Altered Blood Biomarker Profiles in Athletes with a History of Repetitive Head Impacts

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Abstract

The long-term health effects of concussion and sub-concussive impacts in sport are unknown. Growing evidence suggests both inflammation and neurodegeneration are pivotal to secondary injury processes and the etiology of neurodegenerative diseases. In the present study we characterized circulating brain injury and inflammatory mediators in healthy male and female athletes according to concussion history and collision sport participation. Eighty-seven university level athletes (male, n = 60; female, n = 27) were recruited before the start of the competitive season. Athletes were healthy at the time of the study (no medications, illness, concussion or musculoskeletal injuries). Dependent variables included 29 inflammatory and 10 neurological injury analytes assessed in the peripheral blood by immunoassay. Biomarkers were statistically evaluated using partial least squares multivariate analysis to identify possible relationships to self-reported previous concussion history, number of previous concussions and collision sport participation in male and female athletes. Multiple concussions were associated with increases in peripheral MCP-1 in females, and MCP-4 in males. Collision sport participation was associated with increases in tau levels in males. These results are consistent with previous experimental and clinical findings that suggest ongoing inflammatory and cerebral injury processes after repetitive mild head trauma. However, further validation is needed to correlate systemic biomarkers to repetitive brain impacts, as opposed to the extracranial effects common to an athletic population such as exercise and muscle damage.

Introduction

Concern regarding the potential negative health impact of concussions and collision sport participation has led to an increased demand to delineate the pathophysiological mechanisms mediating long-term outcomes [1]. Our current conceptual understanding of concussion

pathophysiology consists of an acute disturbance of neurobehavioral function together with damage to neuronal and glial cells [2]. Symptoms are commonly short-lived and self-limited, resolving within a span of days to weeks [3–5]; however, recent objective advances in neuroimaging and analytical biomarker assessment have documented underlying functional and structural abnormalities persisting beyond symptom resolution [6–8]. Furthermore, evidence is now emerging that suggests concussion, as well as the repetitive head impacts that commonly occur in collision sport participation, may contribute to negative health outcomes such as chronic traumatic encephalopathy (CTE) [9–14]. However, our current understanding of these pathophysiological processes in humans is limited.

Inflammation is an important contributor to both repair and neurodegenerative processes after neurotrauma [15–17]. Resident microglial cells and central nervous system (CNS) invading peripheral immune cells facilitate the acute repair and regeneration of damaged brain tissue via the release of neurotrophic factors and scavenging of debris [18–20]. However, chronic inflammation may also exacerbate neuronal and glial cell injury, leading to further cellular degeneration and culminating in the deposition of neurofibrillary tangles and amyloid plaques [18]. In view of this, human studies have found prolonged neuroinflammation persisting for months to years after moderate and severe traumatic brain injury (TBI) [21–24], and experimental evidence suggests these maladaptive processes may occur through the interaction of inflammatory mediators and glutamate receptors in the CNS [25–28]. Moreover, multiple head impacts may worsen these processes by priming microglial cells, leading to an exaggerated inflammatory reaction upon subsequent trauma [18,29].

Inflammation post-concussion is difficult to characterize due to practical limitations such as the inability to access tissue proximal to the site of injury, and the invasive nature of cerebral spinal fluid (CSF) acquisition [30]. Nevertheless, peripheral blood samples have the potential to provide meaningful information regarding inflammatory processes both in the CNS and periphery in response to brain injury, in a relatively cost-effective, non-invasive manner [18,30,31]. In view of this, recent evidence has shown that increased circulating C-reactive protein levels post-injury are associated with persistent post-concussive syndrome symptoms [32], and coated platelet levels, an inflammatory correlate, are elevated in mild TBI patients up to 9 years post-injury [33].

Historically, one of the limitations in concussion research has been the lack of consideration for potential sex differences. Specifically, there has been a paucity of concussion research on females [34]. Yet, available evidence suggests that females may be at a greater risk for concussion [35,36], report more symptoms post-concussion [35,37], and take longer to recover [35,38]. In addition, it is known that males and females display distinct immunological responses; women exhibit stronger cellular and humoral immune responses, are more prone to many autoimmune diseases, but are less susceptible to various of bacterial, viral, and fungal infections [39,40]. Therefore, the possibility exists that inflammatory related processes occurring chronically after concussion may have sex-specific pathological sequelae.

