Table 2). When using the same threshold of 10.5 pg/mL for younger athletes but a stricter threshold of 15.4 pg/mL for older athletes, 3 participants were no longer considered to have high levels of pTau181 and therefore the cohort was divided into 48/54 (89%) athletes with normal levels of pTau181 and 6/54 (11%) with high levels of pTau181. Aside from the higher pTau181 group having a significantly higher number of years of professional play in comparison to the normal pTau181 group (11.33±3.39 years vs. 6.61±4.21 years; 95% CI 0.85-8.21; p=.02, controlled for age), there were no differences in demographics, neuropsychological or neuropsychiatric scores between the two groups.

Regional brain volumes comparison between normal/high pTau181 groups and healthy controls

When using the abnormal thresholds of plasma pTau181 > 10.5 pg/mL for young athletes and pTau181 > 13.3 pg/mL for older athletes, the regional volumes of CC and entorhinal were significantly decreased in high pTau181 (N=9) in comparison to older healthy controls (N=9), controlled for age (1.57±0.19 vs. 2.02±0.32, p=.002; and 2.07±0.35 vs. 2.82±0.51, p=.003, respectively; Additional File 1, Figure 3A). Additionally, multiple other areas (ie. superior frontal, thalamus, and inferior parietal) had trends for lower regional volumes in the high pTau181 group in comparison to older healthy controls. The normal pTau181 athlete group (N=45) had significantly lower entorhinal volume in comparison to healthy controls (N=27) (2.32±0.32 vs. 2.58±0.44, p=.005), controlled for age. When using the stricter threshold of >15.4 pg/mL for older athletes, the results remained similar with CC and entorhinal volumes significantly lower in the high pTau181 group (N=6) in comparison to the older healthy controls (N=9) (1.54±0.20 vs. 2.02±0.32, p=.009; and 2.04±0.44 vs. 2.82±0.51, p=.01, respectively), controlled for age. Entorhinal volume also remained significantly lower in normal pTau181 (N=48) athlete group in comparison to healthy controls (N=27) (2.30±0.31 vs. 2.58±0.44, p=.003), controlled for age.

WM integrity differences between normal/high plasma pTau181 group and healthy controls

When using the abnormal thresholds of plasma pTau181 > 10.5 pg/mL for young athletes and pTau181 > 13.3 pg/mL for older athletes, CC MD and AxD were significantly higher in the high pTau181 group (N=8) in comparison to older healthy controls (N=9) (0.96±0.04 $x10^{-3}$ mm^2/s vs. 0.90±0.03 $x10^{-3}$ mm^2/s, p=.003; and 1.49±0.04 $x10^{-3}$ mm^2/s vs. 1.41±0.02 $x10^{-3}$ mm^2/s, p<.001, respectively; see Additional File 2 and Figure 3B), controlled for age. In addition, left uncinate AxD was significantly higher in the high pTau181 group (N=8) in comparison to older healthy controls (N=9) (1.29±0.05 $x10^{-3}$ mm^2/s vs. 1.20±0.05 $x10^{-3}$ mm^2/s, p=.004), controlled for age. When using the stricter threshold of >15.4 pg/mL for older athletes, the CC MD, CC AxD, and left uncinate AxD remained significantly higher in the high pTau181 group (N=6) in comparison to older healthy controls (N=9) (0.95±0.04 $x10^{-3}$ mm^2/s vs. 0.90±0.03 $x10^{-3}$ mm^2/s, p=.008; and 1.48±0.05 $x10^{-3}$ mm^2/s vs. 1.41±0.02 $x10^{-3}$ mm^2/s, p<.001; and 1.29±0.06 $x10^{-3}$ mm^2/s vs. 1.20±0.05 $x10^{-3}$ mm^2/s, p=.004, respectively), controlled for age. There were no significant differences between normal pTau181 group and healthy controls regardless of which of the two thresholds for high pTau181 was used.

