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Cerebrospinal Fluid Tau, A β , and sTREM2 in Former National Football League Players: Modeling the Relationship between Repetitive Head Impacts, Microglial Activation, and Neurodegeneration

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Abstract

INTRODUCTION—Cerebrospinal fluid (CSF) protein analysis may facilitate detection and elucidate mechanisms of neurological consequences from repetitive head impacts (RHI), such as chronic traumatic encephalopathy (CTE). We examined CSF concentrations of total tau (t-tau), phosphorylated tau (p-tau), and A β ₁₋₄₂, and their association with RHI in former National Football League (NFL) players. The role of microglial activation (using sTREM2) was examined as a pathogenic mechanism of CTE.

METHODS—Sixty-eight former NFL players and 21 controls underwent lumbar puncture to quantify t-tau, p-tau₁₈₁, A β ₁₋₄₂, and sTREM2 in the CSF using immunoassays. The cumulative head impact index (CHII) estimated RHI.

RESULTS—No between group differences for CSF analytes emerged. In the former NFL players, the CHII predicted higher t-tau concentrations (p=0.041) and higher sTREM2 levels were associated with higher t-tau concentrations (p=0.009).

DISCUSSION—In this sample of former NFL players, greater RHI and increased microglial activation were associated with higher CSF t-tau concentrations.

Keywords

Cerebrospinal fluid; A β ; sTREM2; chronic traumatic encephalopathy; microglial activation; Alzheimer's disease; repetitive head impacts; concussion; subconcussive

1. INTRODUCTION

Chronic traumatic encephalopathy (CTE) is a neurodegenerative disease associated with exposure to repetitive head impacts (RHI), such as those incurred through contact sports (e.g., tackle football, boxing) and combat military service involving blast exposures[1–4]. In a recent convenience sample of 202 deceased tackle football players, CTE was neuropathologically diagnosed in 177 participants, including 110 of 111 former National Football League (NFL) players[4]. CTE cannot yet be diagnosed during life and the mechanisms by which exposure to RHI transitions to neurodegeneration are unknown. To address these knowledge gaps, current research is focused on the development of *in vivo* biomarkers for CTE[5,6]. *In vivo* fluid biomarkers play a critical role in the clinical diagnosis and study of mechanistic pathways of other neurodegenerative diseases, such as

Alzheimer's disease (AD)[7]. Clinically useful fluid biomarkers for concussion and acute traumatic brain injury (TBI) are now emerging[8].

The pathognomonic diagnostic lesion of CTE is the perivascular deposition of abnormal phosphorylated tau (p-tau) in neurons and astroglia at the base of the cortical sulci[9]. If present, A β plaques are sparse and diffuse and related to age[4,10]. Positron emission tomography (PET) imaging of paired helical filament tau is currently under investigation as diagnostic biomarkers to identify and grade tau pathology in patients with suspected CTE and may have value for CTE diagnosis, particularly when used in conjunction with A β PET. PET imaging, however, is time-demanding and expensive. Although lumbar puncture is often viewed as invasive, cerebrospinal fluid (CSF) concentrations of tau (total tau [t-tau], p-tau) and A β represents a pragmatic biomarker that provides a direct assessment of the central nervous system (CNS). CSF concentrations of A β , t-tau, and p-tau are core biomarkers for AD[7,11–13]. As in AD[7], CSF p-tau₁₈₁ may be a specific marker of intraneuronal tau pathology and CSF t-tau may be a marker of neurodegeneration in CTE. Because tau is predominantly expressed in neuronal axons, elevated CSF t-tau concentrations are usually interpreted to reflect general neuronal injury. However, CSF t-tau is non-specific (e.g., it can be temporarily increased following stroke or acute TBI) [14–16], particularly in the absence of neuropathological examination. Elevated CSF t-tau concentrations could reflect downstream CTE-related neurodegeneration and/or neuronal injury related to long-term neurological consequences associated with RHI (e.g., axonal degeneration). Nevertheless, the specific relationship between RHI and later life CSF tau, as well as A β is unknown.

There is evidence that RHI and CTE are associated with increases in microglial activation[17,18]. In 66 deceased American football players with autopsy-confirmed CTE, more years of football play predicted CD68 (a marker of microglial activation), which partially mediated the effect of RHI on p-tau[18]. Tackle football players with autopsy-confirmed CTE have been shown to exhibit elevated CSF CCL11 (a marker of neuroinflammation), which was related to RHI[19]. Molecular neuroimaging studies further demonstrate chronic microglial activation (e.g., PET measures of TSPO) in active, recently retired, and older former NFL players[20,21].

Soluble CSF concentrations of the triggering receptor expressed on myeloid cells 2 (sTREM2) may serve as an additional *in vivo* biomarker of microglial activation in CTE. TREM2 is expressed on microglia in the CNS and modulates microglial activation[22,23]. *TREM2* variants predict increased risk for other neurodegenerative diseases (e.g., frontotemporal dementia, AD)[24], with odds ratios for AD risk similar to *apolipoprotein E*, although the allele frequency in the population is low[25,26]. sTREM2 can be detected in the CSF and higher concentrations are believed to reflect increased microglial activation[27–31]. CSF sTREM2 has been the focus of several recent clinical research studies of AD. sTREM2 correlates with CSF markers of AD t-tau, p-tau[27–29,32], as well as with CSF glial protein YKL-40[27,28]. sTREM2 concentrations, however, vary with disease progression. AD cohorts have exhibited elevated[27,32], reduced[33], or normal levels[34] of sTREM2. When disease stage is considered, sTREM2 levels are higher in the early stages of the AD continuum[28,29,33] and can be increased five years before symptom onset[35].

