metabolism in human repetitive concussion

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ABSTRACT

Objective: To determine whether postconcussion syndrome (PCS) due to repetitive concussive traumatic brain injury (rcTBI) is associated with CSF biomarker evidence of astroglial activation, amyloid deposition, and blood–brain barrier (BBB) impairment.

Methods: A total of 47 participants (28 professional athletes with PCS and 19 controls) were assessed with lumbar puncture (median 1.5 years, range 0.25–12 years after last concussion), standard MRI of the brain, and Rivermead Post-Concussion Symptoms Questionnaire (RPQ). The main outcome measures were CSF concentrations of astroglial activation markers (glial fibrillary acidic protein [GFAP] and YKL-40), markers reflecting amyloid precursor protein metabolism ($A\beta38$, $A\beta40$, $A\beta42$, $sAPP\alpha$, and $sAPP\beta$), and BBB function (CSF:serum albumin ratio).

Results: Nine of the 28 athletes returned to play within a year, while 19 had persistent PCS >1 year. Athletes with PCS >1 year had higher RPQ scores and number of concussions than athletes with PCS <1 year. Median concentrations of GFAP and YKL-40 were higher in athletes with PCS >1 year compared with controls, although with an overlap between the groups. YKL-40 correlated with RPQ score and the lifetime number of concussions. Athletes with rcTBI had lower concentrations of $A\beta40$ and $A\beta42$ than controls. The CSF:serum albumin ratio was unaltered.

Conclusions: This study suggests that PCS may be associated with biomarker evidence of astroglial activation and β-amyloid ($A\beta$) dysmetabolism in the brain. There was no clear evidence of $A\beta$ deposition as $A\beta40$ and $A\beta42$ were reduced in parallel. The CSF:serum albumin ratio was unaltered, suggesting that the BBB is largely intact in PCS. *Neurology® 2017;88:1400–1407*

GLOSSARY

Aβ = β-amyloid; **AD** = Alzheimer disease; **APP** = amyloid precursor protein; **BBB** = blood–brain barrier; **cTBI** = concussive traumatic brain injury; **CTE** = chronic traumatic encephalopathy; **DSM-IV** = *Diagnostic and Statistical Manual of Mental Disorders, 4th edition*; **GFAP** = glial fibrillary acidic protein; **LP** = lumbar puncture; **NFL** = neurofilament light; **PCS** = postconcussion syndrome; **rcTBI** = repetitive concussive traumatic brain injury; **RPQ** = Rivermead Post-Concussion Symptoms Questionnaire; **sAPPα** = α-cleaved soluble amyloid precursor protein; **sAPPβ** = β-cleaved soluble amyloid precursor protein; **TBI** = traumatic brain injury.

Concussion is a type of mild traumatic brain injury (TBI) (concussive TBI [cTBI]) caused by rapid rotational acceleration of the head, which causes the brain to deform, resulting in tissue damage, particularly to vulnerable white matter axons.[1] While most individuals with cTBI recover completely within days to weeks, about 10%–15% of individuals display persistent neurobehavioral symptoms for more than 3 months, a condition referred to as postconcussion syndrome (PCS).[2] A proportion of individuals exposed to repetitive concussive TBI (rcTBI) may develop a progressive neurodegenerative condition referred to as chronic traumatic encephalopathy (CTE).[3,4] CTE is characterized by tau and, less consistently, β-amyloid (Aβ) pathology, which are also histologic hallmarks of Alzheimer disease (AD).[5,6] Evidence from animal models and limited clinical studies of moderate to severe TBI suggest that astrogliosis, increased

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inflammatory activity, as well as disruption of the blood–brain barrier (BBB) are additional important processes in TBI.[7–10]

A PCS diagnosis is mainly based on self-reported clinical symptoms.[11] In contrast, a CTE diagnosis can only be made postmortem.[3,4,12,13] Recently, we showed that PCS due to rcTBI is associated with increased CSF concentrations of neurofilament light (NFL).[14]

Considering the literature on disease mechanisms other than axonal injury in cTBI, we tested the following specific hypotheses: (1) PCS due to rcTBI is associated with CSF signs of astroglial activation, (2) PCS due to rcTBI is associated with CSF signs of amyloid burden, and (3) rcTBI is associated with impaired BBB, as reflected by elevated CSF:serum albumin ratio. The study was designed in compliance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement.[15]

METHODS Study population. In this multicenter cross-sectional study, we enrolled 28 professional athletes (26 professional ice hockey players, 2 professional soccer players) with PCS following rcTBI and 19 neurologically healthy controls without a history of concussion between September 2014 and June 2016. The controls were healthy volunteers (mostly students) without known history of brain trauma or any other neurologic disease who were recruited through advertisement at the University of Gothenburg, Sweden.