Relationship between baseline plasma pTau181 and longitudinal neuroimaging measures

In the subset of retired athletes who returned for their 2-year follow-up visit [N=21; age (52.95±13.12 years)], baseline plasma pTau181 did not predict longitudinal change in fornix FA, CC MD, CC AxD, and left uncinate AxD, or entorhinal volume over 2 years, controlled for age.

[18F]-AV1451 PET-tau subset

A subset of retired contact sport athletes (23/54, 43%) had [18F]-AV1451 PET-tau scan completed and were negative for AD-typical pattern of tau ligand binding[38,39]. Comparison between this subset of retired contact sport athletes and healthy controls did not reveal any significant differences in demographics, neuropsychological measures, and plasma levels of NfL and t-tau (see Figure 1B). Consistent with the analysis on the full cohort, this subset of retired athletes when compared to healthy controls had higher plasma pTau181 levels (9.36±3.78 pg/mL vs. 6.08±2.47 pg/mL; 95% CI 1.54-5.03; adjusted p<.003) and PAI aggression scores (52.32±9.22 vs. 42.83±5.96; 95% CI 5.21-13.77; adjusted p<.003).

Discussion

The present study showed elevated plasma pTau181 levels in retired athletes compared to healthy controls. In the cross-sectional cohort, higher plasma pTau181 levels was associated with lower fornix integrity. When comparing

high and normal high pTau181 retired players, the high pTau181 group had significantly lower CC and entorhinal cortex volumes, and lower CC and left uncinate integrity compared to age matched healthy controls. This is in keeping with our previously published results of lower uncinate integrity related to increased impulsivity in retired contact sports athletes[40]. However, decreased entorhinal cortex volume was also found in the normal pTau181 group compared to healthy controls, making this finding non-specific to the high pTau181 group. Numerous studies have reported CC abnormalities in sport related concussion and reduced CC integrity persists long after the incident concussion, and is related to post-concussion symptoms[41–43]. None of the retired athletes had AD-typical PET-tau tracer retention, but this subset's plasma pTau181 levels were also significantly elevated in comparison to healthy controls. Longitudinally, baseline plasma pTau181 levels did not predict regional brain volumes or WM integrity changes over 2 years. This may be because the delayed neurodegeneration that can occur after multiple concussions is heterogeneous and can be associated with various other pathologies in addition to CTE, as seen in the participant who was later neuropathologically diagnosed with mixed CTE/AD/FTLD-TDP.

When comparing high pTau181 group to age matched older healthy controls, there were additional brain regions that were trending towards lower volumes in the high pTau181 group. These trends were not found in retired athletes with normal pTau181 levels. This suggests that our analysis is likely underpowered due to small sample size and pTau181 may be useful for identifying those with brain abnormalities related to repeated concussion.

Insoluble tau deposits are the underlying pathological substrate behind many neurodegenerative diseases including AD and CTE, and can also be one of the multiple pathological causes of frontotemporal dementia. However, the isoform composition of the pathogenic tau aggregates differs across diseases and the plasma pTau181 appears to be a marker of mixed 3R/4R tauopathy as seen in AD[11] or rare *MAPT* mutations with mixed 3R/4R tau pathology[15]. In comparison, autopsy data from frontotemporal dementia associated with insoluble deposits of either 3R or 4R tau pathology had lower levels of plasma pTau181[11]. While the pathological substrate of CTE is also a mixed 3R/4R tauopathy[20], the fold of the tau filaments in CTE is distinct from those in AD[44]. Based on the outcomes of our study, we cannot conclude that pTau181 is a biomarker of CTE-related tau pathology but our results do suggest that pTau181 is abnormal in those with repeated concussions even if their PET Tau scans are not consistent with AD. This adds to the growing body of evidence suggesting specificity of plasma pTau181 to mixed 3R/4R tau pathology but not tau aggregates found in other tauopathies including CBD, and FTLD[10,15,45,46]. The heterogeneity of pathologies in those with repeated concussions, as well as the patchy distribution of pathology unlike in AD make biomarker discovery more difficult in CTE[2,3].