Analysis of CSF t-tau, p-tau, and A β may be a pragmatic method to detect long-term neurological consequences associated with RHI, including early CTE pathology. Evaluation of sTREM2 levels, in conjunction with CSF t-tau, p-tau, and A β , may also provide insight into the role of microglial activation in the pathogenesis of CTE. This study compared CSF t-tau, p-tau₁₈₁, A β ₁₋₄₂ and sTREM2 between 68 symptomatic former NFL players at high risk for CTE and 21 same-age asymptomatic controls without a history of contact sports or head trauma. The relationship between estimated exposure to RHI (using a cumulative head impact index [CHII][36]) and CSF t-tau, p-tau₁₈₁, A β ₁₋₄₂, and sTREM2 was investigated in the former NFL players. The study used simultaneous equations regression models to examine the relationships among RHI, sTREM2, t-tau, p-tau, and A β ₁₋₄₂.

2. METHODS

2.1. Participants and Study Design

The current sample included 68 former NFL players who participated in the National Institutes of Health-funded study, known as, “Diagnosing and Evaluating Traumatic Encephalopathy using Clinical Tests” (DETECT). The present sample had complete data for all CSF analytes examined. The purpose of the DETECT study is to identify possible *in vivo* biomarkers for CTE. Inclusion criteria for the former NFL players were: male, ages 40–69, a minimum of two seasons in the NFL and a minimum of twelve years of organized football, and self-reported complaints of cognitive, behavior, and/or mood symptoms at the time of screening. A history of concussion within one year from study enrollment was an exclusion criterion and the overall mean (SD) of years since last concussion in the former NFL players was 21.80 (11.37). The sample also included 21 controls who were of the same-age as the former NFL players, had no history of TBI or participation in contact sports, and who reported being asymptomatic at the time of screening. Exclusion criteria for all participants included MRI and/or lumbar puncture contraindications, presence of another CNS disease, and/or a primary language other than English. Participants completed a single two- to three-day study visit, which involved administration of neuropsychological tests, neurological and psychiatric evaluations, lumbar puncture, and history interview, and other examinations not pertinent to the current study. Additional descriptions of the DETECT Study have been reported previously[5,6,37]. All study protocols were approved by the Boston University Medical Center Institutional Review Board. Participants provided written informed consent prior to participation.

2.2. Measures

2.2.1. CSF Analytes—CSF (15–20mL) was obtained by lumbar puncture (LP) in the morning after overnight fasting. LPs were performed by the study neurologist (S.F.) using an atraumatic 25-gauge Sprotte needle at either L3/L4 or L4/L5. After aspiration, approximately 10mL of CSF was deposited into a polypropylene transfer tube and frozen at –80°C. Aliquots were shipped to the University of Pennsylvania for batch analysis of t-tau, p-tau₁₈₁, and A β ₁₋₄₂. Methods of CSF analysis of t-tau, p-tau₁₈₁, and A β ₁₋₄₂ are described elsewhere[38–40]. Briefly, p-tau₁₈₁, t-tau, and A β ₁₋₄₂ levels were measured using the multiplex xMAP Luminex platform (Luminex Corp, Austin, TX) with Fujirebio (INNO-BIA AlzBio3; Ghent, Belgium; for research use—only reagents) immunoassay kit-based reagents.

The Fujirebio kit reagents included well-characterized capture monoclonal antibodies specific for A β ₁₋₄₂ (4D7A3), t-tau(AT120), and p-tau₁₈₁ (AT270), each chemically bonded to unique sets of color-coded beads, and analyte-specific detector antibodies (HT7, 3D6). Calibration curves were produced for each biomarker using aqueous buffered solutions that contained the combination of three biomarkers at concentrations ranging from 56 to 1,948 pg/ml for recombinant tau, 27 to 1,574 pg/ml for synthetic A β ₁₋₄₂ peptide, and 8 to 230pg/ml for a tau synthetic peptide phosphorylated at the threonine 181 position (i.e., p-tau₁₈₁ standard).