Taking into account that only 10%–15% of individuals with cTBI may develop PCS,[2] and of these only a small number of cases may develop PCS for more than 1 year, the sample size of 28 was considered to be relatively adequate for this pilot study.

Selection of participants. The diagnosis of concussion was made according to the latest diagnostic guidelines on sports-related concussion and players with concussion were managed according to these guidelines.[16,17] The diagnosis of PCS was based on DSM-IV criteria.[18] The inclusion criteria were (1) persistent postconcussion symptoms for more than 3 months following rcTBI at the time of inclusion in the study, (2) consent to undergo lumbar puncture (LP), (3) no contraindications to LP (normal coagulation parameters, focal neurologic sign, papilledema, reduced consciousness, infection at puncture site), and (4) no evidence of structural damage on conventional MRI (T1/T2 and fluid-attenuated inversion recovery).

The inclusion criteria for healthy controls were (1) age >18 years, (2) no history of known head trauma, (3) no history of neurologic or psychological condition, and (4) no contradictions to LP as stated above. At inclusion and at the end of the study, the participants underwent neuropsychological assessment with the Rivermead Post-Concussion Symptoms Questionnaire (RPQ).[19]

Standard protocol approvals, registrations, and patient consents. The regional ethics committee at the University of Gothenburg, Sweden, approved the study. Written informed consent was obtained from all participants.

Biochemical procedures. Blood samples were collected by venipuncture into gel-separator tubes for serum and centrifuged within 20–60 minutes. LP was performed in the lateral decubitus position, through L3-L4 or L4-L5, between 10:00 AM and 2:00 PM. Atraumatic 20-G (Sprotte) needles were used for all the LPs. None of the participants was fasting. A total of 8–10 mL CSF was collected in a single polypropylene tube from each participant. The CSF was gently mixed and a cell count was performed to exclude blood contamination. Thereafter, the CSF was centrifuged (2,000g at 4°C for 10 minutes) and the supernatant was aliquoted in 0.5 mL portions in polypropylene screw cap cryo tubes that 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 neurologic injury.

CSF glial fibrillary acidic protein (GFAP) concentration was measured using a previously described in-house ELISA procedure.[20] CSF chitinase-3-like protein 1 (CHI3L1 or YKL-40) concentration was measured using a commercial ELISA (R&D Systems, Minneapolis, MN). CSF Aβ38, Aβ40, and Aβ42 concentrations were measured using the 6E10-based Abeta Triplex method on a Meso Scale Discovery platform (MSD, Gaithersburg, MD). CSF α- and β-cleaved soluble amyloid precursor protein (sAPPα and sAPPβ) concentrations were measured using sandwich ELISAs (IBL International GmbH, Hamburg, Germany).

All samples were analyzed on one occasion using the same batch of reagents by board-certified laboratory technicians who were blind to the clinical information of each individual. CSF GFAP data for 16 of the 28 patients with PCS due to rcTBI and 15 of the 19 healthy controls were reported in our previous study.[21]

Statistical analysis. We used χ^2 test to examine differences in categorical variables between the rcTBI vs the control group. For the rcTBI vs the control group comparisons, the Mann-Whitney U test was used. The Kruskal-Wallis analysis of variance was performed for the multiple group comparisons. To examine the potential influence of age on the results, we also performed analysis of covariance of log-transformed continuous data with and without age as a covariate. The Spearman rank correlation examined the relationship between changes in various biomarker levels and age, and lifetime concussion events as well as RPQ score. Partial correlations examined associations between CSF biomarkers and lifetime concussion and RPQ score, adjusted for age. We checked model assumptions by inspecting residuals (normality, histograms, q-q plots, and homogeneity of variance). All tests were 2-sided and statistical significance was determined at $p < 0.05$. Corrections for multiple group comparison were done using Dunn or Tukey post hoc tests. All statistical calculations were performed using GraphPad Prism 6.0 (GraphPad Inc., San Diego, CA) and R (v 3.0.3, The R Foundation for Statistical Computing).