Plasma NfL levels were significantly associated with CC and bilateral hippocampi volumes, as well as fornix integrity although not significantly different between athletes and controls. These results are consistent with previously reported literature, where plasma NfL levels were associated with concussion-related changes in brain structure and able to differentiate players that returned to play from those who did not[6,7]. Again, highlighting the heterogeneity of outcomes amongst those who suffer concussions. Since in our study baseline plasma pTau181 did not predict longitudinal atrophy and WM changes, it would suggest that plasma NfL may have more prognostic value in retired contact sports athletes but is less specific given that it is elevated in all neurodegenerative diseases. Finally, t-tau did not relate to any neuroimaging or neuropsychological outcomes, and this was expected because plasma t-tau was only reported to be elevated in acute concussions where it predicted prolonged slowed recovery[7,47].

The current study has limitations including the small sample size and a low number of female retired athletes, which limits generalizability of results. The longitudinal subset had a follow-up of only 2 years, which is a short time for examining longitudinal changes in neurodegenerative diseases. Only a small portion of retired athletes had completed PET-tau analysis and therefore we cannot completely rule out AD. Finally, the lack of brain pathology tissue in our cohort does not allow us to infer the specificity of plasma pTau181 to neurofibrillary tangles of pathogenic tau in CTE.

Conclusions

Plasma pTau181 may be an additional marker to detect neuropathological changes in retired athletes at risk for neurodegeneration. However, more work is required to determine if it has utility as an *in vivo* biomarker for CTE.

List of abbreviations

AxD (Axial Diffusivity)

CC (Corpus Callosum)

CFL (Canadian Football League)

Cg (Cingulum)

CU (Cognitively Unimpaired)

CTE (Chronic Traumatic Encephalopathy)

DTI (Diffusion Tensor Imaging)

DWI (Diffusion Weighted Imaging)

FA (Fractional Anisotropy)

FTLD (Frontotemporal Lobar Degeneration)

ICV (Intracranial Volume)

MD (Mean Diffusivity)

NfL (Neurofilament Light Chain)

PAI (Personality Assessment Inventory)

PET (Positron Emission Tomography)

pTau181 (Tau Phosphorylated at Threonine 181)

RD (Radial Diffusivity)

RAVLT (Rey Auditory Verbal Learning Test)

RVDLT (Rey Visual Design Learning Test)

SUVRs (Standardized Uptake Value Ratios)

SLF (Superior Longitudinal Fasciculus)

SDMT (Symbol Digit Modalities Test)

T-tau (Total Tau)

TMT (Trail Making Test)

UNC (Uncinate)

WMH (white matter hyperintensities)

WCST (Wisconsin Card Sorting Test)

Declarations

Ethics approval and consent to participate

This study was approved by the Research Ethics Board of the University Health Network and written consent was obtained from all participants.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyses during the current study are available from the corresponding author on reasonable request.

Competing interests

HZ has served at scientific advisory boards for Eisai, Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics, Nervgen, AZTherapies and CogRx, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work).

KB has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu. Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program.

GGK has served at advisory board for Biogen.

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Author's Contributions

A.V. analyzed the data, interpreted the data, drafted the manuscript for intellectual content; F.T. analyzed the data, interpreted the data; N.M. analyzed the data, interpreted the data; M.O. analyzed the data, interpreted the data; A.T. major role in the acquisition of data; M.K. major role in the acquisition of data; R.W. interpreted the data, revised the manuscript for intellectual content; P.R. interpreted the data, revised the manuscript for intellectual content; S.H. interpreted the data, revised the manuscript for intellectual content; R.G. acquisition of data, interpreted the data, revised the manuscript for intellectual property; B.C. acquisition of data, interpreted the data, revised the manuscript for intellectual property; K.B. analyzed the data, interpreted the data, drafted the manuscript for intellectual content; H.Z. analyzed the data, interpreted the data, drafted the manuscript for intellectual content; T.K. analyzed the data, interpreted the data, drafted the manuscript for intellectual content; D.M. interpreted the data, revised the manuscript for intellectual content; L.N.H. analyzed the data, interpreted the data, drafted the manuscript for intellectual content; G.G.K. analyzed the data, interpreted the data, drafted the manuscript for intellectual content; K.D.D. interpreted the data, revised the manuscript for intellectual content; C.T. major role in acquisition of data, interpreted the data, revised the manuscript for intellectual content; M.C.T. major role in acquisition of data, interpreted the data, drafted the manuscript for intellectual content, revised the manuscript for intellectual content.