Additional samples of CSF were shipped to University College London (UK) for batch analysis of sTREM2. Methods for analysis of CSF sTREM2 analysis were adapted from Kelinberger et al.[33] The antibodies validated in Kleinberger et al.[33] were used (i.e., goat polyclonal against the N-terminus of human TREM2; 1:1,000 – 1:2,000, R&D Systems, AF1828). Streptavidin-coated 96-well plates (Meso-Scale discovery (MSD) Maryland, U.S.) were blocked for 1 hour at 4°C in block buffer (0.5% bovine serum albumin (BSA) and 0.05% Tween 20 in PBS (pH 7.4)). The plates were incubated with the biotinylated polyclonal goat anti-human TREM2 capture antibody (0.25 μ g/ml R&D Systems, Minnesota U.S) diluted in block buffer for 1 hour at room temperature. They were subsequently washed four times with wash buffer (0.05% Tween 20 in PBS) and incubated overnight at 4°C with CSF diluted 1:4 in assay buffer, 0.025% BSA and 0.05% Tween 20 in PBS (pH 7.4) or a standard curve constructed from recombinant human TREM2 protein (4000 –62.5pg/ml (Sino Biological Inc. Beijing, China) diluted in assay buffer. Plates were again washed three times with wash buffer before incubation for 1 hour at room temperature with the detector antibody monoclonal mouse anti-human TREM2 antibody (1 μ g/ml Santa Cruz Biotechnology, Texas, U.S). After three additional washing steps, plates were incubated with the secondary antibody (SULFO-TAG–labeled anti-mouse secondary antibody, MSD) and incubated for 1 hour in the dark. Lastly, plates were washed three times with wash buffer followed by two washing steps in PBS alone. The electrochemical signal was developed by adding MSD Read buffer (1 in 2) and the light emission measured using the MSD SECTOR Imager 6000. The concentration of sTREM2 was calculated using a five-parameter logistic curve fitting method with the MSD Workbench software package. Intra-assay cvs were < 10%, and all samples were measured on the same day using the same reagents.

2.2.2. Cumulative Head Impact Index (CHII)—The CHII quantified estimated exposure to RHI[36]. The CHII is based on reported number of football seasons played, position[s] played, and levels played (e.g., youth, high school, college), as well as estimated head impact frequencies derived from published helmet accelerometer studies. The CHII was initially developed in former high school and college football players because helmet accelerometer studies at the professional football level have not been published or made available. For the current sample of former NFL players, college level estimates of head impact frequencies were applied for estimation of professional level head impact frequencies. A higher CHII reflects greater exposure to RHI. Previous research in the DETECT sample supports the sensitivity of the CHII to neurological outcomes[5,41].

2.2.3. Cognitive and Neuropsychiatric Function—A neuropsychological test battery and semi-structured interviews and self-report measures of neuropsychiatric function were administered to participants on a separate day from the LP. For a full list of the tests administered as part of DETECT, see Alosco et al.[37] To limit the number of analyses, a subset of measures was selected *a priori* to include tests routinely used in the clinical evaluation of neurodegenerative disease, as well as those that assess clinical functions presumed to be impaired in CTE and included in provisional clinical research diagnostic criteria for CTE[42]. This included measures of depression (Center for Epidemiologic Studies Depression Scale [CES-D]), behavioral regulation (Behavior Rating Inventory of Executive Function-Adult version [BRIEF-A] Behavioral Regulation Index [BRI]), episodic memory (Neuropsychological Assessment Battery [NAB] List Learning [LL] Delayed Free Recall), and executive function (Trail Making Test Part B [TMT B]). Raw scores from the neuropsychological tests were transformed to standard scores using normative data that accounted for age, sex, and/or education. For analyses that included clinical measures, the sample size was reduced to 66 former NFL players due to exclusion of two participants for evidence of intentional symptom exaggeration; sample size for TMT B was reduced to 65 former NFL players due to missing data.

2.3. Statistical Analyses

Multivariate linear mixed-effects models examined differences between former NFL players and controls in t-tau, p-tau₁₈₁, p-tau₁₈₁/t-tau, A β ₁₋₄₂, and sTREM2. Linear mixed-effect models reduce type I error, as they account for correlations between groups and outcomes from the same participant. The models were adjusted for age and body mass index (BMI). Race was not included as a covariate in the between group analyses because only one control was African American, precluding the ability to obtain reliable estimates of the differential effects of race between the study groups. Bootstrap analysis was performed on 500 replicates to control for type I error, account for potential non-linear relationships, and increase statistical power. The between group results presented are those from the bootstrap analysis.

Additional statistical analyses were conducted only on data from the former NFL player group. Independent regression analyses examined the relationship between exposure to RHI (using the CHII) and the CSF analytes of t-tau, p-tau₁₈₁, p-tau₁₈₁/t-tau, and A β ₁₋₄₂. These analyses were adjusted for age, BMI, and race. For relationships that emerged significant, the role of sTREM2 was examined using simultaneous equations regression models. Simultaneous equations regression modeling is a form of structural equation modeling that limits endogeneity and evaluates for the presence of potential feedback loops between multiple outcomes[43]. The predictor variables included age, BMI, race, and the CHII. Outcome variables included sTREM2 and CSF t-tau, p-tau₁₈₁, p-tau₁₈₁/t-tau, and A β ₁₋₄₂; again, only the analyte(s) that demonstrated a significant relationship with the CHII in the independent regression model was (were) examined as an outcome, along with sTREM2, in the model. The model tested for direct and total effects (i.e., direct + indirect) of the predictor variables on the outcomes (i.e., the direct and total effects of age, BMI, race, and CHII on the CSF analytes), as well as for direct effects between the outcomes. In initial models, age, BMI, race, and the CHII were estimated to predict all outcome variables. To

maintain model parsimony, only pathways with a $p < 0.10$ were retained. Finally, independent regressions controlling for age, BMI, and race were used to examine the effects of t-tau, p-tau₁₈₁, p-tau₁₈₁/t-tau, A β ₁₋₄₂, and sTREM2 on each of the clinical measures, i.e., NAB LL Delayed Recall, TMT-B, BRIEF-A BRI, and CES-D.