Thus, in this study we set out to examine a panel of systemic brain injury markers and inflammatory mediators in a sample of male and female athletes to characterize the relationship between these biological indices, concussion history, and collision sport participation.

Methods

Participants

Participants were recruited from University of Toronto intercollegiate "varsity" athletic teams between August 2014 and December 2015. A member of the research team provided an overview of the study and requested consent to obtain blood samples and use the Sport Concussion

Assessment Tool 3 (SCAT3) results for research purposes. Medical history was obtained by the team's therapist/trainer, followed by administration of the SCAT3. Sixteen teams (8 male, 8 female) were contacted for research purposes, including the following sports: basketball, baseball, field hockey, football, ice hockey, lacrosse, rugby, soccer, wrestling and volleyball. Athletes were excluded if they suffered from seasonal allergies, cold, infection, disclosed any inflammatory-related health conditions, were taking any medications other than birth control at the time of the study, or had musculoskeletal injuries (9 subjects). Study procedures were approved by the Health Sciences Research Ethics Board, University of Toronto (protocol reference # 27958), and all participants provided written informed consent prior to the study.

Measures

Sport Concussion Assessment Tool 3 (SCAT3): The SCAT3 combines aspects of several previously published concussion tools into eight components designed to assess concussion symptoms (number endorsed and severity), cognition (Sideline Assessment of Concussion or SAC and Maddocks questions), balance (firm conditions of the Balance Error Scoring System or BESS), Glasgow Coma Scale (GSC) and neurological signs (physical signs, coordination) [41]. Each of the eight components are scored and recorded. The symptom score is comprised of a 22-item post-concussion symptom scale using a seven-point Likert scale rating. Symptom severity is obtained by summing the rated symptom score for each symptom [39]. This symptom scale has been shown to be reliable and valid for the assessment of both symptom presence and severity [37,41,42].

Blood Sample Collection

Venous blood samples were drawn from athletes after consent was obtained and prior to the beginning of the competitive varsity season. Samples were drawn into a 10-mL K_2EDTA (with 4mM sodium metabisulfite [$Na_2S_2O_5$]) or 4-mL non-additive (Vacutainer, Becton Dickinson, NJ, USA) tube. Within one hour, specimens were centrifuged at 1600 x g for 15 minutes at 4°C, and the plasma supernatant was aliquoted and frozen at -70°C until analysis.

Biomarker Analysis

Twenty-eight of the thirty-nine markers were analyzed using MSD® 96-Well MULTI-ARRAY/-SPOT® V-plex Human Immunoassay Kits purchased from MSD (MD, USA), and run on a Meso-Scale Discovery (MSD®) Sector imager™ 6000 with Discovery Workbench software (version 3.0.18). A prototype assay panel of eleven additional neuroinjury markers including total tau, glial fibrillary acidic protein (GFAP), s100 calcium-binding protein (s100) B, neuron specific enolase (NSE), Neurogranin (NRGN), creatine kinase-BB isoenzyme (CKBB), visinin-like protein (VILIP)-1, von Willebrand factor (vWF), brain derived neurotrophic factor (BDNF), peroxiredoxin (PRDX)-6, and monocyte chemoattractant protein (MCP)-1, was assessed by multiplexed immunoassay [43].

Statistical Analyses

Demographic and descriptive statistics were completed on male and female athletes by student's independent *t*-test Mann Whitney *U*, or χ^2, where appropriate. For dichotomized analysis of collision vs. non collision sports, collision sports were delineated as sports with purposeful contact as an inherent part of the game, and included men's ice hockey, football, rugby, lacrosse, and women's rugby [44]. All other sports, including those where inadvertent contact may occur (soccer, basketball), were considered non-collision sports [44]. For all