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Table 1. Cross-sectional comparisons between full cohort of retired contact sports athletes and healthy controls.

	Retired Athletes (N=54)	**Healthy Controls (N=27)**	**Unadjusted p (adjusted p)**	**95% CI**
Sex (Males:Females)	52M:2F	27M	.55	
Age (years)	53.40 (13.14)	49.32 (12.16)	.18	-1.94 to 10.10
Education (years)	15.72 (1.84)	16.67 (2.35)	.05	-1.90 to 0.01
Sports played	Football: 43; Hockey: 9; Boxing: 2	NA		
Number of concussions	Min=0; Max=30; Median=4	NA		
Professional play (years)	Min=0; Max=16; Median=7	NA		
Memory measures				
RAVLT trials 1-5 z-score	0.08 (0.98)	0.30 (0.82)	.32 (N.S.)	-0.66 to 0.22
N	52	27		
RAVLT long-delay recall z-score	-0.29 (1.10)	0.07 (1.06)	.18 (N.S.)	-0.87 to 0.16
N	52	27		
RVDLT trials 1-5 z-score	0.03 (1.20)	0.10 (1.21)	.78 (N.S.)	-0.69 to 0.52
N	52	27		
Attention & Speed of Processing measures				
TMT part A t-score	55.26 (12.11)	51.85 (14.89)	.27 (N.S.)	-2.76 to 9.58
N	53	27		
Digit span forward %	60.34 (31.71)	50.41 (30.95)	.19 (N.S.)	-4.88 to 24.74
N	53	27		
Stroop color naming test t-score	47.42 (5.82)	46.91 (6.72)	.73 (N.S.)	-2.38 to 3.39
N	53	27		
Stroop word reading test t-score	49.43 (5.50)	50.39 (8.20)	.54 (N.S.)	-4.03 to 2.12
N	53	27		
SDMT written z-score	0.28 (0.78)	0.38 (0.90)	.59 (N.S.)	-0.49 to 0.28
N	52	27		
SDMT oral z-score	0.57 (0.99)	0.66 (0.97)	.70 (N.S.)	-0.55 to 0.37
N	53	27		
Executive Function measures				
TMT part B t-score	55.74 (11.06)	51.78 (13.24)	.16 (N.S.)	-1.61 to 9.53
N	53	27		
Digit span backwards %	62.34 (27.78)	59.56 (25.42)	.66 (N.S.)	-9.93 to 15.50
N	53	27		
WCST % error t-score	51.51 (8.13)	50.19 (6.63)	.48 (N.S.)	-2.34 to 4.98
N	53	26		
WCST % perseverative error t-score	52.06 (8.92)	48.58 (7.453)	.09 (N.S.)	-0.57 to 7.53
N	53	26		
WCST % non-perseverative error t-score	49.28 (7.47)	49.92 (6.55)	.71 (N.S.)	-4.07 to 2.79
N	53	26		
Neuropsychiatric outcomes				
PAI depression t-score	51.30 (13.17)	44.31 (9.43)	.01 **(.03)**	0.99 to 12.12
N	53	26		
PAI anxiety t-score	47.40 (8.11)	42.15 (8.95)	.02 (N.S.)	0.79 to 8.57
N	53	26		
PAI aggression t-score	50.15 (10.22)	43.42 (5.91)	<.001 (<**.003**)	2.84 to 11.18
N	53	26		
Blood Plasma Biomarkers				
pTau181 (pg/mL)	8.94 (5.08)	6.00 (2.53)	.006 **(.02)**	0.87 to 5.01
N	54	27		
NfL (pg/mL)	11.29 (6.73)	10.58 (6.58)	.65 (N.S.)	-2.43 to 3.86
N	53	27		

