JOURNAL OF NEUROTRAUMA 35:2351–2356 (October 15, 2018)

DOI: 10.1089/neu.2017.5553

Longitudinal Performance of Plasma Neurofilament Light and Tau in Professional Fighters: The Professional Fighters Brain Health Study

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Abstract

The aim of this study is to evaluate longitudinal change in plasma neurofilament light (NF-L) and tau levels in relationship to clinical and radiological measures in professional fighters. Participants (active and retired professional fighters and control group) underwent annual blood sampling, 3-Tesla magnetic resonance imaging (MRI) brain imaging, computerized cognitive testing, and assessment of exposure to traumatic brain injury. Plasma tau and NF-L concentrations were measured using Simoa assays. Multiple linear regression models were used to compare the difference across groups in regard to baseline measurements, whereas mixed linear models was used for the longitudinal data with multiple measurements for each participant. Plasma samples were available on 471 participants. Baseline NF-L measures differed across groups ($F_{3,393} = 6.99$; $p = 0.0001$), with the active boxers having the highest levels. Higher NF-L levels at baseline were correlated with lower baseline MRI regional volumes and lower cognitive scores. The number of sparring rounds completed by the active fighters was correlated with NF-L (95% confidence interval, 0.0116–0.4053; $p = 0.0381$), but not tau, levels. Among 126 subjects having multiple yearly samples, there was a significant difference in average yearly percentage change in tau across groups ($F_{3,83} = 3.87$; $p = 0.0121$). We conclude that plasma NF-L and tau behave differently in a group of active and retired fighters; NF-L better reflects acute exposure whereas the role of plasma tau levels in signifying chronic change in brain structure over time requires further study.

Keywords: mild traumatic brain injury; neurofilament light; tau

Introduction

Repetitive head impacts (RHIs) can result in long-term neurological injury and are a risk factor for chronic traumatic encephalopathy (CTE).[1,2] A vulnerable neural element to RHI is the axon.[2] Neurofilament light (NF-L) and tau are two well-established biochemical markers of axonal injury.[3] Both NF-L and tau levels have been reported to rise after concussion.[4–6] However, more chronic elevations of NF-L levels have been reported in individuals with prolonged post-concussive symptoms and increased tau levels in those with self-reported history of mild traumatic brain injury (TBI).[7,8]

The use of these markers to follow or predict progressive neurological injury has not been investigated as thoroughly. In cross-sectional studies, higher levels of NF-L have been associated with several neurodegenerative conditions, including progressive supranuclear palsy, amyotrophic lateral sclerosis, and Alzheimer's disease.[9–11] Moreover, although tau deposits in the form of neurofibrillary tangles are considered the pathological hallmark of CTE, there are no known peripheral indicators of brain tau accumulation in the blood of those at increased risk of CTE.

There has yet to be any published reports of NF-L and tau levels in blood or cerebrospinal fluid (CSF) over multiple years in those who have been exposed to RHI. Utilizing a well-characterized cohort of professional fighters, both active and retired, from the Professional Fighters Brain Health Study (PFBHS), we explore the longitudinal performance of plasma NF-L and tau and their relationship to clinical and radiological evidence of impairment.

Methods

The PFBHS is a cohort study of active and retired professional fighters (boxers and mixed martial artists [MMA]), along with age-

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and education-matched controls. Active fighters were required to have at least one professional fight within 2 years of enrollment and be training with the intent to compete; information about the study was disseminated by the Nevada Athletic Commission, fight promoters, and local training facilities. Retired fighters were included if they had been boxers, had a minimum of 10 professional fights, no sanctioned fights for at least 2 years, and did not intend to return to competition (there were too few retired MMA fighters to include as a separate group). Control subjects were recruited from outreach efforts in the community and could not have any past history of neurological disorders, TBI, military service, or participation at a high school level or higher in a combat sport or a sport in which TBI can be anticipated to occur, such as football, wrestling, hockey, rugby, soccer, or rodeo. Enrollment in the PFBHS began in 2011 and has been continuous since then. Each participant is seen on an annual basis, and for active fighters, not sooner than 45 days from a sanctioned fight. The PFBHS was approved by the Cleveland Clinic Institutional Review Board, and written informed consent was obtained from all participants. Methods of recruitment and study procedures have been described previously.[12]

At each visit, blood sampling is obtained, along with a battery of other tests including magnetic resonance imaging (MRI) brain imaging, computerized cognitive testing, and exposure information. At the baseline visit, participants answer questionnaires with the assistance of the study coordinator that collect information on demographics; educational attainment; medical history including concurrent illnesses and prescribed medications; previous TBI, both related and unrelated to athletic activities; and previous involvement in other contacts sports. Number of professional fights was ascertained by review of commonly recognized databases (boxrec.com for boxers, sherdog.com for MMA fighters). Information on the amount of sparring the participant has engaged in, as well as whether there has been any concussions or TBIs within the 2 weeks preceding the study visit is obtained through self-report.

