Neuroinflammatory Markers in Former Athletes with Repetitive Head Impacts

Lian Lopes Troncoso, PhD[1,2], Mozhgan Khodadadi, MA[1], Chloe Anastassiadis, MSc[1,2], Simrika Thapa, PhD[1,2], Vishaal Sumra, MSc[1,2], Nusrat Sadia[1], Faiza Robbani[4], Pia Kivisäkk, MD, PhD[5,6], Steven E. Arnold, MD[5,6], Robin Green, PhD[1], Charles Tator, MD, PhD[1,3,4] and Maria Carmela Tartaglia, MD, FRCPC[1,2,4]

(1)Canadian Concussion Centre, Toronto Western Hospital, University Health Network, Toronto, ON, Canada, (2)Tanz Centre for Research in Neurodegenerative Diseases, University of Toronto, Toronto, ON, Canada, (3)Department of Surgery, University of Toronto, Toronto, ON, Canada, (4)Krembil Brain Institute, University Health Network (UHN), Toronto, ON, Canada, (5) Harvard Medical School, Boston, Massachusetts, USA, (6) Department of Neurology, Massachusetts General Hospital, Boston, Massachusetts, USA.

Introduction

Background

- Repetitive Head Impacts (RHI), common in contact sports, are associated with neuroinflammation and increased risk for neurodegenerative diseases, including Chronic Traumatic Encephalopathy (CTE).
- Neuroinflammatory changes have been observed in individuals with RHI even before substantial tau pathology is detected, suggesting that immune dysregulation may represent an early event in RHI-related brain injury.
- However, the inflammatory pathways underlying these early alterations remain poorly characterized. Identifying neuroinflammatory markers in individuals with RHI could support detection and mechanistic understanding of RHI-related brain injury.

Objectives

- To identify CSF protein co-expression modules associated with RHI exposure.
- To characterize hub proteins within RHI-associated modules.
- To use machine learning to identify proteins most informative for cognitive and structural outcomes.

Methods

Participants

- **Participants**: 35 former contact sports athletes with a history of repetitive head impacts (RHI) (34M/1F, mean age 55.4) and 26 HC (14M/12F, mean age 63.9) recruited through the CCC brain monitoring research program
- **Clinical assessments**: Cognitive scores (memory & executive function), concussion history
- **Imaging**: Brain MRI (cerebral white matter volume and hippocampal volume)
- **CSF collection**: Lumbar puncture.

MRI

- Structural T1-weighted MRI scans.
- Hippocampal and cerebral white matter volumes extracted for analysis.

Proteomic Profiling

- Olink Explore Inflammation I & II panels.
- Proximity Extension Assay (PEA)-based multiplex proteomics.
- 737 inflammatory proteins measured.
- **425** proteins passed quality control.
- Results expressed as NPX (log_2-scaled normalized protein expression)

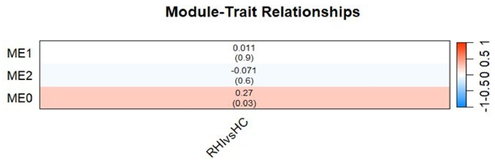
Statistical Analyses

- Weighted Gene Co-expression Network Analysis (WGCNA) to identify coordinated CSF protein modules associated with RHI.
- Random forest regression to identify multivariable protein signatures associated with cognition and MRI measures.
- Significant associations evaluated using false discovery rate (FDR) correction

Results

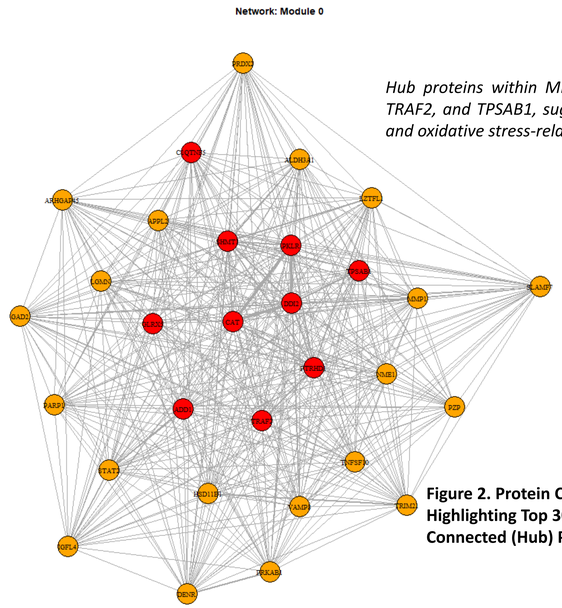
Table 1. Demographics and Clinical Features of former contact sports athletes with RHI and HC Groups

	HC (n = 26) (Mean ± SD)	RHI (n = 35) (Mean ± SD)	
Age (years)	63.9 ±8.5	55.4 ±13.8	**(p<0.05)**
Sex (M/F)	14/12	34M/1F	
Memory function score	NA	-0.23 ±1.2	
Executive function score	NA	0.2 ± 0.8	
Number of concussions	NA	8.9 ± 8.2	
White matter volume (cm^3)	NA	505.3 ± 48.8	
Hippocampal volume (cm^3)	NA	8.4 ± 0.9	
CSF NfL (pg/mL)	668.3 ±274.3	960.3 ±847.3	**(p=0.07)**



WGCNA identified one protein co-expression module associated with RHI exposure (ME0; r = 0.27, p = 0.03). The module included 134 proteins involved in oxidative stress, inflammation, and cellular metabolism.

