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Neurochemical Aftermath of Repetitive Mild Traumatic Brain Injury

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IMPORTANCE Evidence is accumulating that repeated mild traumatic brain injury (mTBI) incidents can lead to persistent, long-term debilitating symptoms and in some cases a progressive neurodegenerative condition referred to as chronic traumatic encephalopathy. However, to our knowledge, there are no objective tools to examine to which degree persistent symptoms after mTBI are caused by neuronal injury.

OBJECTIVE To determine whether persistent symptoms after mTBI are associated with brain injury as evaluated by cerebrospinal fluid biochemical markers for axonal damage and other aspects of central nervous system injury.

DESIGN, SETTINGS, AND PARTICIPANTS A multicenter cross-sectional study involving professional Swedish ice hockey players who have had repeated mTBI, had postconcussion symptoms for more than 3 months, and fulfilled the criteria for postconcussion syndrome (PCS) according to the *Diagnostic and Statistical Manual of Mental Disorders* (Fourth Edition) matched with neurologically healthy control individuals. The participants were enrolled between January 2014 and February 2016. The players were also assessed with Rivermead Post Concussion Symptoms Questionnaire and magnetic resonance imaging.

MAIN OUTCOMES AND MEASURES Neurofilament light protein, total tau, glial fibrillary acidic protein, amyloid β, phosphorylated tau, and neurogranin concentrations in cerebrospinal fluid.

RESULTS A total of 31 participants (16 men with PCS; median age, 31 years; range, 22-53 years; and 15 control individuals [11 men and 4 women]; median age, 25 years; range, 21-35 years) were assessed. Of 16 players with PCS, 9 had PCS symptoms for more than 1 year, while the remaining 7 returned to play within a year. Neurofilament light proteins were significantly increased in players with PCS for more than 1 year (median, 410 pg/mL; range, 230-1440 pg/mL) compared with players whose PCS resolved within 1 year (median, 210 pg/mL; range, 140-460 pg/mL) as well as control individuals (median 238 pg/mL, range 128-526 pg/mL; $P = .04$ and $P = .02$, respectively). Furthermore, neurofilament light protein concentrations correlated with Rivermead Post Concussion Symptoms Questionnaire scores and lifetime concussion events ($\rho = 0.58$, $P = .02$ and $\rho = 0.52$, $P = .04$, respectively). Overall, players with PCS had significantly lower cerebrospinal fluid amyloid-β levels compared with control individuals (median, 1094 pg/mL; range, 845-1305 pg/mL; $P = .05$).

CONCLUSIONS AND RELEVANCE Increased cerebrospinal fluid neurofilament light proteins and reduced amyloid β were observed in patients with PCS, suggestive of axonal white matter injury and amyloid deposition. Measurement of these biomarkers may be an objective tool to assess the degree of central nervous system injury in individuals with PCS and to distinguish individuals who are at risk of developing chronic traumatic encephalopathy.

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Supplemental content

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Traumatic brain injury (TBI) represents a leading cause of mortality and morbidity worldwide, with 1.6 to 3.6 million sports-related TBIs occurring annually alone in the United States and additional large numbers occurring in civilian and military personnel.[1] Concussion is a type of mild TBI (mTBI) that is caused by rapid acceleration, deceleration, and rotational forces to the head that cause the brain to deform, resulting in stretching of individual neurons, glial cells, and blood vessels.[2]

Symptoms of mTBI usually resolve within days to weeks, but in 10% to 15% of individuals, neurological symptoms persist for more than 3 months.[3] The presence of neurological symptoms lasting more than 3 months in individuals who had an mTBI is referred to as postconcussion syndrome (PCS).[3] A proportion of individuals exposed for repeated mTBI episodes may develop a progressive neurodegenerative condition referred to as chronic traumatic encephalopathy (CTE).[4,5] Chronic traumatic encephalopathy shares similar histopathological changes with other neurodegenerative disorders, particularly Alzheimer disease (AD), including hyperphosphorylation of tau protein in neurofibrillary tangles and, in a proportion of cases, also deposition of amyloid β (Aβ) in diffuse plaques.[6,7]

The relationship between repeated mTBIs and development of PCS is poorly understood, specifically to which degree such long-lasting symptoms are caused by neuronal damage and to which degree psychogenic or psychosocial mechanisms contribute. Notably, there are no objective biomarkers to quantify neuronal damage or other types of central nervous pathology in individuals with PCS. Postconcussion syndrome diagnosis is mainly based on self-reporting clinical symptoms, while CTE diagnosis can only be made post mortem.[4,5,8,9] Furthermore, the relation between PCS and future development of CTE is unknown.