Cognitive function was assessed by a computer-based battery that consists of four subtests of the CNS Vital Signs (CNS Vital Signs LLC, Morrisville, NC), including verbal memory, symbol digit coding, Stroop, and a finger-tapping test. CNS Vital Signs offers robust and reliable measurements of cognition, which are computerized, but are supervised by a technician.[13] Results from these tests are used to make up scores in various clinical domains: verbal memory, processing speed, psychomotor speed, and reaction time.

A high-resolution T1-weighted anatomical MRI was obtained on all fighters at each visit. A 3-Tesla MRI unit (Verio; Siemens, Munich, Germany) with a 32-channel head coil was used to acquire structural three-dimensional T1-weighted magnetization prepared rapid acquisition gradient echo images (repetition time msec/echo time msec, 2300/2.98; resolution, $1 \times 1 \times 1.2\ mm^3$).[14,15] Volumes of the hippocampus and amygdala and subcortical gray matter, including thalamus, caudate, and putamen, along with corpus callosum, were calculated using the automated full brain segmentation process in FreeSurfer software (version 5.3.0; http://surfer.nmr.mgh.harvard.edu). These regions have been shown in pathological series and our previous work to be effected in those with extensive RHI.[1,14] The volumes of each structure were measured in both hemispheres separately and adjusted for total intracranial volume.

The blood samples were collected in ethylenediaminetetraacetic acid tubes and centrifuged at 3200 rpm for 10 min to separate plasma from blood cells. The supernatant was aliquoted in 2-mL portions that were immediately frozen and stored at −80°C pending analysis. Plasma tau and NF-L concentrations were measured using ultrasensitive single-molecule array (SiMoA) assays as previously described in detail.[16,17] For NF-L, two quality control (QC) levels were run in duplicates in the beginning and the end of each run. For QC with concentration 11.8 pg/mL, repeatability was 8.1% and intermediate precision was 14.7%. For QC with concentration 108.4 pg/mL, repeatability was 7.0% and intermediate precision was 13.5%. For Tau, two QC levels were run in duplicates in the beginning and the end of each run. For QC with concentration 2.5 pg/mL, repeatability was 9.0% and intermediate precision was 10.1%. For QC with concentration 28.7 pg/mL, repeatability was 7.9% and intermediate precision was 9.9%. The lower limits of quantification for tau and NF-L were 1.22 and 6.7 pg/mL, respectively.

All analyses were performed by board-certified laboratory technicians who were blinded to clinical data.

Statistical analysis

Descriptive statistics were computed for continuous outcomes (e.g., age) with mean and standard deviation (SD) and dichotomized outcomes (e.g., sex) with proportions. Multiple linear regression models were used to compare the difference across groups with regard to the baseline measurement after controlling for the confounding factors in the study: age, race, and education. Total intracranial volume was additionally controlled for volumetric analysis. For the post-hoc test to compare the pair-wise group differences, the Tukey-Kramer approach was used to adjust the multiple comparisons. For the longitudinal data with multiple measurements for each participant, a mixed linear model was used in this repeated-measures design. This model is able to capture the dependence of the measurements from the same participant. The model included the main effects of group and time and the confounding factors as aforementioned. All tests were two-sided at the significant level of 0.05. SAS statistical software (Version 9.4; SAS Institute Inc., Cary, NC) was used for data analysis.

Results

Plasma samples were available on 471 participants, with 126 having two or more measurements spanning an average of 1.64 years (range, 1–5). The characteristics of the study group are described in Table 1. Baseline results reflect the entire group of participants, whereas the longitudinal results only include those with two or more measurements.