3. RESULTS

Table 1 summarizes sample characteristics, and Table 2 provides descriptive statistics for CSF concentrations in the sample. The multivariate linear mixed-effect models showed no differences between the former NFL players and controls in t-tau ($p=0.18$), p-tau₁₈₁ ($p=0.79$), p-tau₁₈₁/t-tau ($p=0.06$), A β ₁₋₄₂ ($p=0.08$), or sTREM2 ($p=0.50$). In the former NFL players, independent regressions showed a statistically significant association between the CHII and t-tau ($p=0.041$). See Figure 1. Higher CHII scores were associated with higher levels of t-tau (Table 3). There were no effects between the CHII and p-tau₁₈₁ ($p=0.13$), p-tau₁₈₁/t-tau ($p=0.61$), A β ₁₋₄₂ ($p=0.75$), or sTREM2 ($p=0.54$).

To examine the role of microglial activation (sTREM2) in the relationship between RHI and t-tau, a simultaneous equations regression model with t-tau and sTREM2 as the outcome variables was conducted in the former NFL players. Age, BMI, race, and the CHII were included as predictors. In the initial model, the p-values for paths between age and t-tau, as well as between BMI and sTREM2 were greater than 0.10 and were removed from the final model. Based on the independent regression models (see above), the CHII was modeled to have a direct effect on t-tau. The model fit was excellent (Goodness of Fit=0.9995). All direct and total effects from the model are presented in Table 4. There was a statistically significant direct effect of sTREM2 on t-tau ($p=0.009$), such that higher concentrations of sTREM2 were associated with higher concentrations of t-tau (Figure 2). There was a statistically significant total effect for the CHII on t-tau ($p=0.030$), meaning that the relationship between the CHII and t-tau was strengthened by sTREM2.

See Table 5 for clinical test scores in the former NFL players and asymptomatic controls. Independent regressions showed that t-tau and sTREM2 were not associated with any of the cognitive or neuropsychiatric function tests ($p > 0.10$). Therefore, simultaneous equations regression models with clinical measures included as additional outcomes were not performed. However, lower A β ₁₋₄₂ was associated with worse performance on the NAB LL Delayed Recall ($\beta=0.06$, $t=2.59$, $p=0.012$). Higher levels of p-tau₁₈₁ also correlated with worse depression severity on the CES-D ($\beta=0.33$, $t=2.04$, $p=0.046$).

4. DISCUSSION

In this sample of 68 symptomatic former NFL players, greater exposure to RHI was associated with higher CSF concentrations of t-tau, but not p-tau₁₈₁, p-tau₁₈₁/t-tau, or A β ₁₋₄₂. Higher sTREM2 concentrations were associated with higher t-tau concentrations, and sTREM2 strengthened the relationship between exposure to RHI and t-tau. Although sTREM2 and t-tau were not associated with neurobehavioral test performance, lower A β ₁₋₄₂ levels were associated with worse episodic memory and higher levels of p-tau₁₈₁ correlated with greater symptoms of depression. There were no statistically significant differences in

concentrations for any of the CSF analytes between former NFL players and same-age asymptomatic controls without a history of contact sports or head trauma. Overall, this study provides evidence that exposure to RHI is associated with later life CSF t-tau concentrations in former professional football players and that microglial activation may play a role in that relationship.

Exposure to RHI and CSF t-tau and p-tau₁₈₁ Concentrations

The present association between exposure to RHI and CSF t-tau is consistent with our previous research in the DETECT sample that found a significant positive relationship between RHI and *plasma* t-tau[5]. Tau is predominantly expressed in neuronal axons and elevated CSF t-tau is hypothesized to be a marker of neurodegeneration[7,11–13]. An acute mild TBI (concussion) leads to a ‘cascade’ of neurometabolic events due, in part, to diffuse shearing and tearing of axons[44]. CSF t-tau levels have been shown to be increased acutely following concussion or contact sports play, likely representing axonal injury[45,46]. Repeated aggravation of CNS injury from RHI, which involves repeated concussive and subconcussive brain trauma with minimal recovery between head impacts[36], may limit axonal recovery to result in long-term elevations in t-tau. In general, CSF t-tau is a non-specific marker and, given the absence of neuropathological examination in this *in vivo* study, it is not known if the current relationship between RHI and CSF t-tau is indicative of long-standing t-tau elevation from RHI-associated neuronal injury from the time of play, or later-life t-tau elevation secondary to neurodegeneration from CTE. The lack of differences in t-tau between the former NFL players and controls argues against a neurodegenerative process, as does the lack of relationship between CSF t-tau and neurobehavioral test performance. However, CSF t-tau concentrations have been shown to be only mildly increased in other primary tauopathies (i.e., frontotemporal dementia) when compared to non-demented controls[47]. Overall, the clinical implications of CSF t-tau in the setting of CTE remains inconclusive.

