Supplementary Online Content

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eMethods.
eTable. Demographic and Clinical Characteristics of Participants at Inclusion.

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethod

Biochemical procedure, neurogranin assay

CSF Ng concentrations were measured using an in-house developed ELISA employing two monoclonal antibodies. In brief, the monoclonal antibodies were developed by immunizing 8-week-old Balb/c mice with the KLH-conjugated peptide Ng52–75 or Ng 63-75 (Caslo, ApS Denmark) in complete Freund's adjuvant (Sigma). Ng22 (with the epitope 63-75) and Ng2 (with the epitope 52-63) were selected for the final assay set up and the method protocol was developed as follows. Nunc maxisorp 96-well microliter plates were coated with 3μg/mL of Ng22 in bicarbonate buffer (pH 9.6) and incubated over night at + 4°C. After washing with PBS (0.01 M phosphate buffer, 0.14 M NaCl, pH 7.4)-Tween (0.05%) the plates were blocked with 200 μL PBS-Tween (0.05%) with 1% BSA. After the second wash, 100 μL of calibrators (full length 1-78 neurogranin (Caslo)), quality control CSF samples and CSF samples was loaded in each well and incubated over night at + 4°C. After washing, 100 μL of the detection antibody, biotinylated Ng2, diluted to 2.7μg/mL in PBS-Tween (0.05% BSA) with 1% BSA, was incubated at room temperature for 1 hour, shaking. After washing, 100μL Enhanced Streptavidin-HRP (Kem-En-Tec Diagnostics) was added in a 1:20000 dilution in PBS-Tween (0.05%) with 1% BSA and incubated for 30 min. After a final wash, 100 μL of substrate (TMB One Substrate, Kem-En-Tec Diagnostics) was incubated in the dark for 20 minutes and the reaction was quenched with 100 μL 2 M H_2SO4. The plate was read at 450 nm (reference wavelength 650 nm) using an ELISA plate reader (Vmax, Molecular Devices, USA) and the calibration curve was plotted using a 4-parameter curve and calculations were made using SoftMax. The assay ranged between 6400 pg/mL and 50 pg/mL, where 50 pg/mL was set as the limit of quantification. Within and between plate coefficients of variability were < 5% and < 13%, respectively.

eTable . Demographic and clinical characteristics of participants at inclusion[a]		
Variable	**PCS (n = 16)**	**Controls (n = 15)**
Age, year	31 (22-53)	25 (21-35)
Sex, male No. (%)	16 (100)	11 (73)
Time since recent concussion, month	4 (3-144)	0
Lifetime number of concussions	6 (2-20)	0
Post-lumbar headache	2	4
Total Rivermead Post-Concussion Symptoms Questionnaire score, range, 0-64	22 (8-35)	0 (0-0)
Abbreviations: PCS = post-concussion syndrome [a]All continuous variable are shown as median (range) unless denoted otherwise.		