Baseline NF-L or Tau measures across the four study groups were compared by using the multiple linear regression model, with group as the primary variable of interest while controlling for other covariates: age and race. For the post-hoc test to compare the pair-wise group differences, the Tukey-Kramer approach was used to adjust the multiple comparisons for the p value and the confidence interval (CI).[18] There was a significant difference in baseline NF-L measures across groups ($F_{3,393} = 6.99$; $p = 0.0001$), with the active boxer fighters having higher levels than the active MMA fighters (95% CI, 2.27–12.85; $p = 0.0015$) and controls (95% CI, 3.91–16.87; $p = 0.0002$; Fig. 1A). Mean baseline NF-L levels for the active boxers was 21.55 pg/mL (standard error [SE] = 1.85), active MMA 14.58 pg/mL (SE = 0.86), retired fighters 15.12 pg/mL (SE = 3.30), and controls 11.27 pg/mL (SE = 1.40). Tau levels at baseline were slightly higher in active fighters than controls, but did not reach statistical significance (Fig. 1B).

There was a significant relationship between the number of sparring rounds completed by the active fighters within the 2 weeks before drawing the plasma sample with NF-L (95% CI, 0.0116–0.4053; $p = 0.0381$), but not tau, after controlling for age and race. This was particularly prominent in the active boxers (95% CI, 0.0696–0.8003; $p = 0.0206$).

Similarly, higher levels of baseline NF-L were associated with lower baseline volumes of thalamus, hippocampus, and central and posterior corpus callosum on MRI imaging, after controlling for age, race, education, number of fights, and total intracranial volume

Table 1. Characteristics of Study Cohort

	Boxer ret	*Boxer act*	*MMA act*	*Control*
Male	50 (96%)	110 (94%)	152 (90%)	69 (87%)
Female	2 (4%)	7 (6%)	17 (10%)	10 (13%)
Education	13.00 (2.60)	12.92 (2.42)	14.24 (2.46)	13.63 (2.60)
Age, years	48.00 (10.26)	30.38 (6.86)	29.59 (4.77)	30.78 (10.01)
Ethnicity				
African American	13 (25%)	32 (27%)	42 (25%)	7 (9%)
White	34 (65%)	68 (58%)	105 (63%)	59 (76%)
Asian	1 (2%)	4 (3%)	6 (4%)	5 (6%)
Other	4 (8%)	13 (11%)	14 (8%)	7 (9%)
No. fights	38.15 (20.23)	14.66 (12.86)	12.53 (12.29)	0
Years of fights	11.70 (5.27)	5.94 (4.52)	5.61 (4.22)	0

Characteristics of the study cohort including retired boxers (Boxer ret), retired MMA fighters (MMA ret), active boxers (Boxer act), active MMA fighters (MMA act), and control subjects.

MMAQ, mixed martial arts.

(Table 2). Higher baseline NF-L levels were associated with lower baseline performance in the domains of psychomotor speed ($r=-0.1219$; $p=0.0203$) and processing speed ($r=-0.1097$; $p=0.0378$) on computerized cognitive testing after controlling from age, race, education, and number of fights. However, no correlation was noted between baseline tau level and performance on baseline cognitive testing.