Unlike CSF t-tau, p-tau is considered to be a specific marker of intraneuronal tau pathology. We did not find group differences in p-tau₁₈₁ and there was not a statistically significant association between RHI and p-tau₁₈₁. RHI may initiate the physiological events necessary for the eventual development of CTE, but once CTE p-tau pathology manifests, RHI may not be related to the CTE-specific p-tau pathology. Additionally, the presence of CTE neuropathology in this clinical cohort is unknown, and the severity of p-tau pathology may be too limited to result in changed CSF p-tau (or t-tau) levels in these former NFL players. Further, although CSF p-tau₁₈₁ is an established biomarker for NFT pathology in AD[7] (and FTLD-TDP[48]), p-tau₂₃₁ has been shown to be superior to p-tau₁₈₁ in the clinical and neuropathological diagnostic prediction of AD[49]. Normal CSF p-tau levels have also been reported in various patient populations with dementia, e.g., Lewy body dementia, frontotemporal dementia[50]. Each neurodegenerative tauopathy, including CTE, may be characterized by distinct p-tau isoforms or species not uniformly detected by CSF assays.

Microglial Activation and Neuronal Injury

In this sample of former NFL players, increased microglial activation (i.e., sTREM2) was associated with t-tau. Increased microglial activation has been found in living and deceased,

active and retired, tackle football players[18–21] and is associated with the development of dementia[18]. Remote exposure to RHI was not directly associated with current microglial activation (i.e., sTREM2) in this sample of NFL players who stopped playing football years ago. RHI may initiate microglial activation (as suggested by recent experimental evidence[51]), but this activation may diminish after exposure to RHI has ended, and then increase again when neurodegeneration begins. This is similar to research in AD, which suggests that sTREM2 levels may be dependent on disease stage[28,29,33,35]. That being said, evidence from research among deceased football players with neuropathologically-confirmed CTE suggests that RHI from tackle football may lead to chronic microglial activation, leading to the formation of p-tau as NFTs[18,19]. Mouse models of RHI and CTE have further linked microglial activation with p-tau pathology[52–56], and also found that an induced head impact resulted in microgliosis, as measured by TREM2, and phosphorylated tauopathy[51]. Microglial activation may thus contribute to the pathogenesis of CTE.

Because the current study is cross-sectional, it is plausible that there is a bidirectional relationship between CSF t-tau and sTREM2. Cherry et al. found a bidirectional relationship between p-tau pathology and microglial activation in their sample of 66 deceased tackle football players with CTE[18]. Once neuronal injury from RHI or CTE begins, an additional microglia response may be initiated that could further promote neuronal injury. Regardless of when microglial activation begins (or what causes it), chronic microglial activation can be detrimental due to prolonged exposure to inflammatory cytokines that can result in neuronal toxicity and eventual neurodegeneration[54–58]. Indeed, increased sTREM2 levels strengthened the effect of RHI on t-tau in this sample of former NFL players, and the presence of microglial activation may modify the relationship between RHI and later-life neurological outcomes. Longitudinal studies that follow former football players throughout their life are needed to elucidate directionality in the relationships among RHI, sTREM2, and t-tau.

Group Differences and Clinical Function

The former NFL players exhibited statistically significant worse clinical test performance than the asymptomatic controls, with the exception of a statistical trend for Trail Making Test Part B. This is likely a manifestation of sample selection as the controls were required to be asymptomatic at the time of telephone screening whereas the former NFL players were required to have self-reported complaints of cognitive, behavior, or mood disturbances. The normative T-scores (and the established cut score of 16 for the CES-D) are a more accurate reflection of the clinical status in this sample of former NFL players. On average, the former NFL players reported clinically meaningful symptoms of depression (based on the average CES-D score of 21). In contrast, scores on the other neuropsychiatric measures and performance on the cognitive tests were all within normal limits (based on normative T-scores).

Although there were no between-group differences in $A\beta_{1-42}$, lower $A\beta_{1-42}$ levels were associated with worse episodic memory in the former NFL players, and RHI exposure was not associated with $A\beta_{1-42}$. $A\beta$ plaques are not a diagnostic neuropathological feature of CTE and, if present, the plaques are often sparse and diffuse, strongly associated with older

age, and accelerate the clinical and pathological course of CTE[3,4,10]. The relationship between TBI and A β accumulation has been examined by several studies using transgenic mice susceptible to AD pathology (due to mutations of the A β precursor protein) subjected to controlled cortical impact[59–61]. Some of these studies provide evidence for reduced or no accumulation of A β months after the TBI[59,60]. Interestingly, however, other evidence in transgenic mice with mutations of the amyloid precursor protein suggests that repetitive mild TBI may accelerate A β deposition[62], and this is similar to what has been observed in CTE[10]. Future research on A β _{1–42} deposition in the setting of CTE is needed.

The current study did not find group differences for p-tau₁₈₁ and t-tau (as discussed above), or sTREM2. Total tau and sTREM2 were not associated with clinical function, and higher p-tau₁₈₁ levels were *only* associated with greater symptoms of depression (a core clinical feature of CTE)[42]. Again, the presence or severity of CTE pathology in this sample is unknown and there is likely substantial variability across participants in pathology. P-tau₁₈₁ burden and/or neuronal injury may be below clinical threshold (as evidence by the clinical test performance in the former NFL players) or only a small subset have meaningful pathology[5]. The disease stage of the sample (which is unknown) may have particularly important implications for sTREM2. For example, when disease stage is considered, sTREM2 levels are higher in the early stages of the AD continuum, perhaps even before symptom onset[28,29,33,35]. Interestingly, microglial activation seems to have opposing effects on A β and p-tau in AD, as it may facilitate clearance of A β [63–65] and promote p-tau pathogenesis[54–56,58].