analyses, individual biomarker values were excluded if they were above or below the manufacturers' recommended level of quantitation for each analyte, or displayed a coefficient of variance >25% between duplicates. Because multiple 96-well plates were analyzed, inter-plate variance was accounted for; plates were only included in the statistical analysis if the inter-plate variance was <20%, calculated from internal control samples acquired on each plate. Biomarkers were not included in the multivariate analysis if >30% of the data points were missing in any group. Multivariate analysis was conducted using a partial least squares discriminant (PLS-DA). PLS-DA is a supervised technique used to objectively characterize the covariance between a set of predictor variables and binary response variables [45,46]. A PLS-DA output provides model prediction accuracy (Accur) and posterior probability (PProb). Briefly, these indices measure how accurately a fitted model can predict a binary outcome based solely on predictor variables. Accur is evaluated by assigning each subject to the outcome group with the most similar mean PLS score; 1 = correctly predicted, and 0 = incorrectly predicted. This provides a simple, robust metric of prediction, which does not depend on a specific probability model. PProb is the likelihood of the PLS model identifying the correct outcome conditional on the observed subject scores, under a Gaussian noise model. This provides an alternative probabilistic measure that accounts for uncertainty in the PLS model and observed data. With numerous response variables, the PLS analysis yields the fraction of variance explained. Fraction of variance reflects the proportion of total inter-subject variability in biomarker data that is described by the PLS component of interest. In the current study, covariance between peripheral blood biomarkers (predictor variables) and both concussion history and collision sport participation (response variables) was assessed separately in male and female athletes. Missing biomarker values were imputed using the k-means nearest-neighbour method [47], and were rank-transformed to ensure robustness against non-normality. Significant biomarker loadings were identified by performing bootstrap resampling on subjects (1000 iterations) to obtain empirical p-values, which were then corrected for multiple comparisons at a false discovery rate (FDR) of 0.05. For PLS plots, variable loadings are represented as bootstrap ratios (i.e., the bootstrapped mean / standard error), which are z-scored statistics reflecting the reliability of variable contributions. Descriptive and univariate statistics were completed using Stata Version 14.1 (StataCorp, TX, USA). Multivariate analyses were conducted using in-house software developed for Matlab, Version R2015b (Matworks, Natick MA). All data were visualized using GraphPad Prism Version 6.0f (GraphPad Inc., CA, USA).

Results

Demographics and Clinical Characteristics

A total of 87 athletes were included in the study (male, n = 60; female, n = 27). Athlete characteristics and concussion history are listed in Table 1. Briefly, athletes were of similar age, and we observed no significant differences in medical history and SCAT3 symptoms at the time of the study. There were no differences between male and female athletes regarding concussion history, number of previous concussions, and days since last concussion. As expected, a significantly higher proportion of males played in collision sports as compared to their female counterparts (63.9% vs. 7.4%, respectively).

Systemic inflammatory marker analysis

A list of all biomarkers with corresponding median values and the percent of samples detectable in the plasma for each analyte are listed in Table 2. No significant differences were identified between male and female athletes who did not participate in collision sports or who had no previous history of concussion (data not shown).

Table 1. Athlete demographics and characteristics.

Characteristic	Male (n = 60)	Female (n = 27)	P value
Age (years)	19.5 ± 2.0	19.5 ± 1.8	0.86
Concussion history–n (%)	23 (38.3)	12 (44.4)	0.55
Days since last concussion–median (IQR)	793 (420–1249)	552 (375.5–714.5)	0.170
Number of previous concussions	0.64 ± 1.0	1.1 ± 1.7	0.619
0 –n (%)	37 (61.7)	15 (55.6)	
1 –n (%)	12 (20.0)	5 (18.5)	
2 –n (%)	8 (13.3)	3 (11.1)	
≥ 3 –n (%)	3 (5.0)	4 (14.8)	
Collision sport participation–n (%)	39 (65.0)	2 (7.4)	**<0.001**
Medical history–n (%)			
Migraines	2 (3.3)	0 (0.0)	0.156
Learning disability	1 (1.7)	0 (0.0)	0.203
Depression/Anxiety or other psychiatric disorders	1 (1.7)	2 (7.4)	0.226
Family history of psychiatric illness	12 (20.0)	7 (25.9)	0.247
SCAT3 symptom scores			
Total symptoms	3.4 ± 3.6	3.8 ± 3.0	0.350
Symptom severity	5.4 ± 7.0	6.0 ± 4.7	0.15

Unless otherwise stated, results are reported as the mean ± standard deviation (SD).

Demographic and characteristic differences between male and female athletes were assessed by χ^2, Mann-Whitney U, or independent student's t-test, where appropriate.