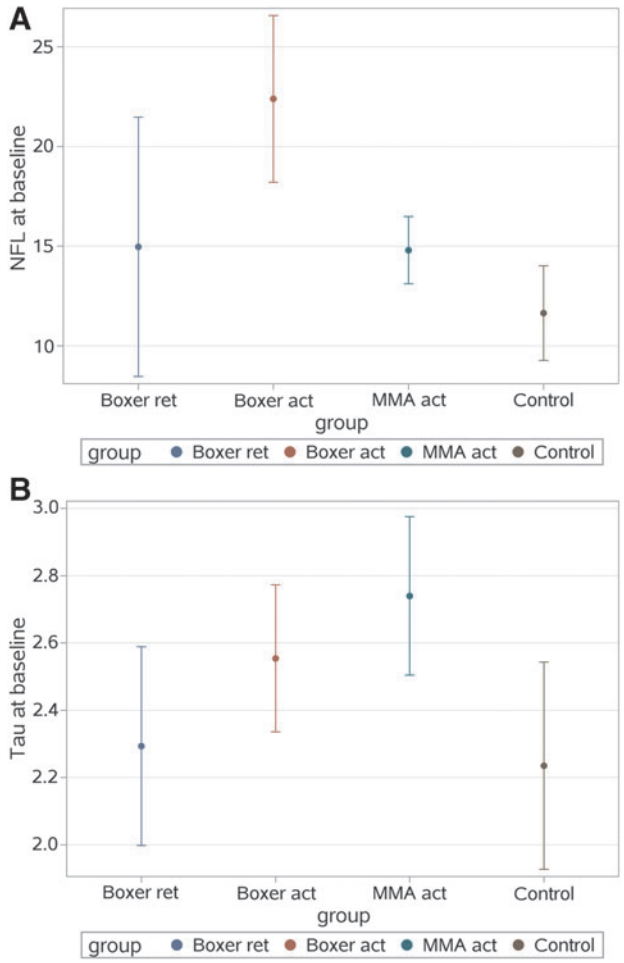

FIG. 1. Baseline NF-L and tau levels. Baseline (**A**) neurofilament light and (**B**) tau levels (pg/mL) among retired fighters, active boxers, active mma fighters and controls (mean and 1 standard error of mean). act, active; MMA, mixed martial arts; NFL, neurofilament light.

A multiple linear model was used to assess the average yearly percentage change of tau or NF-L among the four groups, by controlling for age, race, education, and baseline measurement. Tau and NF-L levels remained relatively constant over 2 years in the controls and retired fighters; in the control subjects with at least two measurements, the average change between baseline and last measurement was 0.30 pg/mL (−1.38, −0.84). The active MMA fighters as a group showed a rise in tau over time, which was not observed as prominently with the boxers (Fig. 2). There was a significant difference in average yearly percentage change in tau across groups ($F_{3,83}=3.87$; $p=0.0121$), with the active MMA fighters having greater increases in tau levels than controls (95% CI, 0.0811–1.0369; $p=0.0152$). However, there was no relationship between increasing tau levels and change in either MRI volumetric measures or performance on cognitive testing.

Discussion

There are currently no validated biomarkers that are known to reliably reflect underlying neuronal injury from RHIs. One potential method would be to measure a constituent that is released from damaged neurons in the brain and make its way into the blood.

Because of advances in ultrasensitive assays, it is now possible to detect brain-derived substances in blood; literature is accumulating

Table 2. Baseline NF-L and Regional Volumes

MRI regional volumes	*NF-L at baseline*		*Tau at baseline*	
	Partial correlation	p *value*	*Partial correlation*	p *value*
left_thalamus_proper	−0.153	0.005	0.014	0.805
right_thalamus_proper	−0.138	0.012	0.006	0.908
left_hippocampus	−0.125	0.022	0.002	0.978
right_hippocampus	−0.115	0.036	−0.053	0.333
cc_central	−0.109	0.047	−0.029	0.592
cc_posterior	−0.110	0.045	−0.053	0.331

Correlations between baseline neurofilament light and tau levels with baseline MRI-based regional volumes adjusted for age, education, and number of professional fights. cc central = central corpus callosum; cc posterior = posterior corpus callosum.

NF-L, neurofilament light; MRI, magnetic resonance imaging.

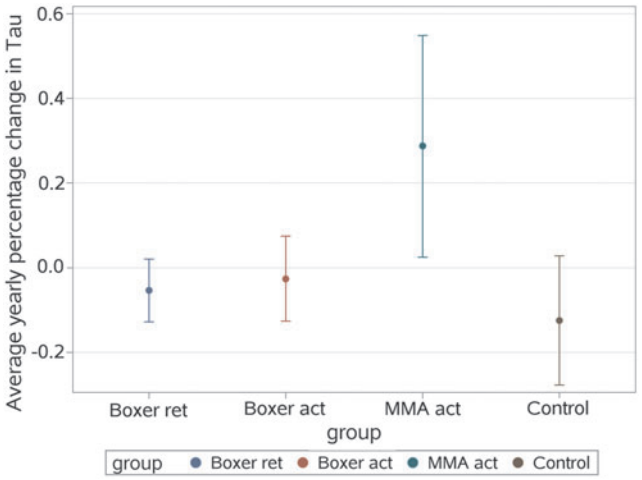

FIG. 2. Longitudinal tau levels. Average yearly percentage change in plasma tau levels (pg/mL) among controls, retired fighters, active MMA fighters, active boxers (mean and 1 standard error of mean). act, active; MMA, mixed martial arts.

on two markers of axonal injury, NF-L and tau.[3,4,6–8] However, previous reports have evaluated these markers either in a cross-sectional manner or pre- and post-concussion.