Limitations

sTREM2 is believed to reflect microglial activation, but the exact biological role(s) of sTREM2 remains unclear[24]. There are multiple *TREM2* variants that influence ligand binding and each variant may play a unique role in response to pathology and to the different types of pathologies. Other CSF analytes of neuroinflammation (e.g., CCL11, CD68) not examined in this study could provide additional insight into the dynamic role of neuroinflammation in CTE. In this sample of former NFL players, college level estimates of head impact frequencies were applied for estimation of professional level head impact frequencies because helmet accelerometer data at the professional level have not been published or made public. These methods likely underestimated the extent of RHI in the former NFL players and its relationship with the CSF analytes.

Controls were required to be asymptomatic and without a history of head trauma at the time of telephone screening, thereby limiting the external validity of analyses examining group differences (namely in regards to clinical test performance). The objective of the participant selection method was to obtain a control sample at very low risk for CTE to compare to the former NFL players presumably at high risk for CTE, to facilitate biomarker development. Despite these efforts, the controls had comparable—and even non-significantly higher absolute values of—CSF analyte concentrations compared to former NFL players. The control group was small and demographically homogenous (all but one of the controls were Caucasian). The race disparity between the groups is noteworthy given that in the former NFL players, Caucasian race and older age were associated with higher sTREM2; race

further correlated with the p-tau/t-tau ratio and, in the full model, older age correlated with higher CSF t-tau. Because African American race is associated with increased risk for AD, it would have been expected that African Americans have higher sTREM2. There appears to be different *TREM2* coding variants that confer risk for AD in African Americans versus Caucasians[66]. In general, race is becoming increasingly recognized as a potential modifier of clinical outcomes related to concussion[67] and exposure to RHI[41]. Future research that recruits larger and demographically-and clinically-diverse (e.g., symptomatic and asymptomatic) controls, in addition to other contact and non-contact sport athletes, is needed to elucidate CSF tau and sTREM2 concentrations in former NFL players.

It is premature to state that the current findings do or do not provide support for any of the CSF analytes examined as biomarkers for CTE. The development of meaningful fluid biomarkers requires several critical steps[68]. Foremost, this study was without neuropathological examination and the presence of a competing neurodegenerative disease, such as AD, is possible. Biomarker development and validation for CTE will occur through studies that prospectively collect biomarker data (e.g., CSF tau, sTREM2) in living participants who agree to brain donation and then examine the association with post-mortem CTE neuropathology. Such prospective data is unlikely to be available in the near future. In the meantime, PET tau and amyloid imaging may clarify the biomarker utility of CSF t-tau, p-tau, A β , and sTREM2 in the setting of CTE. Studies examining biomarker development and validation in CTE using PET tau and amyloid imaging are currently underway.

CONCLUSIONS

In this sample of former NFL players, estimated cumulative exposure to RHI and microglial activation (based on CSF sTREM2) were associated with later-life CSF t-tau concentrations. Microglial activation may be involved in the pathogenesis of long-term neurological consequences associated with exposure to RHI, like CTE. Prospective clinicopathological research, in addition to studies that use PET tau and amyloid imaging are needed to elucidate the clinical and diagnostic implications of CSF t-tau, p-tau₁₈₁, and sTREM2 in the setting of CTE.

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Abbreviations

CSF	cerebrospinal fluid
CTE	chronic traumatic encephalopathy
AD	Alzheimer's disease

RHI	repetitive head impacts
t-tau	total tau
p-tau	phosphorylated tau
Aβ	beta-amyloid
sTREM2	soluble triggering receptor expressed on myeloid cells 2

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HIGHLIGHTS

- Repetitive head impacts (RHI) were positively associated with CSF total tau in former NFL players
- sTREM2 was positively associated with CSF total tau in former NFL players
- sTREM2 strengthened the relationship between exposure to RHI and CSF total tau