The cerebrospinal fluid (CSF) is in direct contact with the brain parenchyma and is a suitable biofluid to monitor biochemical changes in the central nervous system. Cerebrospinal fluid biomarkers reflecting amyloid and tau pathology have been validated extensively in the context of AD.[10-14] In 2015, independent reports have shown that neurodegenerative disorders, mainly AD, also are associated with increased levels of CSF neurogranin (Ng), which reflect synaptic degeneration and loss[15-17] as well as increased CSF neurofilament light protein (NF-L), reflecting injury to large-caliber myelinated axons in the white matter.[18,19]

Considering that CTE shares many neuropathological changes with AD, we hypothesized that PCS would also display similar pathophysiology, at least in CSF.

We specifically tested the following hypotheses: (1) PCS is associated with axonal injury and astrogliosis, as reflected by increased CSF concentrations of the axonal proteins total tau (T-tau) and NF-L as well as the astroglial protein glial fibrillary acidic protein, and (2) PCS is associated with increased amyloid burden and tau pathology as well as synaptic loss, as reflected by altered CSF concentrations of the 42-amino acid variant of Aβ (Aβ1-42), phosphorylated tau (P-tau), and the synaptic biomarker Ng.

Key Points

Question Is repeated mild traumatic brain injury associated with cerebrospinal fluid biomarkers of amyloid pathology, tau pathology, and synaptic loss?

Findings In a cross-sectional study, among 16 professional ice hockey players who have had repeated mild traumatic brain injury and fulfilled the criteria for postconcussion syndrome compared with 15 neurologically healthy control individuals, we observed increased cerebrospinal fluid neurofilament light protein and reduced amyloid β levels. Our findings provide neurochemical evidence of white matter injury and amyloid deposition.

Meaning Measurement of these biomarkers may be an objective tool to assess the degree of brain injury in individuals with postconcussion syndrome and to distinguish individuals who are at risk of developing progressive neurodegeneration.

Methods

Study Population

In this multicenter cross-sectional study, we enrolled 16 male professional ice hockey players with prolonged postconcussive symptoms for more than 3 months and 15 neurologically healthy control individuals between January 2014 and February 2016. None of the participants had been part of any previous investigation from our group. The study was approved by the Ethics Committee for Medical Research at the University of Gothenburg, Sweden. Written informed consent was obtained from all participants.