Total Tau (pg/mL)	2.00 (0.59)	1.87 (0.50)	.32 (N.S.)	-0.13 to 0.39
N	54	27		

Values are presented as mean (standard deviation). CI, confidence interval; RAVLT, Rey Auditory Verbal Learning Test; RVDLT, Rey Visual Design Learning Test; SDMT, Symbol digit modalities test; TMT, Trail making test; WCST, Wisconsin card sorting test; PAI, Personality assessment inventory. Independent student t-test, Fisher's exact test; unadjusted significance set at $p<0.05$ (2-sided). Bonferroni adjusted p-values significant at $p<0.05$.

Table 2. Cross-sectional comparisons between normal pTau181 and high pTau181 groups.

	Normal plasma pTau181 group (N=45)	**High plasma pTau181 group (N=9)**	**Unadjusted p (adjusted p)**	**95% CI**
Sex (Males:Females)	43M:2F	9M	1.00	
Age (years)	51.32 (12.86)	63.78 (9.48)	**.01**	-21.54 to -3.37
Education (years)	15.82 (1.92)	15.22 (1.39)	.38	-0.75 to 1.95
Sports played	Football: 34; Hockey: 9; Boxing: 2	Football: 9		
Number of concussions	Min=0; Max=16; Median=4	Min=1; Max=30; Median=4	.62	-8.50 to 5.39
Professional play (years)	Min=0; Max=15; Median=7	Min=4; Max=16; Median=9		
Memory measures				
RAVLT trials 1-5 z-score	0.03 (1.01)	0.31 (0.84)	.43 (N.S.)	-1.01 to 0.44
N	43	9		
RAVLT long-delay recall z-score	-0.27 (1.13)	-0.37 (1.02)	.81 (N.S.)	-0.72 to 0.92
N	43	9		
RVDLT trials 1-5 z-score	0.17 (1.35)	-0.73 (0.93)	.06 (N.S.)	-0.05 to 1.86
N	43	9		
Attention & Speed of Processing measures				
TMT part A t-score	56.61 (11.44)	48.67 (13.80)	.07 (N.S)	-0.75 to 16.65
N	44	9		
Digit span forward %	60.36 (33.30)	60.22 (23.97)	.99 (N.S.)	-19.96 to 20.24
N	44	9		
Stroop color naming test t-score	47.56 (5.57)	46.72 (7.25)	.70 (N.S.)	-3.47 to 5.14
N	44	9		
Stroop word reading test t-score	49.34 (4.76)	49.89 (8.64)	.79 (N.S.)	-4.62 to 3.53
N	44	9		
SDMT written z-score	0.30 (0.79)	0.19 (0.75)	.71 (N.S.)	-0.47 to 0.68
N	43	9		
SDMT oral z-score	0.68 (1.00)	0.04 (0.79)	.08 (N.S.)	-0.07 to 1.35
N	44	9		
Executive Functioning measures				
TMT part B t-score	56.11 (11.39)	53.89 (9.65)	.59 (N.S.)	-5.95 to 10.40
N	44	9		
Digit span backwards %	61.82 (28.05)	64.89 (27.89)	.77 (N.S.)	-23.65 to 17.51
N	44	9		
WCST % error t-score	50.64 (6.99)	55.78 (11.95)	.24 (N.S.)	-14.43 to 4.15
N	44	9		
WCST % perseverative error t-score	50.84 (7.89)	58.00 (11.60)	.03 (N.S.)	-13.46 to -0.86
N	44	9		
WCST % non-perseverative error t-score	49.32 (7.33)	49.11 (8.58)	.94 (N.S.)	-5.33 to 5.75
N	44	9		
Neuropsychiatric outcomes				
PAI depression t-score	52.48 (13.55)	45.56 (9.79)	.15 (N.S.)	-2.65 to 16.50
N	44	9		
PAI anxiety t-score	47.61 (8.25)	46.33 (7.68)	.67 (N.S.)	-4.72 to 7.28
N	44	9		
PAI aggression t-score	50.20 (10.82)	49.89 (7.04)	.93 (N.S.)	-7.27 to 7.90
N	44	9		
Blood Plasma Biomarkers				
NfL (pg/mL)	11.05 (6.95)	12.47 (5.79)	.57 (N.S.)	-6.40 to 3.56
N	44	9		
Total Tau (pg/mL)	2.04 (0.56)	1.79 (0.72)	.25 (N.S.)	-0.18 to 0.68
N	45	9		