From a longitudinal cohort study of active and retired professional fighters, we now report the behavior of plasma tau and NF-L over multiple years. In individuals no longer, or never, exposed to RHIs, both of these markers remain relatively stable over several years. On the other hand, active fighters have elevated NF-L levels, possibly related to acute exposure to RHIs, as suggested by the correlation between the amount of sparring done in close proximity to when the plasma sample was obtained. Tau levels were not correlated to amounts of sparring, and were found to increase primarily in MMA fighters, over a few years.

Before discussing how our findings fit into the current body of literature on NF-L and tau, it is important to note the characteristics of the cohort. The active professional fighters in the study are exposed to RHIs to variable degrees depending on the amount and intensity of sparring they are doing with others and in preparation for a fight; the amount of activity for each individual fighter may vary from year to year. Further, active boxers and MMA fighters, while both exposed to RHI, likely differ in the amounts and type of RHI. Thus, although we record self-reports of sparring amounts over the time between annual visits and within 2 weeks of blood sampling, as well as verify the number of professional fights they had, we are unable to ascertain the absolute amount of RHIs a participant is exposed to. The retired professional fighters in the cohort have, by criteria, had a minimum of 10 professional fights and not had any sparring or fights for at least 2 years. Many of the retired fighters had numerous professional fights and are quite symptomatic, with 28% scoring at least 2 SDs below average for their age on baseline cognitive testing. However, there is no way to know what, if any, neuropathological process they may have.

We, like others, found that NF-L and tau levels obtained cross-sectionally were, as a group, higher in active fighters than controls.[6] Whereas other studies have reported long-term elevations in plasma tau in individuals exposed to RHI, our retired fighters had measurements that were very similar to controls.[7]

The observation that NF-L, but not tau, levels at baseline were strongly correlated with the amount of sparring the participant reported in the 2 weeks preceding sampling underscores the differences in these two markers. NF-L is highly expressed in large-caliber myelinated axons of the white matter.[3,19,20] Tau is a central nervous system (CNS)-enriched protein with greater expression in unmyelinated cortical axons.[21,22] Blood NF-L levels have been shown to correlate better with CSF concentrations than tau.[6,23,24] Previous studies in amateur boxers have reported relatively higher increases in CSF levels of NF-L than tau, with only NF-L levels remaining elevated over a 14-day rest period.[5] In addition, over a season of collegiate football, there was no significant change observed in plasma tau levels in a small group of players sampled serially.[25] Thus, plasma measures of NF-L may be more sensitive than tau to detect damage associated with recent repetitive subconcussive impacts. In addition, the strong relationships between baseline NF-L levels and various subcortical regional volumes may reflect accumulated chronic injury to white matter tracts and resultant inflammation or Wallerian degeneration of the structures they innervate.[26]

The role of blood biomarkers to monitor cumulative neuronal brain injury over time has not been previously examined; the results of this study shed some initial light on the subject. Plasma tau and NF-L are generally stable in not only individuals never exposed to RHI, but also in our retired boxers, many of whom had extensive exposure in the past. And because a significant number of retired boxers were expressing symptoms and signs of cognitive, mood, and motoric impairment, it could be speculated that these plasma markers may not be very useful in identifying individuals that may be harboring CTE pathology (though post-mortem diagnostic confirmation would be needed). Other forms of tau may perform differently for longitudinal monitoring of trauma-related brain disorders. Aggregation of hyperphosphorylated tau (P-tau) in cortical areas is a characteristic of CTE; a recent report indicated the P-tau and P-tau/total tau ratio outperformed total tau levels as a diagnostic marker in acute TBI, as well as showing sustained elevations up to 176 days post-injury.[(27)] In addition, exosomal tau measurement in plasma has been suggested as a potential biomarker for CTE,[(28)] but these results need validation in independent cohorts.

On the other hand, a number of active professional fighters showed an increase in tau levels over time. The significance of this finding is uncertain; one could speculate that there may be a window of time in those exposed to RHI where there is either increased expression or reduced clearance of tau occurring in the brain. Whether these individuals are at higher risk of developing a progressive neurodegenerative condition down the line is uncertain and awaits longitudinal follow-up of this group.