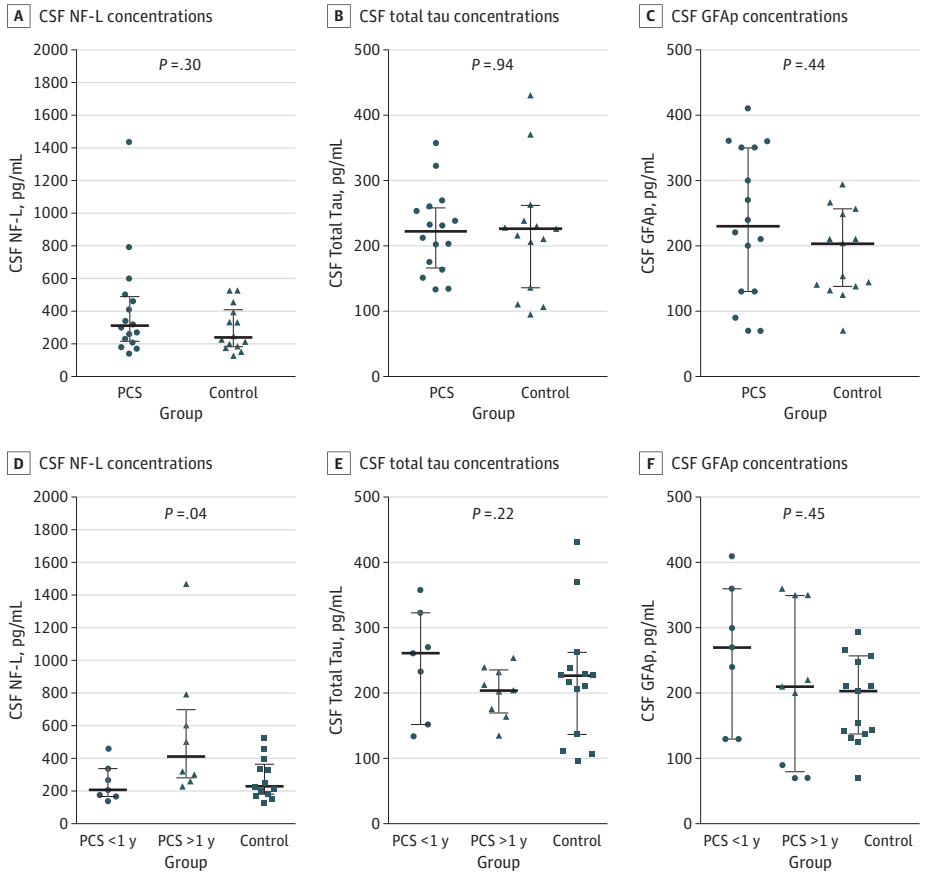
Selection of Participants

The diagnosis of concussion was made according to the latest diagnostic guidelines on sports-related concussion, and players with concussion were treated according to these guidelines.[20,21] The diagnosis of PCS was based on *Diagnostic and Statistical Manual of Mental Disorders* (Fourth Edition) criteria.[22] The inclusion criteria were (1) persistent postconcussion symptoms for more than 3 months following mTBI; (2) no contraindications to lumbar puncture (LP) (decreased platelet count [$<50 \times 10^3/\mu L$; to convert to $\times 10^9$ per liter, multiply by 1], focal neurologic sign, papilledema, reduced consciousness, and infection at puncture site); and (3) no evidence of structural damage on conventional magnetic resonance imaging (T1/T2 and fluid-attenuated inversion recovery).

The inclusion criteria for control participants were (1) older than 18 years, (2) no history of known head trauma, (3) no history of neurological or psychological condition, and (4) no contradictions to LP as stated earlier.

At inclusion, the participants underwent neuropsychological assessment with the Rivermead Post Concussion Questionnaire (RPQ),[23] and the PCS group was also followed up with repeated RPQ assessment at the end of the study. One of the players agreed to undergo repeated LPs at inclusion (5 months after last concussion), 11 months, 17 months, and 23 months after the injury.

Figure 1. Cerebrospinal Fluid (CSF) Biomarkers Reflecting Axonal and Astroglial Injury



Concentrations of neurofilament light (NF-L) protein were increased in the postconcussion syndrome (PCS) group vs control group (A). There was no significant difference in the level of total tau (B) and glial fibrillary acidic protein (GFAp) (C). Players with PCS for more than 1 year had increased concentration of NF-L compared with players whose PCS resolved within 1 year after injury as well as control individuals (D). There was no significant difference in the levels of total tau (E) and GFAp (F) at either group or subgroup level as well as compared with control individuals. One of the 15 control participants had increased CSF NF-L concentration (1491 pg/mL) for unknown reasons and was excluded from the statistical analyses. Values are presented as medians; error bars indicate interquartile range.

Biochemical Procedures

Cerebrospinal fluid was collected in polypropylene tubes by LP through the lumbar nerve 3 to 4 or lumbar nerve 4 to 5 interspace. All CSF samples were stored at −80°C pending analysis. The participants were examined physically and neurologically before LP. All were healthy and showed no signs of focal neurological injury.

Cerebrospinal NF-L concentrations were measured using a commercial enzyme-linked immunosorbent assay (NF-L enzyme-linked immunosorbent assay; Uman Diagnostics) as described previously.[24] Cerebrospinal fluid glial fibrillary acidic protein concentrations were measured using a previously described in-house enzyme-linked immunosorbent assay procedure.[25] Cerebrospinal fluid concentrations of Aβ1-42, T-tau, and P-tau were measured with INNOTEST (Fujirebio Diagnostics). Cerebrospinal fluid Ng concentrations were measured using an in-house–developed enzyme-linked immunosorbent assay using 2 monoclonal antibodies, described in detail in the eMethods of the Supplement.

All samples were analyzed at the same time using the same batch of reagents by board-certified laboratory technicians who were blind to clinical information.