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Multivariate analysis. PLS analysis of the covariance between peripheral blood biomarkers and athlete characteristics is shown in Fig 1. No individual biomarkers were significantly correlated to previous concussion history in either male (model PProb = 0.50, Accur = 0.48) or female (model PProb = 0.41, Accur = 0.35) athletes (Fig 1A). Similarly, when further stratified, compared to athletes with no concussion history, athletes with one previous concussion displayed no significant differences in biomarker levels (males–model PProb = 0.47, Accur = 0.46; females–model PProb = 0.46, Accur = 0.46) (Fig 1B). However, in athletes with multiple previous concussions vs. those with no previous concussions (males–model PProb = 0.53, Accur = 0.51; females–model PProb = 0.43, Accur = 0.44), female athletes had significantly higher MCP-1 (median conc.; 96.4 vs. 69.3 pg/mL) levels, while male athletes had significantly higher MCP-4 (median conc.; 48.3 vs. 26.1 pg/mL) (Fig 1C). See S1 Table. for plasma concentrations of all biomarkers according to concussion history.

PLS analysis of the covariance between systemic biomarkers, and both collision sport participation and previous concussion history in males is shown in Fig 2. Only collision sport participation significantly co-varied with increases in tau (median conc; 33.9 vs. 20.8 pg/mL in non-collision sport athletes). See S2 Table. for plasma concentrations of all biomarkers according to collision sport participation.

Discussion

In this study we identified differences in the systemic biomarker profiles of male and female athletes who sustained multiple previous concussions, and in males who participate in collision sports. We included blood samples from athletes with no inflammatory-related conditions, musculoskeletal injuries or concussion symptoms prior to the start of the competitive season.

Table 2. List of biomarkers analyzed.

Markers (pg/mL)*	% Quantifiable[a]	Median (IQR)
Cytokines		
IL-1α	27.6	—
IL-1β	0	—
IL-2	0	—
IL-4	1.1	—
IL-5	0	
IL-6	2.3	—
IL-7	59.8	2.6 (2.0–3.7)
IL-10	6.9	—
IL-12p40	97.7	121.4 (93.1–146.2)
IL-12p70	0	—
IL-13	0	—
IL-15	100	2.3 (2.0–2.7)
IL-16	70.1	259.2 (198.5–369.0)
IL-17A	1.1	—
TNF-α	96.5	1.8 (1.5–2.2)
TNF-β	0	—
GM-CSF	0	—
VEGF	87.3	36.5 (28.0–55.6)
IFN-γ	16.1	—
Chemokines		
Eotaxin	91.9	77.7 (62.7–94.3)
Eotaxin-3	69.3	22.2 (18.5–31.3)
IP-10	77.0	202.6 (159.7–257.1)
IL-8	82.8	1.9 (1.5–2.7)
MCP-1	96.6	86.8 (72.4–109.4)
MCP-4	94.2	26.5 (19.5–38.3)
MDC	98.8	807.2 (706.3–989.1)
MIP-1α	4.6	—
MIP-1β	95.4	37.9 (30.3–49.6)
TARC	88.5	43.1 (27.3–55.5)
Neuroinjury Markers		
s100B	85.0	707.1 (603.0–896.1)
GFAP	59.8	75.6 (63.2–98.1)
NSE (ng/mL)	100	1.5 (1.2–2.1)
Neurogranin (ng/mL)	100	7.8 (4.5–11.8)
CKBB	11.5	—
VILIP-1	8.0	—
Tau	98.8	24.0 (18.5–32.9)
vWF (μg/mL)	96.5	38.2 (23.3–53.5)
BDNF	100	856.4 (566.4–2022.7)
PRDX-6 (ng/mL)	100	26.4 (18.6–33.2)

Interleukin (IL)-1α, -1β, -2, -4, -5, -6, -7, -10, -12p40, -12p70, -13, -15, -16, -17A, tumor necrosis factor (TNF) -α, -β, granulocyte macrophage colony-stimulating factor (GM-CSF), vascular endothelial growth factor (VEGF), interferon-gamma (IFN-γ), eotaxin, eotaxin-3, interferon gamma-induced protein (IP) -10, IL-8. monocyte chemoattractant protein (MCP)-1, -4, macrophage derived chemokine, (MDC), macrophage inflammatory protein (MIP)-1α, -1β, thymocyte- and activation-regulated chemokine (TARC), s100 calcium binding protein beta (s100B), glial fibrillary acidic protein (GFAP), neuron specific enolase (NSE), creatine kinase-BB isoenzyme (CKBB), visinin-like protein (VILIP-1), von Willebran factor (vWF), brain derived neurotrophic factor (BDNF), peroxiredoxin (PRDX) -6.