Longitudinal measures of both NF-L and tau levels were not associated with cognitive performance in the active fighters or the number of professional fights they had. It may be that the computerized cognitive tests utilized in the study were not sensitive enough to detect what, at best, may be small changes in a young, healthy cohort. Moreover, simply considering number of fights may not adequately capture the amount of subconcussive exposure each fighter is sustaining while training. Finally, why MMA fighters were more likely than boxers to show longitudinal elevations of tau is unclear. If anything, boxers generally are on the receiving end of more punches than MMA fighters. Further exploration of other potential factors that differentiate the boxers and MMA fighters is needed to explain this finding.

The strength of this study is the relatively large number of well-characterized subjects that are, or have been, exposed to numerous RHIs. Moreover, this is the first study, to our knowledge, to measure plasma tau and NF-L serially over a period of years. On the

other hand, several limitations need to be mentioned. For one, we cannot be certain that all of the tau and NF-L we measure in plasma is from brain origin. Tau can be detected in the liver, kidney, and testis.[29] One could imagine that fighters, particularly MMA fighters that often sustain blows to the unprotected body by punches and kicks, could sustain blunt trauma to these organs. However, samples were not obtained close to a sanctioned competition. In addition, a previous report showed that plasma tau did not increase on participation in an ice hockey game without incident concussions.[4] NF-L is also expressed in peripheral nerves, although the robust correlation of plasma with CSF NF-L concentration observed in numerous studies suggests that most NF-L in plasma is CNS derived.[30,31] Further, blood NF-L levels correlate with severity of TBIs during a bout in amateur boxers.[6] Another limitation is that we cannot be certain about the precise amount of exposure to RHI each active fighter experienced; sparring amounts preceding blood sampling are self-reported, and the intensity of sparring is variable between subjects. Finally, given that the cohort in the PFBHS and, more specifically, those for whom longitudinal blood sampling was obtained is not a random sample, it is possible that they may differ from the general population of fighters in some yet to be determined way. Because we have rolling enrollment in the study, and given the transient nature of active fighters, many participants have yet to return for follow-up visits.

In conclusion, plasma NF-L and tau levels have slightly different behaviors in a group of active and retired fighters. Whereas both measures are relatively stable over time in individuals not actively exposed to TBI, NF-L may better reflect the neural effects of acute exposure. Further longitudinal follow-up is needed to understand the implications of increasing plasma tau levels over time.

Author Disclosure Statement

Charles Bernick receives funding support for the Professional Fighters Brain Health Study from UFC, Bellator/Spike TV, Haymon Boxing, Top Rank Promotions, and UCLA Dream fund; he has been a speaker for Allergan pharmaceuticals. Henrik Zetterberg has served at advisory board meetings for Eli Lilly and Roche Diagnostics; has received travel support from TEVA; and is a cofounder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg. Kaj Blennow has served as a consultant or at advisory boards for Alzheon, BioArctic, Biogen, Eli Lilly, Fujirebio Europe, IBL International, Merck, Pfizer, and Roche Diagnostics and is a cofounder of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg.