Statistical Analysis

For the PCS vs control group comparison, the Mann-Whitney *U* test was used. The Dunn correction was performed for all multiple comparisons. The Spearman rank correlation coefficient (r_s) was used for analyses of correlation between changes in various biomarker levels and lifetime concussion events as well as RPQ score. All tests were 2-sided, and statistical significance was determined at *P* less than .05. All statistical calculations were performed using GraphPad Prism 6.0 (GraphPad Inc).

Results

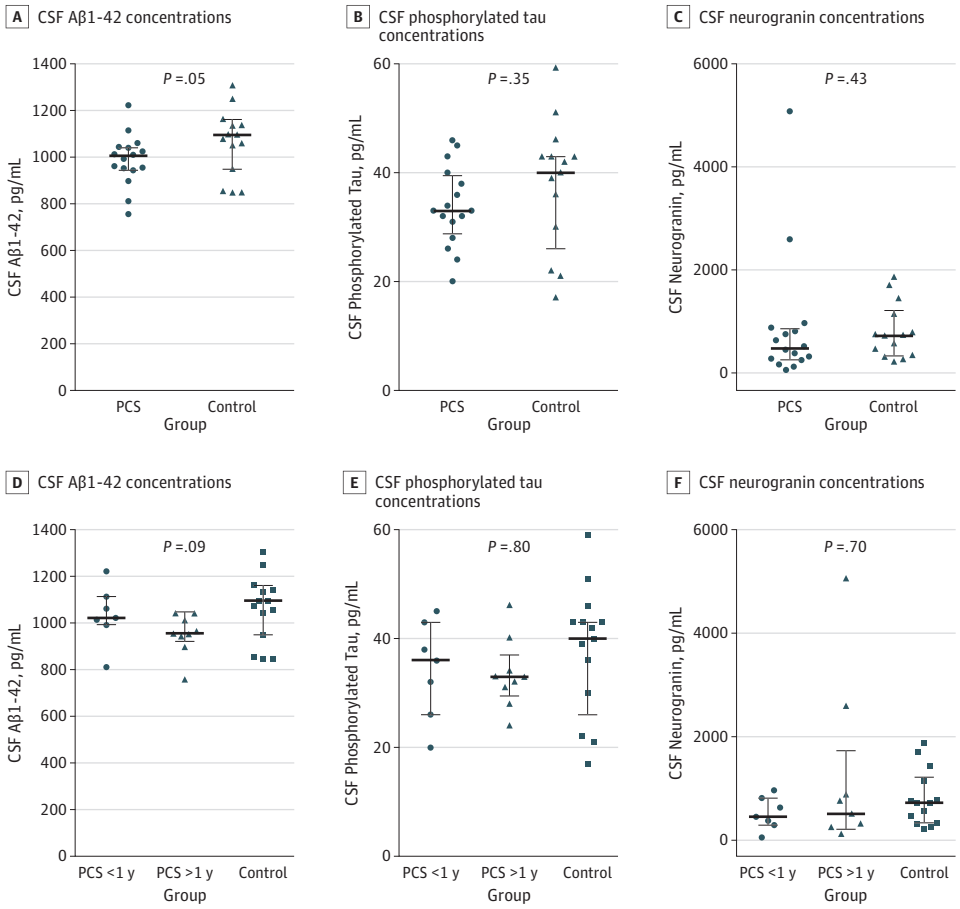
Characteristics of the Participants

Sixteen players with PCS (median age, 31 years; range, 22-53 years) and 15 neurologically healthy control individuals (median age, 25 years; range, 21-35 years) were enrolled between January 2014 and February 2016 (eTable in the Supplement). The median time between most recent concussion and LP was 4 months (range, 3-144 months; eTable in the Supplement). Nine of the 16 players had persistent PCS symptoms for more than 1 year and retired from the game, while 7 players returned to the game within 1 year from the injury.

Axonal White Matter Damage in Players With Persistent PCS

Cerebrospinal fluid concentrations of NF-L were increased in the PCS group compared with the control group, but the increase did not reach statistical significance (**Figure 1**A). How-

Figure 2. Cerebrospinal Fluid (CSF) Biomarkers Reflecting Amyloid and Tau Pathology and Synaptic Loss



The postconcussion syndrome (PCS) group had significantly (P = .05) lower amyloid β 1-42 (Aβ1-42) than the control group (A). Also, concentrations of Aβ1-42 (C) were lower in players with PCS for more than 1 year vs PCS for less than 1 year; however, this was not significant (P = .09). There was no difference in the phosphorylated tau levels at either group (B) or subgroup level (D). There was also no significant difference in the concentrations of neurogranin at either group (C) or subgroup level (F). Values are presented as medians; error bars indicate interquartile range.