* = all markers reported as pg/mL unless otherwise stated

[a] = Biomarkers were included if replicates had less than a 25% CV, were within the LLOQ and ULOQ, and had an inter-plate variance of less than 20% as measured by internal controls.

"—" = below assay quantitation in ≥50% of samples analyzed.

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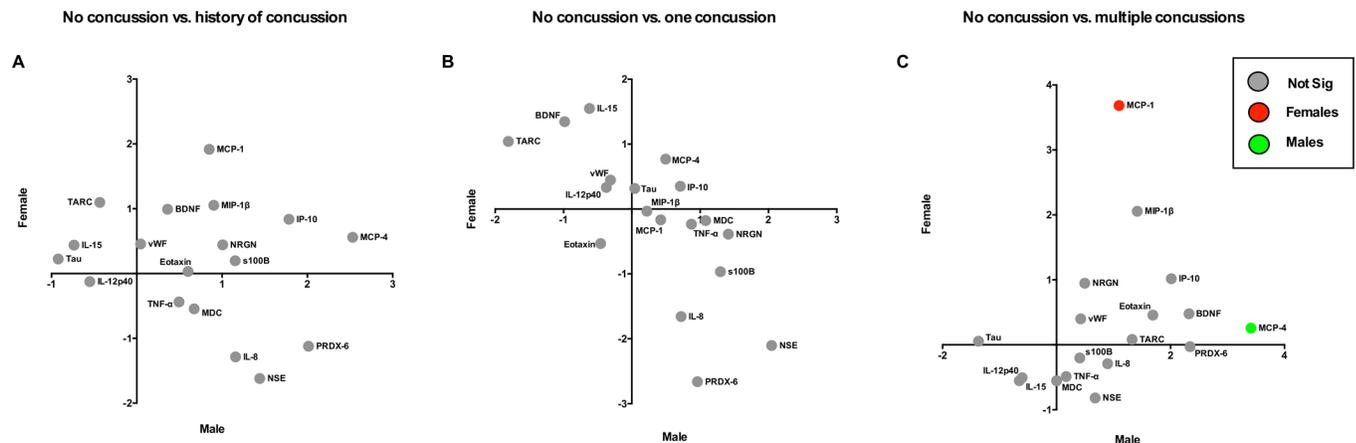
Biomarker contributions to concussion history

Fig 1. Biomarker covariance with concussion history in athletes. *brain injury markers*: s100 calcium-binding protein B (s100B), neuron specific enolase (NSE), Neurogranin (NRGN), tau, von Willebrond factor (vWF), brain derived neurotrophic factor (BDNF), peroxiredoxin (PRDX)– 6; *inflammatory markers*: interleukin (IL) -12p40, -15, tumor necrosis factor (TNF) -α, IL-8, monocyte chemoattractant protein (MCP)-1, -4, interferon gamma induced protein (IP) -10, macrophage derived chemokine (MDC), macrophage inflammatory protein (MIP)-1β, thymus and activation regulated chemokine (TARC), eotaxin. Blood biomarker contributions are displayed on the x-axis for males, and y-axis for females, in (A) healthy athletes with vs. without a history of concussion, (B) healthy athletes with a single previous concussion vs. no history of concussion, and (C) healthy athletes with multiple previous concussions vs. no history of concussion. Dots represent z-scores derived from individual bootstrapped loadings divided by the standard error of the mean. FDR = 0.05.

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To our knowledge, this is the first report to combine an array of brain injury-related and inflammatory indices chronically after sport concussion in male and female athletes.