References

1. McKee, A.C., Cantu, R.C., Nowinski, C.J., Hedley-Whyte, E.T., Gavett, B.E., Budson, A.E., Santini, VE, Lee, H.S., Kublius, C.A., and Stern, R.A. (2009). Chronic traumatic encephalopathy in athletes: progressive tauopathy after repetitive head injury. J. Neuropathol. Exp. Neurol. 68, 709–735.
2. DeKosky, S.T., Blennow, K., Ikonomovic, M.D., and Gandy, S. (2013). Acute and chronic traumatic encephalopathies: pathogenesis and biomarkers. Nat. Rev. Neurol. 9, 192–200.
3. Zetterberg, H., and Blennow, K. (2016). Fluid biomarkers for mild traumatic brain injury and related disorders. Nat. Rev. 12, 563–574.
4. Shahim, P., Tegner, Y., Wilson, D.H., Randall, J., Skillbäck, T., Pazooki, D., Kallberg, B., Blennow, K., and Zetterberg, H. (2014). Blood biomarkers for brain injury in concussed professional hockey players. JAMA Neurol. 71, 684–692.
5. Neselius, S., Brisby, H., Theodorrson, A., Blennow, K., Zetterberg, H., and Marcusson, J. (2012). CSF-biomarkers in Olympic boxing: diagnosis and effects of repetitive head trauma. PLoS One 7, e33606.
6. Shahim, P., Zetterberg, H., Tegner, Y., and Blennow, K. (2017). Serum neurofilament light as a biomarker for mild traumatic brain injury in contact sports. Neurology 88, 1788–1794.
7. Olivera, A., Lejbman, N., Jeromin, A., French, L.M., Kim, H.S., Cashion, A., Mysliwiec, V., Diaz-Arrastia, R., and Gill, J. (2015). Peripheral total tau in military personnel who sustain traumatic brain injuries during deployment. JAMA Neurol. 72, 1109–1116.
8. Shahim, P., Tegner, Y., Gustafsson, B., Gren, M., Arlig, J., Olsson, M., Lehto, N., Engstrom, A., Hoglund, K., Portelius, E., Zetterberg, H., and Blennow, K. (2016). Neurochemical aftermath of repetitive head traumatic brain injury. JAMA Neurol. 73, 1308–1315.
9. Rojas, J.C., Karydas, A., Bang, J., Tsai, R.M., Blennow, K., Liman, V., Kramer, J.H., Rosen, H., Miller, B.L., Zetterberg, H., and Boxer, A.L. (2016). Plasma neurofilament light chain predicts progression in progressive supranuclear palsy. Ann. Clin. Transl. Neurol. 3, 216–225.
10. Lu, C.H., Macdonald-Wallis, C., Gray, E., Pearce, N., Petzold, A., Norgren, N., Giovannoni, G., Frattia, P., Sidle, K., Fish, M., Orrell, R., Howard, R., Talbot, K., Greensmith, L., Kuhle, J., Turner, M.R., and Malaspina, A. (2015). Neurofilament light chain: a prognostic biomarker in amyotrophic lateral sclerosis. Neurology 84, 2247–2257.
11. Mattsson, N., Andreasson, U., Zetterberg, H., and Blennow, K. (2017). Association of plasma neurofilament light with neurodegernation in patients with Alzheimer's disease. JAMA Neurol. 74, 557–566.
12. Bernick, C., Banks, S., Phillips, M., Lowe, M., Shin, W., Obuchowski, N., Jones, S., and Modic, M. (2013). Professional fighters brain health study: rationale and methods. Am. J. Epidemiol. 178, 280–286.
13. Gualtieri, C.T., and Johnson, L.G. (2006). Reliability and validity of a computerized neurocognitive test battery, CNS Vital Signs. Arch. Clin. Neuropsychol. 2, 623–643.
14. Bernick, C., Banks, S.J., Shin, W., Obuchowski, N., Butler, S., Noback, M., Phillips, M., Lowe, M., Jones, S., and Modic, M. (2015). Repeated head trauma is associated with smaller thalamic volumes and slower processing speed: the Professional Fighters' Brain Health Study. Br. J. Sports Med. 49, 1007–1011.
15. Mishra, V.R., Zhuang, X., Sreenivasan, K.R., Banks, S.J., Yang, Z., Bernick, C., and Cordes, D. (2017). Multimodal MR imaging signatures of cognitive impairment in active professional fighters. Radiology 285, 555–567.
16. Rohrer, J.D., Woollacott, I.O., Dick, K.M., Brotherhood, E., Gordon, E., Fellows, A., Toombs, J., Druyeh, R., Cardoso, M.J., Ourselin, S., Nicholas, J.M., Norgren, N., Mead, S., Andreasson, U., Blennow, K., Schott, J.M., Fox, N.C., Warren, J.D., and Zetterberg, H. (2016). Serum neurofilament light chain protein is a measure of disease intensity in frontotemporal dementia. Neurology 87, 1329–1336.
17. Mattsson, N., Zetterberg, H., Janelidze, S., Insel, P.S., Andreasson, U., Stromrud, E., Palmqvist, S., Bakeer, D., Tan Hehir, C.A., Jeromin, A., Hanlon, D., Song, L., Shaw, L.M., Trojanowski, J.Q., Weiner, M.W., Hansson, O., and Blennow, K. (2016). Plasma tau in Alzheimer disease. Neurology 87, 1827–1835.
18. Shan, G., and Gerstenberger, S. (2017). Fisher's exact approach for post hoc analysis of a chi-squared test. PLoS One 12, e0188709.
19. Skillback, T., Farahmand, B., Bartlett, J.W., Rosén, C., Mattsson, N., Nägga, K., Kilander, L., Religa, D., Wimo, A., Winblad, B., Rosengren, L., Schott, J.M., Blennow, K., Eriksdotter, M., and Zetterberg, H (2014) CSF neurofilament light differs in neurodegenerative diseases and predicts severity and survival. Neurology 83, 1945–1953.
20. Friede, R.L., and Samorajski, T. (1970). Axon calibre related to neurofilaments and miocrotubules in sciatic nerve fibers of rats and mice. Anat. Rec. 167, 379–387.
21. Trojanowski, J.Q., Schuck, T., Schmidt, M.L., and Lee, V.M. (1989). Distribution of tau proteins in the normal human central and peripheral nervous system. J. Histochem. Cytochem. 37, 209–215.
22. Blennow, K., de Leon, M.J., and Zetterberg, H. (2006). Alzheimer's disease. Lancet 368, 387–403.
23. Kuhle, J., Barro, C., Andreasson, U., Derfuss, T., Lindberg, R., Sandelius, Å., Liman, V., Norgren, N, Blennow, K., and Zetterberg, H. (2016). Comparison of three analytical platforms for quantification of the neurofilament light chain in blood samples: ELISA, electrochemiluminescence immunoassay and Simoa. Clin. Chem. Lab. Med. 54, 1655–1661.
24. Zetterberg, H., Wilson, D., Andreasson, U., Minthon, L., Blennow, K., Randall, J., and Hansson, O. (2013). Plasma tau levels in Alzheimer's disease. Alzheimers Res. Ther. 5, 9.