ever, the concentrations of NF-L were significantly higher in the subgroup of players with PCS for more than 1 year (median, 410 pg/mL; range, 230-1440 pg/mL) vs PCS for less than 1 year (median, 210 pg/mL; range, 140-460 pg/mL; P = .02) as well as compared with the control group (median, 238 pg/mL; range, 128-526 pg/mL; P = .02) (Figure 1D). No significant differences were observed in the concentrations of T-tau or glial fibrillary acidic protein (Figure 1B, C, and E).

Reduced CSF Aβ1-42 in PCS

Cerebrospinal fluid concentration of Aβ1-42 was significantly lower in the PCS group (median, 1000 pg/mL; range, 757-1220 pg/mL) compared with the control group (median, 1094 pg/mL; range, 845-1305 pg/mL; P = .05) (**Figure 2**A). The subgroup of players with PCS for more than 1 year had lower Aβ1-42 concentrations (median, 955 pg/mL; range, 757-1040 pg/mL) compared with players whose symptoms resolved within 1 year (median, 1022 pg/mL; range, 810-1220 pg/mL; P = .01), although the change was not statistically significant after correcting for multiple comparisons (Figure 2D). There was a trend toward lower concentrations of P-tau and Ng at group level; however, the differences were not significant (Figure 2B, C, and F).

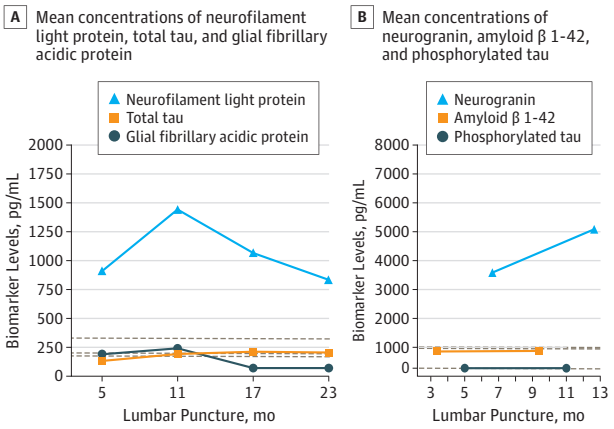
Reduced CSF Aβ1-42 and Increased Ng and NF-L Over Time

Mean CSF NF-L concentration remained more than 2-fold increased compared with the mean of the control participants in the 1 player who underwent repeated lumbar punctures (**Figure 3**A). In addition, the player displayed reduced concentrations of Aβ1-42 compared with the control individuals, while the concentrations of Ng were increased (Figure 3B) at all sampling times. There was also a trend toward higher concentrations of CSF P-tau compared with control individuals (Figure 3B). The concentrations of other measured biomarkers were essentially unchanged (Figure 3B).

Cerebrospinal Fluid NF-L Correlated With Symptom Severity in PCS

As expected, the PCS group had higher RPQ scores (median, 22; range, 8-35) compared with the control group (median,

Figure 3. Longitudinal Biomarker Changes



One of the players was followed up with repeated lumbar punctures (5, 11, 17, and 23 months after most recent concussion). In this player, the mean concentration of neurofilament light protein was elevated compared with the mean of the control group and remained elevated over time, while the levels of total tau and glial fibrillary acidic protein were unchanged (A). Mean concentrations of amyloid β 1-42 were lower at 2 following times compared with the mean of the control group (B). Also, the mean concentration of neurogranin was elevated compared with the mean of the control group (B). Dashed lines indicate mean (SD) of the control group.

0; range, 0-0; $P < .001$). Cerebrospinal fluid NF-L concentrations correlated with RPQ scores ($\rho = 0.58$; $P = .02$) (**Figure 4**A). There was no significant relationship between the concentrations of other measured biomarkers and RPQ (Figure 4B-F).

Cerebrospinal Fluid NF-L and P-tau and Lifetime Concussion Event

Cerebrospinal fluid NF-L correlated with lifetime concussion event ($\rho = 0.52$; $P = .04$) (**Figure 5**A). There was also a significant correlation between CSF P-tau and lifetime concussions ($\rho = 0.55$; $P = .03$) (Figure 5E). No significant relationships were observed between the lifetime concussion events and concentrations of the other biomarkers in the study (Figure 5B-D and F).