We found healthy female athletes with a reported history of multiple concussions had elevated blood MCP-1 levels, while males had elevations in MCP-4. Chemokines are important facilitators of peripheral immune cell migration to the CNS after injury [17], and may contribute to BBB breakdown [48]. Treatments aimed at alleviating inflammation after TBI by inhibiting chemokine recruitment to the brain have been successful in reducing cerebral damage and cognitive deficits in animals [49,50]. Furthermore, MCP-1 and MCP-4 levels are elevated acutely after moderate and severe TBI in humans, and correlated to poor patient outcome [51,52]. While systemic chemokines have not been assessed chronically after concussion, these results are consistent with previous evidence of persistent inflammation months after mild TBI in both animals [31,53] and humans [33]. Admittedly, it is difficult to speculate whether these findings represent detrimental or reparative processes, as chemokines may also aid in neuronal repair and regenerative axonal sprouting [17,54]. Furthermore, it is unclear if MCP-1 and MCP-4 share overlapping or distinct biological actions in response to brain injury. While both molecules are involved in leukocyte recruitment, they may differ in their ability to stimulate other inflammatory mediators; for example, MCP-4 but not MCP-1 is responsible for mediating the production of chemokines IP-10 and the platelet derived chemokine ligand -5, during atherogenesis [55]. Hence, further research is needed to elucidate both the biological sequelae and health consequences of elevated systemic chemokine levels after multiple concussions in males and females.

A second important finding was tau concentrations were higher in male athletes who participate in collision sports compared to non-collision sport athletes. Additionally, when assessed in conjunction with collision sport participation, previous concussion history became a non-significant contributor to biomarker variance. This suggests that the repetitive sub-concussive

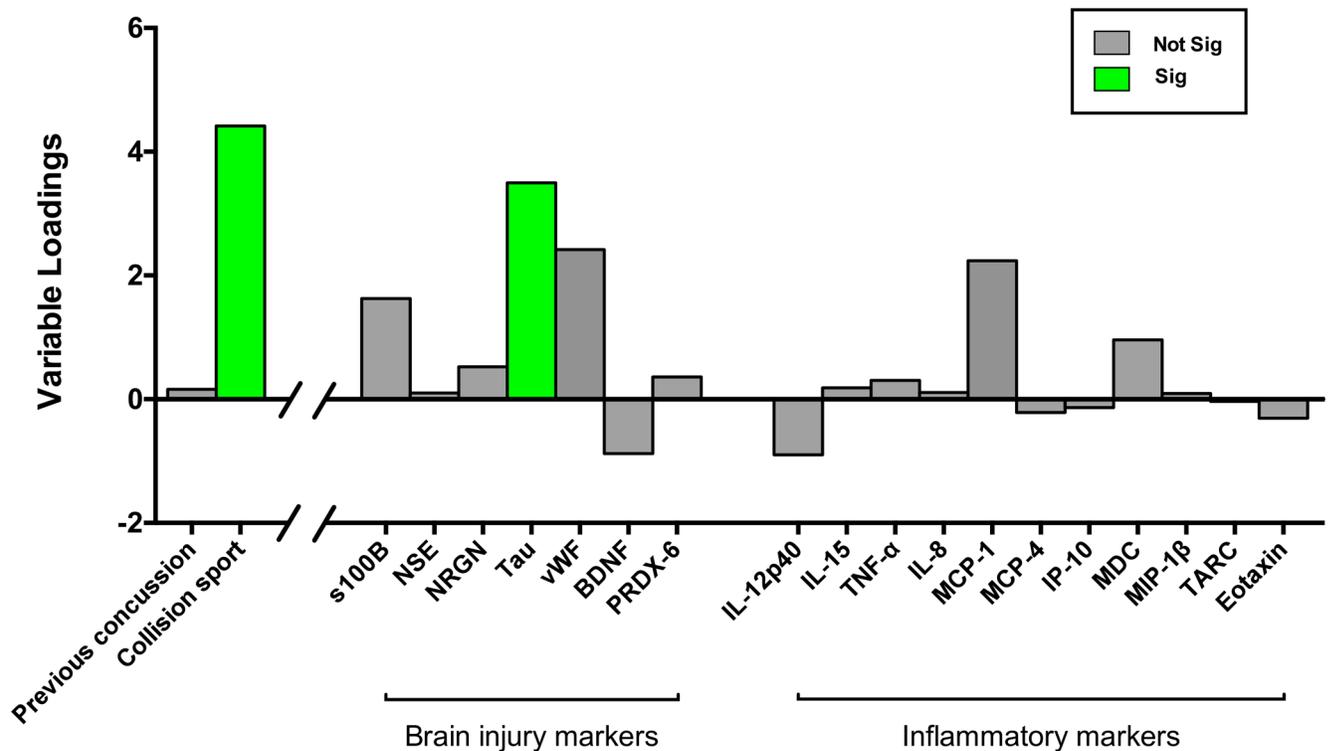## Covariance between biomarkers and head injury characteristics in male athletes