RESULTS Characteristics of the study participants. Twenty-eight professional athletes with rcTBI (median [range] age, 28 [18–52] years) and 19 neurologically healthy controls (25 [21–35] years) were enrolled between September 2013 and June 2016 (table). Age and sex distributions did not differ between the groups ($p = 0.070$ and $p = 0.20$, respectively; table). There was no correlation of any of the markers with age in the control group, while there

Table	Demographic and clinical characteristics of participants at inclusion		
Variables	rcTBI (n = 28)	Controls (n = 19)	p Value
Age, y	28 (18–52)	25 (21–35)	0.078
Male, n (%)	25 (89.2)	15 (78.9)	0.20[a]
Time since recent concussion, y	1.5 (0.25–12)	0	NA
Lifetime number of concussions	5.5 (2–20)	0.0 (0–0)	0.0001
Postlumbar headache, n (%)	2 (7.1)	4 (21.0)	0.18
Total Rivermead Post-Concussion Symptoms Questionnaire score, range 0–64	13 (6–35)	0.0 (0–0)	0.0001

Abbreviations: NA = not applicable; rcTBI = repetitive concussive traumatic brain injury.
All continuous variables are shown as median (range) unless denoted otherwise.
[a] Determined by χ^2 test.

were correlations between age and CSF Aβ38, YKL-40, and sAPPα in the PCS group ($r = 0.43$, $p = 0.021$; $r = 0.70$, $p = 0.001$; and $r = 0.45$, $p = 0.016$, respectively). Correcting for age did not influence any of the results reported below, except for YKL-40. The mean time between the most recent concussion and the LP was 1.5 years (range 0.25–12 years; table). There was no correlation between the time since the last concussion and CSF biomarker concentrations (figure e-1 at Neurology.org). At the end of the study, 9 of the 28 athletes returned to the game within a year, while 19 of them had persistent PCS for more than 1 year, which eventually forced them to retire from the game.

PCS due to rcTBI is associated with biomarker evidence of astroglial activation. CSF GFAP concentration was higher in the rcTBI group as compared to the control group ($p = 0.009$; figure 1A); this change was particularly prominent in athletes with PCS >1 year ($p = 0.0015$; figure 1B). Similar to GFAP, higher concentrations of CSF YKL-40 were measured in athletes with persistent PCS compared with controls ($p = 0.049$), but these results were not significant after correcting for multiple comparisons and age (figure 1, C and D).

PCS due to rcTBI is associated with reduced CSF Aβ. There was no difference in CSF Aβ38 concentration between athletes with rcTBI and controls at either group or subgroup analysis (figure 2, A and B). CSF Aβ40 and Aβ42 concentrations were reduced in athletes with rcTBI compared with controls ($p = 0.0078$ and $p = 0.0009$, respectively; figure 2, C and E). The subgroup of athletes with PCS >1 year had reduced concentrations of Aβ40 and Aβ42 as compared to controls ($p = 0.0073$ and $p = 0.0018$, respectively), but similar concentrations as compared to athletes whose PCS resolved within 1 year (figure 2, D and F). The Aβ42/Aβ40 ratio was lower in athletes with rcTBI as compared to controls ($p = 0.0005$), but did not differ between athletes with PCS >1 year compared with athletes with PCS <1

year ($p = 0.90$; figure 2, G and H). In addition, we measured concentrations of CSF sAPPα and sAPPβ, and found no difference at either group or subgroup level (figure e-2).

CSF:serum albumin ratio in PCS due to rcTBI. CSF: serum albumin ratios were similar in all examined groups (figure e-3).

Persistently increased CSF YKL-40 and persistently decreased Aβ over time. One of the athletes with persistent PCS underwent repeated LPs at 5 and 11 months since the last concussion. There were no clear changes in any of the biomarker concentrations over this time period (figure e-4).