Discussion

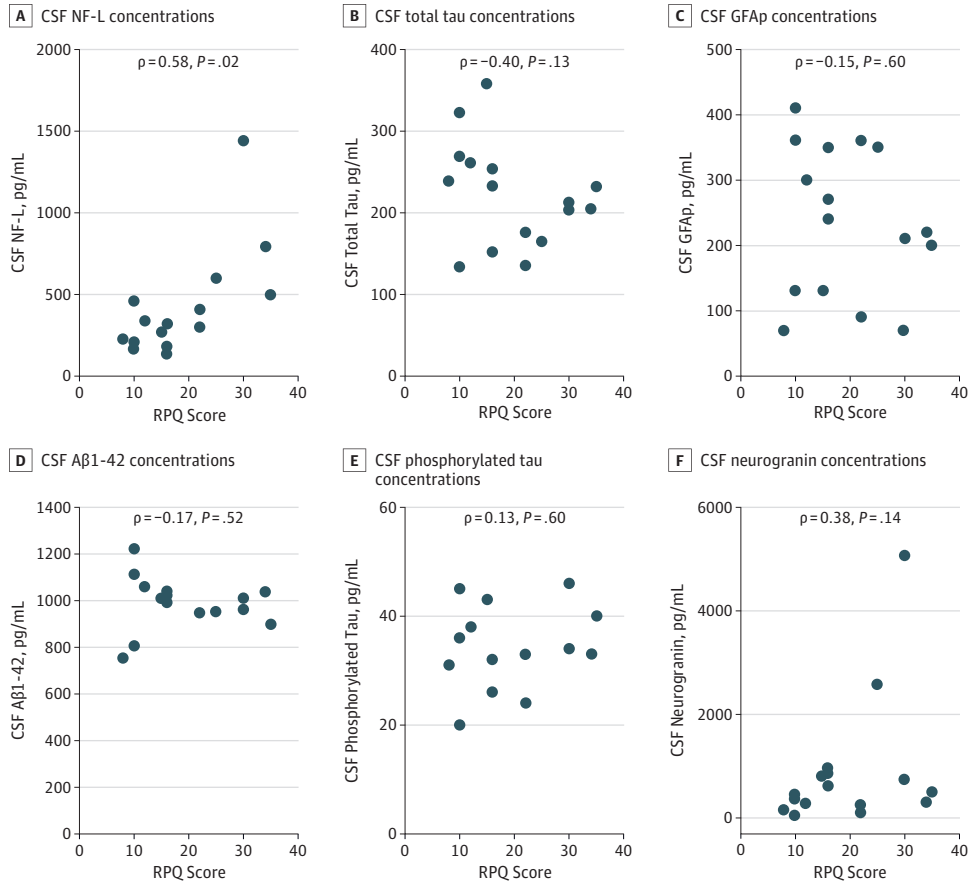
Symptoms of mTBI usually resolve within days to weeks.[3] However, a subgroup of individuals with mTBI, mainly repeated mTBI, display persistent physical, cognitive, and behavioral impairment for months, referred to as PCS.[3] The relation between PCS and development of future CTE is unknown. Thus, improved methods of characterizing neurodegenerative processes triggered by repeated mTBI and identifying individuals at risk of developing PCS or progressive neurodegeneration are needed. To our knowledge, this is the first study to investigate CSF biomarkers reflecting axonal white matter injury, amyloid burden, tau pathology, and synaptic loss in professional ice hockey players who have experienced repeated mTBI and fulfilled the criteria for PCS. We found that (1) a subgroup of individuals with PCS (those with chronic symptoms forcing them to retire) displayed increased concentrations of CSF NF-L compared with those whose PCS resolved within 1 year as well as compared with control individuals; (2) CSF concentrations of NF-L correlated with RPQ scores and lifetime concussion events; (3) overall, the PCS group had lower CSF Aβ1-42 concentrations compared with the control group; and (4) there were no PCS-related changes in CSF T-tau, glial fibrillary acidic protein, and Ng concentrations.