Fig 2. Covariance between biomarkers and head injury characteristics in male athletes. *brain injury markers*: s100 calcium-binding protein B (s100B), neuron specific enolase (NSE), Neurogranin (NRGN), tau, von Willebrond factor (vWF), brain derived neurotrophic factor (BDNF), peroxiredoxin (PRDX)– 6; *inflammatory markers*: interleukin (IL) -12p40, -15, tumor necrosis factor (TNF) -α, IL-8, monocyte chemoattractant protein (MCP)-1, -4, interferon gamma induced protein (IP) -10, macrophage derived chemokine (MDC), macrophage inflammatory protein (MIP)-1β, thymus and activation regulated chemokine (TARC), eotaxin. Bars represent z-scores derived from individual bootstrapped loadings divided by the standard error of the mean. FDR = 0.05.

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impacts associated with collision sport participation may elicit a greater biological response than reported concussion, and could have a distinct pathology. Concern regarding collision sport participation and the potential link to neurodegeneration has been highlighted in recent years as tau-laden plaque depositions have been identified in the brains of post-mortem [11] and living [9] former collision-sport athletes. We found collision-sport participation in male athletes was associated with a 62% increase in peripheral tau levels compared to males who participate in non-collision sports. Previous studies have also found elevated plasma and CSF tau levels in ostensibly non-concussed male boxers [56,57] and in military personnel who sustain multiple mTBI's during deployment [58]. Regarding the latter, tau levels were elevated in soldiers with a self-reported history of concussion, and similar to the current study, participants were sampled within a time-frame of 3 months to 3 years post-injury [58]. While it is unclear if systemic tau is pathologically related to neurodegeneration or cerebral injury, recent findings have specifically identified plasma exosomal tau as a potential CTE biomarker in former professional athletes [59], and have detected associations between plasma tau and clinical conditions such as Alzheimer's Disease [60,61] and mTBI [62]. Taken together our results are

consistent with these previous works, and suggest that systemic tau may be related to repetitive, sub-concussive impacts in male collision-sport athletes.

Brain-borne biomarkers may travel from the CNS into the periphery in at least two distinct fashions, through a disrupted/leaky blood brain barrier (BBB) [48,63], or via the glympathic system [64,65]. Regarding the latter, alterations to glymphatic function caused by clinical maladies including TBI and sleep deprivation, may attenuate the movement of proteins from the brain to the blood [55]. Yet, this process does not affect the passage of molecules across a leaky/damaged BBB [65], and while the athletes evaluated in the current study were not concussed, repetitive head impacts may alter BBB integrity and increase permeability [66,67]. Hence, it is plausible that the biomarkers we identified peripherally may be related to ongoing biological processes linked to repetitive head impacts [68,69].

An important question stemming from these findings is how the observed elevations in these indirect peripheral measures may relate to the biological consequences of repetitive head trauma, as opposed to the effects of confounding factors common to an athletic population such as exercise and/or peripheral injury. We recognize numerous inflammatory mediators, including MCP-1, may be elevated in both the plasma and skeletal muscle for hours after a single bout of exercise [70,71]. Although the time after the last exercise bout and duration/intensity were not recorded, this study was conducted during pre-season training, and we can therefore assume that all athletes (collision and non-collision) had been physical active within 72 h of blood sampling. Hence, any potential confounding effects of exercise are likely common to both groups of athletes. Furthermore, while our study design intentionally excluded athletes with musculoskeletal injuries, the physical demands of collision-sport participation may have the potential to influence biomarker concentrations. For example, tau is expressed in extracranial rat tissues [72], and in the muscle fibers of patients with inflammatory myopathy [73,74]. As tau is released from neurons as a by-product of cell death [75], muscle damage/turnover may result in the extracellular release of tau. Hence, despite being sampled before the onset of the competitive season in athletes absent overt musculoskeletal injuries, we cannot rule out the effect of pre-season training. Future studies are needed to evaluate potential extracranial release of these biomarkers, particularly from damaged/injured muscle tissue.