CSF YKL-40 correlated with symptom severity in PCS due to rcTBI. As expected, the PCS group had higher RPQ score (median 13; range 6–35) as compared to controls (median 0.0; range 0–0, $p < 0.0001$). Further, the subgroup of athletes with PCS >1 year had higher RPQ scores (median 16.5; range 6–35) as compared to the athletes whose PCS resolved within a year (median 8; range 7–16, $p = 0.0009$; figure 3A). CSF YKL-40 concentrations correlated with RPQ scores after adjusting for age (unadjusted $r = 0.44$, $p = 0.020$; adjusted $r = 0.33$, $p = 0.090$; figure 3B). There were no relationships between any of the other biomarkers and RPQ scores (figure e-5).

CSF YKL-40 correlated with lifetime concussion events. The median lifetime number of concussions in the rcTBI group was 5.5 (range 2–20) (table). The subgroup of athletes with PCS >1 year had higher lifetime number of concussions (median 6.0; range 3–20) than athletes whose PCS resolved within a year (median 4.0; range 2–7, $p = 0.040$; figure 4A). CSF YKL-40 concentrations correlated with the lifetime number of concussions (unadjusted $r = 0.51$, $p = 0.0054$; adjusted $r = 0.40$, $p = 0.038$; figure 4B). Further, there was a correlation between sAPPα and lifetime number of concussions (unadjusted $r = 0.45$, $p = 0.015$; adjusted $r = 0.35$, $p = 0.071$; figure 4C).

There were no relationships between any of the other biomarkers and lifetime number of concussions (figure e-6).

Association between CSF biomarkers for astroglial activation, amyloid deposition, and BBB impairment and CSF NFL. We have previously measured the neuroaxonal injury marker CSF NFL on most of the study participants.[21] CSF Aβ38 and YKL-40 correlated with CSF NFL (figure e-7). Also, there was a borderline significant correlation between CSF:serum albumin ratio and NF-L (figure e-7).

DISCUSSION Symptoms of cTBI usually resolve within days to weeks.[2] However, a subgroup of individuals with cTBI, particularly those with rcTBI,