Figure 1. Network Analysis Identifies an RHI-Associated Protein Module.



Hub proteins within ME0 included CAT, SHMT1, PKLR, GLRX5, TRAF2, and TPSAB1, suggesting coordinated immune-metabolic and oxidative stress-related processes in RHI.

Figure 2. Protein Co-expression Network for Module 0 (ME0) Highlighting Top 30 Hub Proteins, with the Top 10 Most Connected (Hub) Proteins Shown in Red.

➢ **Machine learning signatures**

Table 2. Top Proteins Predicting Memory Function based on Random Forest Feature Importance and Correlation.

Protein	Importance	Correlation	Raw p-value	FDR
IL3RA	0.075057	-0.635	0.000164	0.001636
GAPDH	0.079147	-0.52819	0.002698	0.013492
TTR	0.084025	0.476699	0.007738	0.025793
ADAM12	0.028458	-0.46099	0.010351	0.025879
LCAT	0.019818	-0.411	0.024055	0.048111

Table 3. Top Proteins Predicting Hippocampal volume based on Random Forest Feature Importance and Correlation.

Protein	Importance	Correlation	Raw p-value	FDR
TBCA	0.046967	-0.46625	0.006241	0.029066
FOLH1	0.035763	-0.43638	0.011121	0.029066
PGF	0.030013	-0.5311	0.001472	0.029066
VEGFD	0.024151	-0.45375	0.007996	0.029066
UBXN1	0.021692	-0.45791	0.00737	0.029066
C1QA	0.022333	-0.44406	0.009632	0.029066
PZP	0.017717	-0.43157	0.01215	0.029066
MFAP4	0.017873	-0.42751	0.01308	0.029066
JAM3	0.014908	-0.48728	0.004026	0.029066
UBE2Z	0.017142	0.391374	0.024305	0.048387
CCL7	0.015241	-0.38575	0.026613	0.048387
INHBB	0.013314	-0.37821	0.029991	0.049985

Table 4. Top Proteins Predicting White matter volume based on Random Forest Feature Importance and Correlation.

Protein	Importance	Correlation	Raw p-value	FDR
KLRF1	0.093083	-0.57895	0.000416	0.008314
TNFRSF13C	0.057267	-0.5051	0.002717	0.024757
TLR3	0.025809	-0.48051	0.00465	0.024757
CXCL9	0.016692	-0.47152	0.005605	0.024757
HPCAL1	0.012456	-0.46665	0.006189	0.024757
CCL7	0.012606	-0.43819	0.010753	0.035843
GPI	0.016013	-0.42701	0.013198	0.037708

Key Biological Pathways Identified

- Complement activation (C1QA)
- Chemokine signaling (CCL7, CXCL9)
- Vascular remodeling / BBB dysfunction (PGF, VEGFD, JAM3, MFAP4)
- Innate immune activation (TLR3, KLRF1)
- Metabolic stress and oxidative pathways (GAPDH, GPI)

Conclusion

- Although distinct proteins emerged across analyses, convergent pathways implicated complement activation, chemokine signaling, vascular remodeling, microglial activation, and metabolic dysregulation in athletes with a history of RHI.
- A CSF protein co-expression module associated with RHI highlighted hub proteins involved in immune-metabolic and oxidative stress-related processes.
- These proteins may represent candidate biomarkers of immune-related mechanisms in RHI, but findings from this exploratory cross-sectional study require validation in larger and longitudinal cohorts.

References

1. **McKee et al., 2016** Repetitive Head Impacts and Chronic Traumatic Encephalopathy. *Neurosurgical Clinics of North America.* 2016 Oct;27(4):529–535. doi: 10.1016/j.nec.2016.05.009
2. **Butler et al., 2025** Repetitive head impacts induce neuronal loss and neuroinflammation in young athletes. *bioRxiv* 2024 Mar 28; updated 2025 Feb 10 as 2024.03.26.586815. doi: 10.1101/2024.03.26.586815
3. **Bindra et al., 2025** Neuroinflammatory mechanisms may help identify candidate biomarkers in chronic traumatic encephalopathy (CTE). *Free Neuropathology.* 2025 Jul 14;6:15. doi: 10.17879/freeneuropathology-2025-6382
4. **Cho, 2019** Emerging Roles of Complement Protein C1q in Neurodegeneration. *Aging and Disease.* 2019 Jun 1;10(3):652–663. doi: 10.14336/AD.2019.0118
5. **Nataf et al., 2019** Common Neurodegeneration-Associated Proteins Are Physiologically Expressed by Human B Lymphocytes and Are Interconnected via the Inflammation/Autophagy-Related Proteins TRAF6 and SQSTM1. *Frontiers in Immunology.* 2019 Nov 25;10:2704. doi: 10.3389/fimmu.2019.02704
6. **Zhang et al., 2023** Role of neuroinflammation in neurodegeneration development. *Signal Transduction and Targeted Therapy.* 2023 Jul 12;8(1):267. doi: 10.1038/s41392-023-01486-5

email: lian.lopestroncoso@uhn.ca