For young adults (<60 y.o.) the threshold of >10.5 pg/mL of plasma pTau181 was considered high. For older adults (≥60 y.o.), plasma pTau181 levels of >13.3 pg/mL were considered high. Values are presented as mean (standard deviation). CI, confidence interval; RAVLT, Rey Auditory Verbal Learning Test; RVDLT, Rey Visual Design Learning Test; SDMT, Symbol digit modalities test; TMT, Trail making test; WCST, Wisconsin card sorting test; PAI, Personality assessment inventory. Independent student t-test, Fisher's exact test; unadjusted significance set at $p<0.05$ (2-sided). Bonferroni adjusted p-values significant at $p<0.05$.

Figure 1. Blood plasma comparisons between retired athletes and healthy controls.

Visualized blood plasma (ie. pTau181, NfL, total tau) comparisons: (A) between the full cohort of retired athletes and healthy controls; and (B) between the sub-group of retired athletes who underwent [18F]-AV1452 PET-tau scan and were negative for AD-like pattern of tracer signal and healthy controls. Blood plasma levels of pTau181, NfL, and total tau are represented as pg/mL. NfL, neurofilament light chain; PET, positron emission tomography; N.S., not significant. The notch displays the 95% confidence interval (CI) around the mean. Bonferroni adjusted significance: *P<0.05, **P<0.01, ***P<0.001.

Figure 2. Relationships between plasma markers and neuroimaging measures in the full cohort of retired athletes.

Visualization of significant relationships between plasma markers and neuroimaging measures in the full cohort of retired athletes, controlled for age. (A) Relationships between blood plasma NfL levels and hippocampus and CC volumes. (B) Relationships between blood plasma pTau181 and NfL levels with white matter integrity of fornix. Blood plasma concentrations of pTau181 and NFL are represented as pg/mL; fornix FA is represented as mm^2s^{-1}; regional brain volumes are represented as volume-to-ICV ratios $x10^{-3}$. NfL, neurofilament light chain; CC, corpus callosum; FA, fractional anisotropy; ICV, intracranial volume. Unadjusted p-values are shown, significant at the level of p<.007 for regional brain volumes, and significant at the level of p<.006 for white matter integrity, after Bonferroni correction for multiple comparisons.

Figure 3. Cross-sectional comparisons between groups on neuroimaging measures.

Visualized results of significant cross-sectional neuroimaging comparisons between groups, Bonferroni corrected for multiple comparisons and adjusted for age. (A) CC and entorhinal regional brain volumes comparisons between groups. (B) White matter integrity indices of CC MD, CC AxD, and LUNC AxD comparison between groups. Colors distinguish between normal pTau181 group, high pTau181 group, and healthy controls. The threshold for high plasma pTau181 was the following: (1) for participants <60 y.o. the plasma pTau181 had to be >10.5 pg/mL to be considered high; (2) for participants >60 y.o. the plasma pTau181 had to be >13.3 pg/mL to be considered high. MD and AxD values represented as mm^2/s $x10^{-3}$; regional brain volumes presented as volume-to-ICV ratio $x10^{-3}$. ICV, intracranial volume; CC, corpus callosum; MD, mean diffusivity; AxD, axial diffusivity; LUNC, left uncinate; N.pTau181, normal pTau181; H.pTau181, high pTau181; HCs, healthy controls; N.S., not significant. The notch displays the 95% confidence interval (CI) around the mean. Bonferroni corrected significance is **P<0.01, ***P<0.001.