Figure 1   Postconcussion syndrome (PCS) due to repetitive concussive traumatic brain injury (rcTBI) is associated with biomarker evidence of astroglial activation
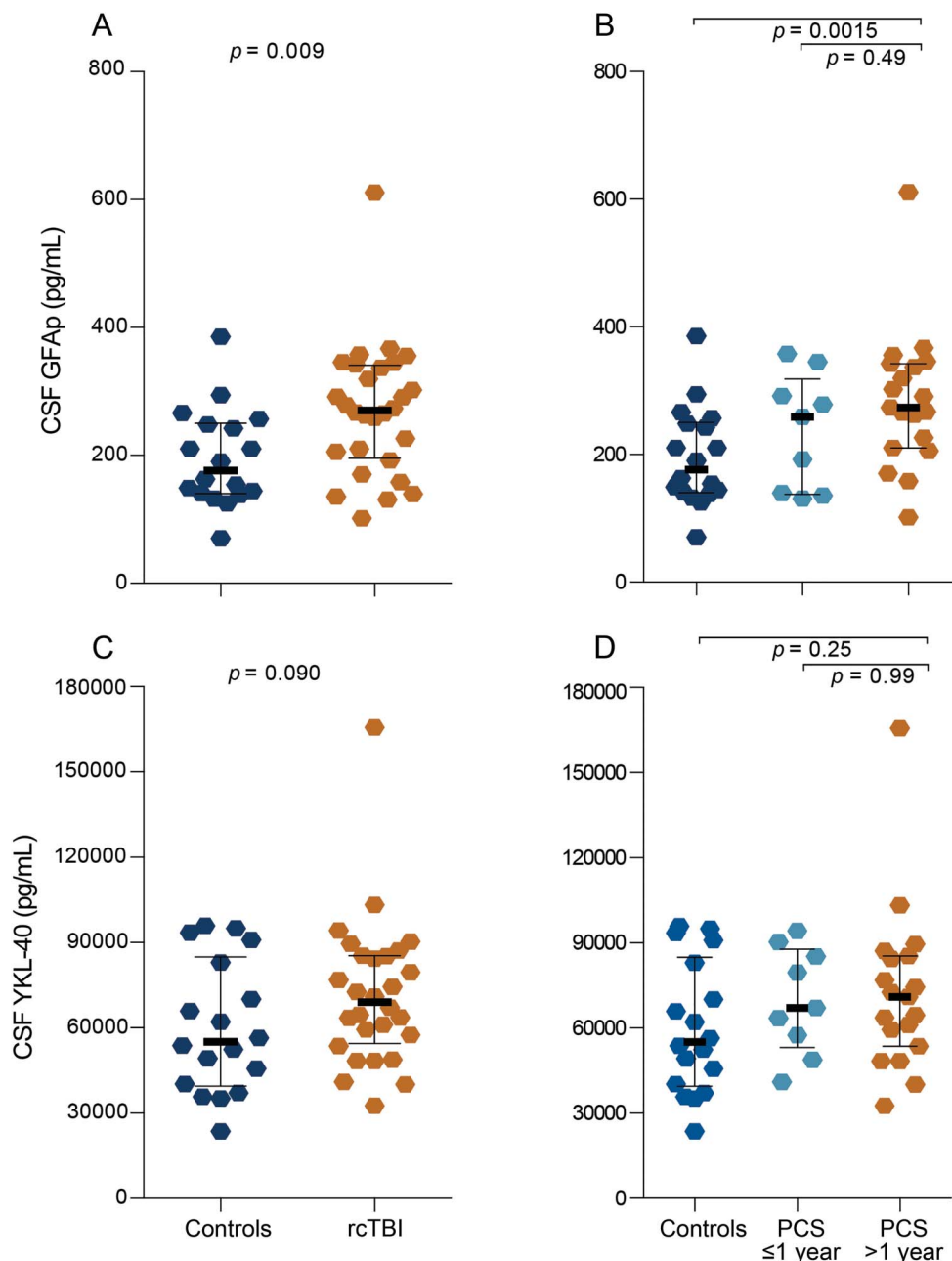
(A) CSF glial fibrillary acidic protein (GFAP) concentration was higher in athletes with rcTBI compared with controls. (B) Athletes with PCS >1 year displayed higher levels of GFAP compared with controls. (C) CSF YKL-40 concentration was also increased in patients with rcTBI compared with controls; however, the increase did not reach statistical significance after correcting for multiple comparison and age. (D) CSF YKL-40 concentrations were not significantly different between groups or subgroups. p Values are adjusted for multiple comparisons. p Values for YKL-40 are also adjusted for age. Values are presented as medians; error bars indicate interquartile ranges.

display persistent behavioral, cognitive, and physical impairment for months, referred to as PCS.[2] A proportion of individuals with PCS may have persistent symptoms beyond months to years, and may develop progressive neurodegeneration or CTE. However, the relationship among concussion, PCS, and future development of CTE is not fully understood. Further, it is unknown whether PCS represents ongoing injury or is a delayed functional outcome following preexisting injury. There are no established objective tools to quantify PCS or identify CTE in living humans, but we recently showed that patients with PCS due to rcTBI had CSF biomarker evidence of axonal injury.[21]

Here, we examined CSF biomarkers reflecting astroglial activation, Aβ metabolism, and BBB integrity in professional athletes who had rcTBI and fulfilled the criteria for PCS. We found that (1) overall, CSF GFAP concentration was higher in patients with rcTBI compared with controls, (2) CSF YKL-40 showed similar changes as GFAP; however, CSF YKL-40 also correlated with RPQ scores and the lifetime number of concussions, (3) CSF concentrations of Aβ40 and Aβ42, but not Aβ38 or sAPPα/β, were

reduced in the rcTBI group, and the lowest concentrations were observed in the subgroup of players with PCS >1 year, and (4) there were no significant changes in the CSF:serum albumin ratio.