Neurofilament light protein is a structural protein that is highly expressed in the large-caliber myelinated subcortical axons of the white matter.[18,19] The findings that NF-L is elevated in CSF of professional athletes with PCS and that CSF concentrations of NF-L correlated with RPQ scores add support for the hypothesis that axonal white matter injury is a primary determinant of outcome following TBI.[26-28] Furthermore, NF-L concentrations correlated with lifetime concussion event in this study. This result is also consistent with earlier studies on amateur boxers with repeated trauma to the head, where the levels of NF-L in CSF increased after bouts, even without knockouts.[29,30] Additionally, a case study on a boxer with mTBI leading to a knockout showed increased CSF NF-L levels over many months following the trauma.[31] Taken together, the findings of this study support the hypothesis that repetitive mTBI may lead to chronic axonal white matter injury.

Tau is a normal axonal protein that is responsible for microtubule assembly and stability and is mainly expressed in unmyelinated cortical axons.[11] In this study, the concentration of CSF T-tau was unchanged. Considering the pathophysiology of mTBI, it is plausible to assume that shorter unmyelinated axons, which do not travel long distance, may only be locally torn as a result of the acceleration and deceleration. Indirectly, the unaltered T-tau levels further support the hypothesis that TBI mainly injures long axons.

Evidence suggests that athletes who have been exposed to repetitive head trauma are at increased risk of developing neurodegenerative changes such as tau pathology and Aβ deposition.[4,5,8,9] Numerous studies have shown that reduced CSF Aβ1-42 levels correlate tightly with positive amyloid positron-emission tomographic findings,[32] and a 2016 study also suggests that the reduction in CSF Aβ1-42 may be an earlier indicator of cerebral Aβ deposition than amyloid positron emission tomography.[33] The finding that CSF Aβ1-42 was lower in players with PCS compared with control individuals and the lowest levels of Aβ1-42 were observed in players with PCS for more than 1 year who were also forced to retire are suggestive of potential early amyloid deposition following mTBI. Our findings are also consistent with postmortem studies of patients with mTBI and a 2016 imaging study of patients with severe TBI.[34] The exact mechanism of what triggers Aβ production is not fully understood. It has been suggested that axonal damage produced at the time of injury may act as an initial trigger for Aβ production and accumulation of amyloid pathology.[35] Additionally, animal models and human autopsy studies

Figure 4. Relationship Between Biomarker Levels and Symptom Severity



Concentrations of neurofilament light protein correlated (ρ = 0.58, *P* = .02) with Rivermead Post Concussion Symptoms Questionnaire (RPQ) score (A). There was no significant correlation between any of the other biomarkers and RPQ score (B-F). Aβ1-42 indicates amyloid β 1-42; CSF indicates cerebrospinal fluid; GFAp indicates glial fibrillary acidic protein; and NF-L indicates neurofilament light protein.

provide evidence that Aβ is produced at the site of axonal injury shortly after TBI.[35]

Trauma to the axons may also cause hyperphosphorylation of tau protein and aggregation into neurofibrillary tangles, which is a histological feature of repetitive mTBI or CTE. However, in this study, the concentration of CSF P-tau remained statistically unchanged. A plausible assumption would be that tau pathology may appear at a later stage or that tau pathology is not as widespread following mTBI or, alternatively, P-tau measured in CSF is not sensitive enough to reflect it. Despite the unchanged concentrations between the PCS and control group, P-tau correlated with the lifetime accumulated concussion score. However, given the modest sample size of this study, this should be interpreted with caution. Longitudinal studies with larger sample sizes and repeated lumbar puncture are needed to clarify any causal relationship between repetitive mTBI and hyperphosphorylation of tau. Such studies should also examine the concentrations of CSF Aβ1-42 and P-tau in the context of genotype, particularly *APOE*,[36] which was not assessed here.