25. Oliver, J., Jones, M., Anzalone, A., Kirk, K.M., Gable, D.A., Repshas, J.T., Johnson, T.A., Höglund, K., Blennow, K., and Zetterberg, H. (2017). A season of American football is not associated with plasma tau levels. J. Neurotrauma 34, 3295–3300.
26. Bishop, C.A., Newbould, R.D., Lee, J.S., Honeyfield, L., Quest, R., Colasanti, A., Ali, R., Mattoscio, M., Cortese, A., Nicholas, R., Matthews, P.M., Muraro, P.A., and Waldman, AD. (2016). Analysis of ageing-associated grey matter volume in patients with multiple sclerosis shows excess atrophy in subcortical regions. Neuroimage Clin. 13, 9–15.
27. Rubenstein, R., Chang, B., Yue, J., Chiu, A., Winkler, E.A., Puccio, A.M., Diaz-Arrastia, R., Yuh, E.L., Mukherjee, P., Valadka, A.B., Gordon, W.A., Okonkwo, D.O., Davies, P., Agarwal, S., Lin, F., Sarkis, G., Yadikar, H., Yang, Z., Manley, G.T., Wang, K.K.W.; and the TRACK-TBI Investigators, Cooper, S.R., Dams-O'Connor, K., Borrasso, A.J., Inoue, T., Maas, A.I.R., Menon, D.K., Schnyer, D.M., and Vassar, M.J. (2017). Comparing plasma phospho tau, total tau, and phospho tau-total tau ratio as acute and chronic traumatic brain injury biomarkers. JAMA Neurol. 74, 1063–1072.
28. Stern, R., Tripodis, Y., Baugh, C., Fritts, N.G., Martin, B.M., Chaisson, C., Cantu, R.C., Joyce, J.A., Shah, S., Ikezu, T., Zhang, J., Gercel-Taylor, C., and Taylor, D.D. (2016). Preliminary study of plasma exosomal tau as a potential biomarker for chronic traumatic encephalopathy. J Alzheimers Dis 51:1099–1109
29. Morris, M., Maeda, S., Vossel, K., and Mucke, L. (2011). The many faces of tau. Neuron 70, 410–426.
30. Disanto, G., Barro, C., Benkert, Naegelin, Y., Schädelin, S., Giardiello, A., Zecca, C., Blennow, K., Zetterberg, H., Leppert, D., Kappos, L., Gobbi, C., and Kuhle, J.; Swiss Multiple Sclerosis Cohort Study Group. (2017). Serum neurofilament light: a biomarker of neuronal damage in multiple sclerosis. Ann. Neurol. 81, 857–870.
31. Ljungqvist, J., Zetterberg, H., and Mitsis, M. (2017). Serum neurofilament light protein as a marker for diffuse axonal injury: results from a case series study. J. Neurotrauma 34, 1124–1127.

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