Trauma to the head may trigger astrogliosis and microglial activation.[22] In this study, we found increased CSF concentrations of GFAP and YKL-40 in athletes with PCS due to rcTBI. Further, YKL-40 concentration correlated with RPQ score and lifetime concussion events. These findings are in concordance with postmortem studies of boxers and military personnel who had repetitive trauma to the head, showing increased astroglial activation.[3,23]
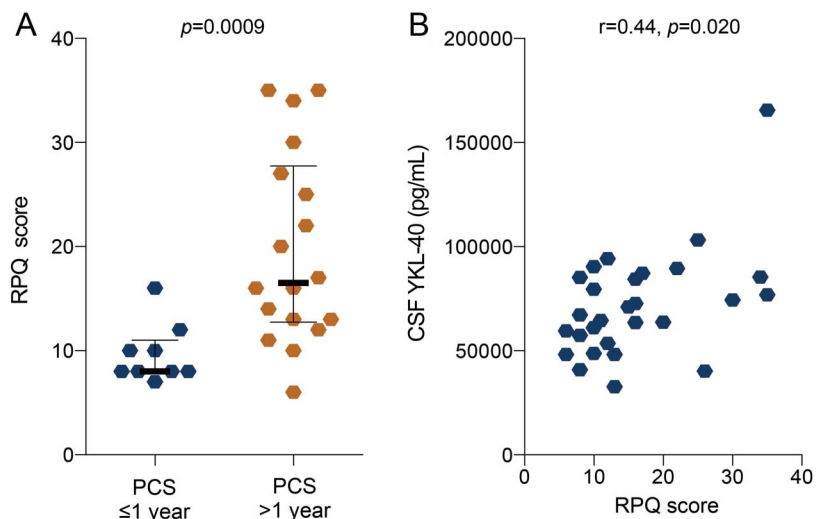
Experimental and postmortem case-control studies suggest that athletes who have been exposed to repetitive head trauma are at increased risk of developing brain pathology, such as Aβ deposition and tau pathology.[3,4,12,13] In the context of AD, numerous studies have shown that reduced CSF Aβ42 concentrations correlate strongly with positive amyloid PET finding,[24] and a recent study also suggests that the reduction in CSF Aβ42 is an earlier indicator of cerebral Aβ deposition than amyloid PET.[25] The findings that CSF Aβ40 and Aβ42 concentrations were

Figure 2 Postconcussion syndrome (PCS) due to repetitive concussive traumatic brain injury (rcTBI) is associated with reduced CSF β-amyloid (Aβ) concentrations
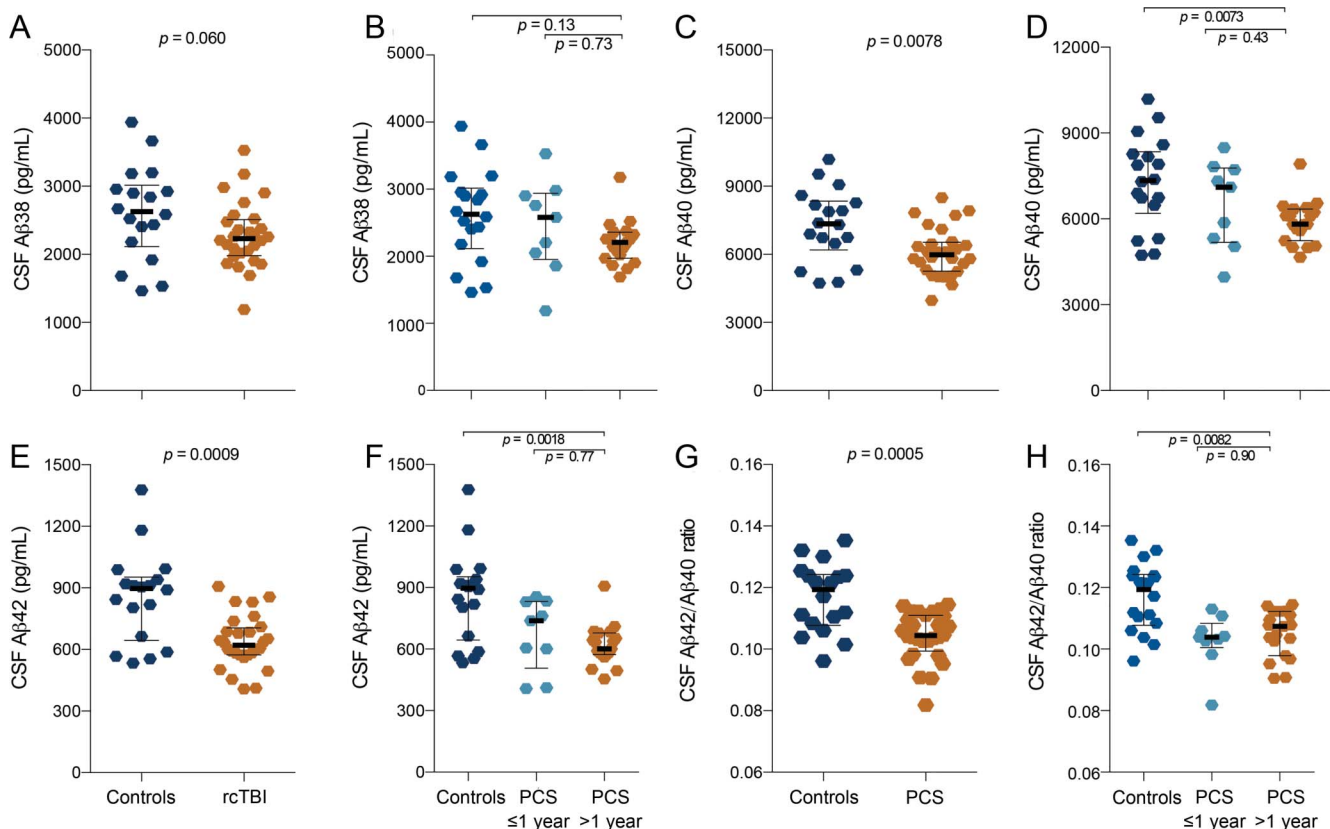
(A–H) CSF concentrations of biomarkers reflect amyloid metabolism in the 28 athletes with PCS due to rcTBI and in a subgroup of athletes with persistent PCS >1 year vs those whose PCS resolved within 1 year. p Values are adjusted for multiple comparisons. p Values for Aβ38 are also adjusted for age. Values are presented as medians; error bars indicate interquartile ranges.