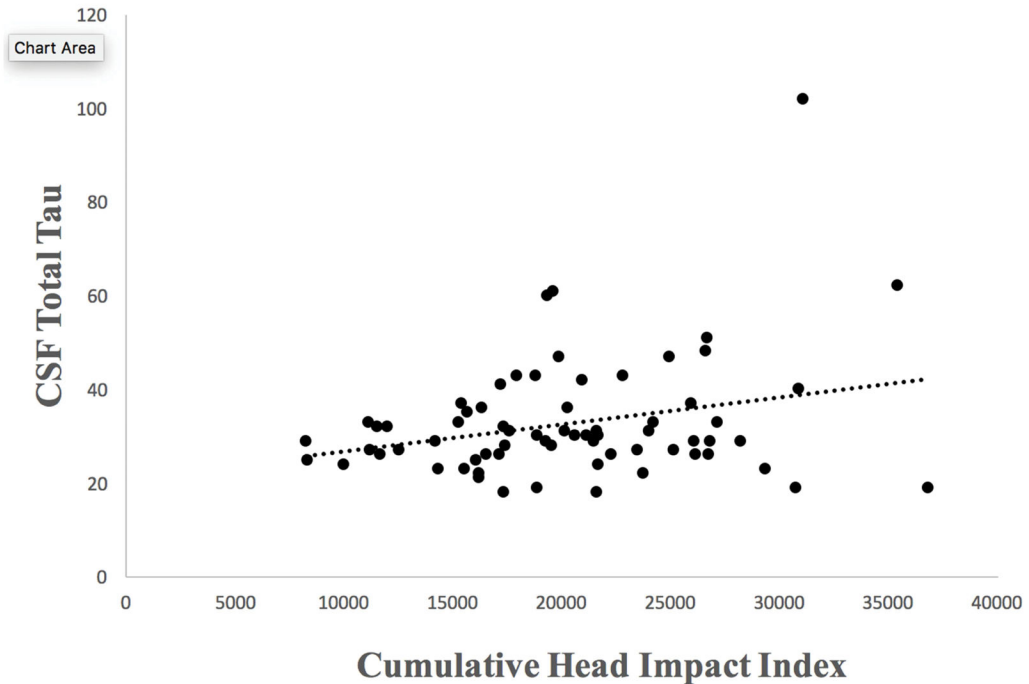


Figure 1. Relationship Between Exposure to Repetitive Head Impacts and CSF Total Tau in Former National Football League Players. As shown in Table 3, independent regressions showed a statistically significant association between the cumulative head impact index and t-tau after controlling for age, BMI, and race ($p = 0.041$). Figure 1 shows the unadjusted relationship between the cumulative head impact index and CSF total tau, which was also statistically significant: $r = 0.27$, $p = 0.024$.

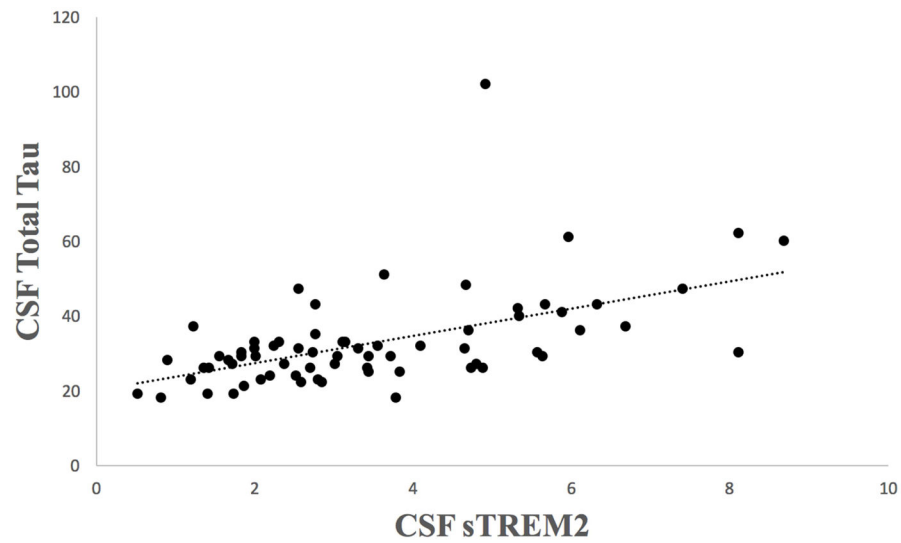


Figure 2. Relationship Between Exposure to CSF TREM2 and CSF Total Tau in Former National Football League Players. Figure shows a scatter plot of the relationship between CSF sTREM2 and total tau. Bivariate correlations showed this to be a statistically significant relationship: $r = 0.54$, $p < 0.001$.

Table 1

Sample Characteristics

	NFL (n = 68)	Controls (n = 21)	p-value
Demographic/Athletic			
Age, mean (SD) years	54.50 (7.95)	57.57 (7.10)	0.12
Education, mean (SD) years	16.44 (0.97)	17.38 (2.22)	0.07
^a African American, n (%)	29 (42.65)	1 (4.76)	0.001
Duration of football play, mean (SD) years	18.67 (3.56)	--	--
Years in the NFL, mean (SD)	8.30 (2.89)	--	--
Cumulative Head Impact Index, mean (SD)	20,304.87 (6195.14)	--	--
Primary Position Group, n (%)			--
Offensive line	20 (29.41)	--	--
Running back	5 (7.35)	--	--
Tight end	5 (7.35)	--	--
Offensive skill	0	--	--
Defensive line	12 (17.65)	--	--
Linebacker	14 (20.59)	--	--
Defensive Back	12 (17.65)	--	--
Body mass index, mean (SD) kg/m ²	32.67 (4.43)	28.36 (3.84)	<0.001

The control group was required to be asymptomatic and have no history of head trauma.

^aIndependent sample t-tests were performed for continuous outcomes and Fisher's Exact Test was used to compare group differences in race.