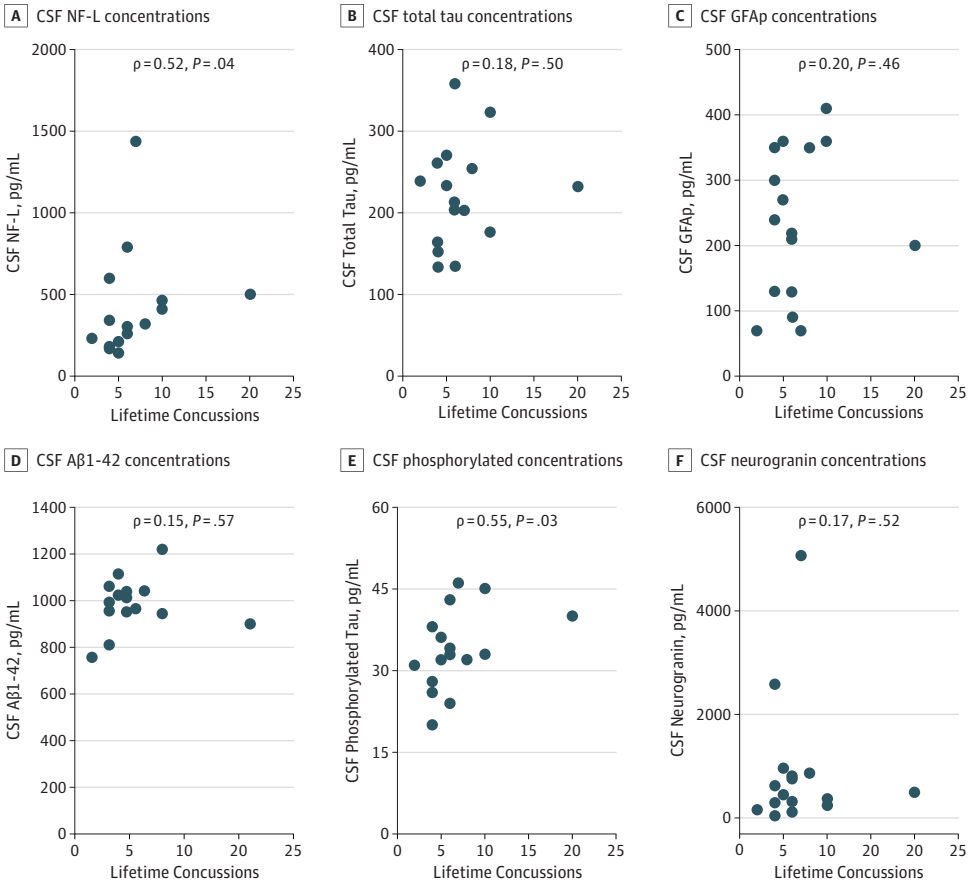
Neurogranin is a postsynaptic protein that has shown diagnostic utility for early symptomatic AD.[15-17] In this study, the concentrations of Ng were essentially unaltered in the PCS group. However, in 1 of the players with persistent PCS who underwent repeated LPs, the mean levels of Ng remained elevated up to 2-fold compared with the mean of the control group. It is plausible to assume that postsynaptic loss may only be evident in individuals with severe PCS.

The main limitation of this study was the modest sample size, which precludes from examining the biomarkers in relation to specific aspects of PCS such as behavioral, cognitive, and/or neurological changes. Also, we did not have diffuse tensor imaging magnetic resonance imaging results or amyloid tau positron emission tomography data for relating the biomarker findings.

Conclusions

Increased CSF NF-L and reduced Aβ1-42 were observed in patients with PCS, suggestive of axonal white matter injury

Figure 5. Relationship Between Biomarker Concentrations and Lifetime Number of Concussions



Neurofilament light protein correlated (ρ = 0.52; *P* = .04) with lifetime concussion event (A). There was also a significant correlation between phosphorylated tau and lifetime concussion event (E). There was no significant relationship between the lifetime concussion event and other biomarkers in the study (B-D and F). Aβ1-42 indicates amyloid β 1-42; CSF indicates cerebrospinal fluid; GFAp indicates glial fibrillary acidic protein; and NF-L indicates neurofilament light protein.

and potential amyloid deposition. Measurement of these biomarkers may be an objective tool to assess the degree of central nervous system injury in individuals with PCS and to distinguish individuals who are at risk of developing persistent PCS or progressive neurodegeneration.

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Study concept and design: Shahim, Tegner, Zetterberg, Blennow.
Acquisition, analysis, or interpretation of data: Shahim, Tegner, Gustafsson, Gren, Ärlig, Olsson, Lehto, Engström, Höglund, Portelius, Zetterberg, Blennow.
Drafting of the manuscript: Shahim, Zetterberg.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: Shahim, Gren, Zetterberg.
Obtained funding: Shahim, Zetterberg, Blennow.
Administrative, technical or material support: Tegner, Gustafsson, Ärlig, Olsson, Lehto, Engström, Höglund, Portelius, Blennow.
Study supervision: Tegner, Zetterberg, Blennow.

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