Figure 3   Relationship between biomarker concentrations and symptom severity

(A) Rivermead Post-Concussion Symptoms Questionnaire (RPQ) scores (range 0–64 points) in athletes with postconcussion syndrome (PCS) >1 year vs athletes whose PCS resolved within 1 year. Values are presented as medians; error bars indicate interquartile ranges. (B) The relationship between YKL-40 and RPQ scores, unadjusted for age (adjusted $r = 0.33$, $p = 0.090$).
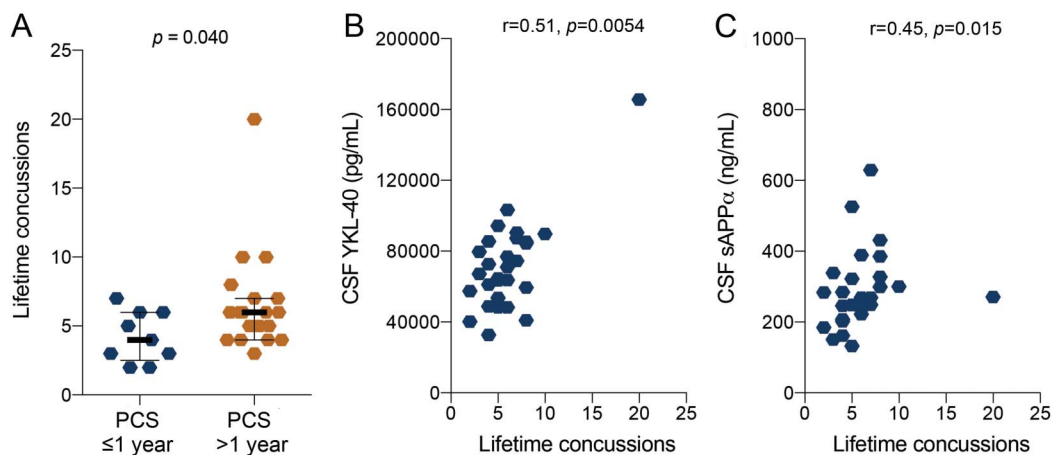
not indicate cerebral Aβ deposition, but rather a more generalized alteration in the Aβ metabolism. The CSF Aβ42/Aβ40 ratio was reduced in the PCS group, but the ratio was still above the cutoff for amyloid positivity we use in clinical laboratory practice (0.09) for most of the athletes with PCS. In addition, we measured concentrations of CSF sAPPα and sAPPβ in order to determine whether the general downregulation of Aβ is due to decreased amyloid precursor protein (APP) expression or processing. However, we did not observe any statistically significant reduction in the concentrations of CSF sAPPα or sAPPβ. Taken together with the Aβ data, at this stage of the disease, this may point towards an alteration of synaptic secretion or γ-secretase-mediated processing of the APP C-terminal fragment, rather than decreased APP expression or altered α- or β-secretase-mediated APP processing. Another potential mechanism we cannot rule out is altered clearance of Aβ peptides from the CNS in the PCS group.