Table 2

Cerebrospinal Fluid Biomarker Concentrations

	t-tau (pg/ml)	p-tau ₁₈₁ (pg/ml)	Aβ ₁₋₄₂ (pg/ml)	p-tau ₁₈₁ /t-tau Ratio	sTREM2 (ng/ml)
NFL Players (n = 68)					
Median	29.50	17.00	362.00	0.57	3.03
Mean (SD)	32.81 (12.97)	18.34 (9.46)	362.75 (8.73)	0.59 (0.27)	3.50 (1.91)
95% CI	29.67, 35.95	16.05, 20.63	345.32, 380.18	0.52, 0.65	3.03, 3.96
Controls (n = 21)					
Median	36.00	22.00	425.00	0.69	4.11
Mean (SD)	36.57 (14.12)	22.62 (9.51)	409.05 (78.74)	0.66 (0.24)	4.50 (1.74)
95% CI	30.15, 43.00	18.29, 26.95	373.20, 444.89	0.55, 0.76	3.71, 5.30

Abbreviations: t-tau = total tau, p-tau₁₈₁ = hyperphosphorylated tau, Aβ = beta-amyloid.

Summary of Linear Regression Examining the Direct Effects of Exposure to Repetitive Head Impacts on Cerebrospinal Fluid Markers of Tau, Amyloid, and sTREM2

Table 3

	t-tau			p-tau ₁₈₁			p-tau ₁₈₁ /t-tau			Aβ ₁₋₄₂			sTREM2		
	beta	SE	P-value	beta	SE	P-value	beta	SE	P-value	beta	SE	P-value	beta	SE	P-value
Age	0.36	0.19	0.056	0.09	0.15	0.552	-0.003	0.004	0.423	-0.49	1.17	0.68	0.08	0.03	0.002
BMI	-0.73	0.38	0.055	-0.23	0.31	0.461	0.003	0.01	0.684	0.08	2.36	0.973	-0.04	0.05	0.388
Race	-3.28	3.37	0.333	4.01	2.79	0.154	0.198	0.07	0.009	-35.51	21.11	0.098	-1.35	0.46	0.004
CHII	0.54	0.26	0.041	0.33	0.22	0.127	0.003	0.01	0.61	0.52	1.64	0.750	0.02	0.04	0.541

Abbreviations: t-tau = total tau, p-tau₁₈₁ = hyperphosphorylated tau, Aβ = beta-amyloid, BMI = body mass index, CHII = cumulative head impact index, SE = standard error. Beta is unstandardized and bolded values highlight statistical significance. For race, 1 = African American and 0 = Caucasian.

Table 4
 Summary of Direct and Total Effects from Simultaneous Equations Regression Model Examining sTREM2 and Total Tau as Outcomes

	Standardized Direct Effects						Standardized Total Effects									
	sTREM2			t-tau			sTREM2			t-tau						
	Beta	SE	t	P	Beta	SE	t	P	Beta	SE	t	P				
Age	0.28	0.12	2.43	0.0149	--	--	--	--	0.35	0.10	3.59	0.0003	0.18	0.08	2.14	0.0323
BMI	--	--	--	--	-0.20	0.12	-1.65	0.10	-0.09	0.10	-0.88	0.38	-0.24	0.12	-1.97	0.0489
Race	-0.28	0.14	-2.02	0.0432	--	--	--	--	-0.35	0.11	-3.15	0.0016	-0.18	0.09	-2.04	0.0411
CHII	--	--	--	--	0.20	0.11	1.80	0.07	0.09	0.10	0.90	0.37	0.25	0.12	2.17	0.0300
sTREM2	--	--	--	--	0.52	0.20	2.61	0.0091	--	--	--	--	0.64	0.37	1.74	0.08
t-tau	0.36	0.36	0.99	0.32	--	--	--	--	0.44	0.55	0.80	0.43	--	--	--	--

Note. In the initial model, the p-values for paths between age and t-tau, as well as between BMI and sTREM2 were greater than 0.10 and were therefore removed from the final model. Based on independent regression, the CHII was only estimated to have a direct effect on t-tau. BMI = body mass index, CHII = cumulative head impact index, t-tau = total tau, SE = standard error. Beta is standardized and bolded values highlight statistical significance. For race, 1 = African American and 0 = Caucasian.

Table 5
Cognitive and Neuropsychiatric Test Performance in the Former NFL Players and Controls

	Former NFL Players (n = 65–66)*	Controls (n = 21)	P-value
Cognitive Function, mean (SD) T-score			
NAB List Learning Delayed Recall	41.92 (13.77)	50.38 (12.64)	0.0415
Trail Making Test Part B	45.12 (15.01)	52.14 (15.45)	0.1076
Neuropsychiatric Status			
CES-D, mean (SD) raw score	21.14 (13.76)	3.71 (4.15)	<0.0001
BRIEF-A BRI, mean (SD) T-score	62.62 (11.49)	47.24 (9.43)	<0.0001

Note. The control group was required to be asymptomatic and have no history of head trauma at the time of recruitment. Mixed-effect models controlling for age and body mass index compared the former NFL players and controls on each of the clinical measures.

* The sample size was reduced to 66 former NFL players due to exclusion of two participants for evidence of intentional symptom exaggeration; sample size for TMT B was further reduced to 65 former NFL players due to missing data.

Abbreviations: NAB: Neuropsychological Assessment Battery; CES-D: Center for Epidemiologic Studies Depression Scale; BRIEF-A: Behavior Rating Inventory of Executive Function-Adult version; BRI: Behavioral Regulation Index