Finally, we observed no difference in the concentration of CSF:serum albumin ratio between the athletes with rcTBI and controls. In acute concussion, CSF:serum albumin ratio is normal, whereas moderate to severe TBI typically has an elevated ratio.[9,10] The results suggest that the BBB remains largely intact in PCS due to rcTBI, although a mild dysfunction cannot be excluded as higher concentrations were observed in athletes with PCS >1 year. It should also be noted that the CSF:serum albumin ratio reflects just one aspect of the BBB: the integrity of the barrier when it comes to restricted passage of medium-sized proteins.

There are limitations to this study. First, the sample size is relatively small, which precludes from

decreased (Aβ38 displayed the same trend) in athletes with PCS due to rcTBI compared with controls, and that the lowest concentrations were observed in players with PCS >1 year, who were also forced to retire, warrant an in-depth discussion. Cerebral Aβ deposition in AD is typified by selective reduction of Aβ42 and unaltered concentrations of Aβ40 and Aβ38.[26] In PCS due to rcTBI, we instead observed a general downregulation of all 3 isoforms, similar to what has been reported in multiple sclerosis and normal pressure hydrocephalus.[27,28] Thus, the results per se do

Figure 4   Relationship between biomarker concentrations and number of lifetime concussions

(A) Number of lifetime concussions in athletes whose postconcussion syndrome (PCS) resolved within a year vs those with persistent PCS >1 year. (B) CSF YKL-40 correlated with lifetime number of concussions (unadjusted $r = 0.51$, $p = 0.0054$; adjusted $r = 0.40$, $p = 0.038$). (C) The CSF α-cleaved soluble amyloid precursor protein (sAPPα) correlated with lifetime number of concussions, unadjusted for age (adjusted $r = 0.35$, $p = 0.071$).

examining the biomarkers in relation to specific aspects of PCS, such as behavioral, cognitive, or neurologic changes. Replication of the findings in larger groups will be important. Second, controls were not matched to the PCS due to rcTBI group; the best controls would have been professional athletes without concussion, however, it is difficult to motivate professional athletes without concussion to undergo LP. The limited age range of the control group is another limitation. In addition, conclusions regarding the precise relationship between rcTBI and PCS cannot be drawn without including a group of concussed athletes who did not develop PCS; a longitudinal study of patients with rcTBI in which PCS is an outcome is warranted.

The data from this study suggest that PCS due to rcTBI is associated not only with axonal injury,[21] but also astroglial activation and Aβ dysmetabolism, apparent in CSF months to years after the last concussion. Whereas these results might not have any immediate clinical implications, they pinpoint astroglial activation and altered Aβ metabolism as being involved in PCS due to rcTBI, which may be relevant to explore further from diagnostic and therapeutic standpoints. The results also suggest that these biomarkers should be examined regarding their accuracy to predict CTE in rcTBI.

AUTHOR CONTRIBUTIONS

Drs. Shahim, Blennow, and Zetterberg had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Drs. Shahim, Tegner, Blennow, and Zetterberg. Acquisition of data: Drs. Shahim, Tegner, and Marklund. Statistical analysis: Drs. Shahim, Blennow, and Zetterberg. Drafting of the manuscript: Drs. Shahim, Blennow, and Zetterberg. Analysis and interpretation of data: Drs. Shahim, Tegner, Höglund, Portelius, Brody Blennow, and Zetterberg. Critical revision of the manuscript for important intellectual content: All authors. Administrative, technical, or material support: Drs. Tegner, Brody, Blennow, and Zetterberg. Obtained funding: Drs. Shahim, Blennow, and Zetterberg. Study supervision: Drs. Tegner, Blennow, and Zetterberg.

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DISCLOSURE

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