Though we did not identify differences in a number of previously identified TBI inflammatory markers such as IL-1β, IL-6 and IL-10, these markers have typically been evaluated in the acute stages after severe TBI [52,76–78]; conversely, our cohort was ostensibly healthy, and the median time from last concussion was approximately two years (Table 1). Furthermore, while numerous cytokines have been found elevated for up to three months after severe TBI [79], few studies have evaluated the chronic inflammatory response after concussion. Yet, Prodan and colleagues found platelet activation in previously concussed military personnel ranging from 6 months to 9 years post-injury [33], and in a follow-up study, identified a positive correlation between this inflammatory correlate and the number of concussions sustained [80]. While these previous works evaluated military personnel and included mechanistically distinct blast-related concussion, the results are consistent with our findings, and suggest that biological perturbations resulting from multiple head injuries are evident systemically up to years after injury.

In the current study, the differences identified in biomarker signatures between male and female athletes after multiple concussions is supportive of the previously noted sex-differences in immunobiology [39,40], and aligned with prior evidence of sex-differences in concussion recovery [3,35,38]. Although it is difficult to speculate on the biological basis of these findings, the potency of male and female sex hormones to differentially mediate inflammatory responses represents a plausible explanation [81]. The sexually dimorphic neurochemical composition of the brain may contribute to divergent responses to brain injury [82], leading to a different

complement of proteins appearing in the blood. However, in addition to the pleiotropic effects of systemic inflammatory indices, and chemokines in particular, the gap in sex-based inflammation research in TBI makes interpretation of our results difficult. Yet, these findings necessitate sex-stratification in future concussion study cohorts, as potentially distinct mechanisms mediating the long-term effects of multiple head impacts may exist.

A limitation of the study was the cross-sectional design, therefore, we lacked the ability to evaluate inflammatory marker levels prior to injury in the athletes with a history of concussion. Furthermore, a larger sample size with additional female athletes who participate in collision sport (i.e., rugby) would allow the evaluation of biomarkers in collision vs non-collision sports. As previously identified, we were unable to control for the potential confounding effects of exercise on biomarker levels; while the homogeneity of our population suggests both collision and non-collision sport athletes were presumably similar in their exercise habits, the ability to quantify the duration and intensity of exercise and how this may have affected any of the markers assessed would have strengthened our results. Finally, while the SCAT3 is the most utilized evaluation tool in the sport context, it is a crude measure of cognitive abilities, and we recognize its comparative limitations to more advanced neuropsychological tests. However, despite these limitations, our results demonstrate potentially sex-specific systemic inflammatory alterations in athletes with multiple previous concussions, and in males who participate in collision sports.

Conclusion

Collision sport participation in male athletes is associated with alterations in brain injury-related and inflammatory blood biomarkers. Specifically, multiple previous concussions are associated with elevations in MCP-1 in female athletes, and MCP-4 in male athletes. Furthermore, collision sport participation displays a greater covariance with systemic biomarkers compared to that of concussion history, and is specifically associated with increases in tau. Future studies are required to identify the source and biological relevance of systemic biomarkers in athletes who have sustained repetitive head trauma and who participate in collision sports, in order to better understand and characterize the potential health consequences. Particular attention should be paid to sex differences, as well as extracranial sources of biomarkers related to muscle damage and exercise.

Supporting Information

S1 Table. Biomarker values according to concussion history.
(DOCX)

S2 Table. Biomarker values in athletes stratified by collision sport participation.
(DOCX)

Acknowledgments

Author Contributions

Conceived and designed the experiments: AD SR MH AB DR. Performed the experiments: AD MH. Analyzed the data: AD MH SR NC. Contributed reagents/materials/analysis tools: MH SR DR AB. Wrote the paper: AD SR DR NC AB MH.

References

1. Blennow K, Hardy J, Zetterberg H. The Neuropathology and Neurobiology of Traumatic Brain Injury. Neuron. 2012; 76(5):886–99. doi: 10.1016/j.neuron.2012.11.021 PMID: 23217738

2. Giza CC, Hovda DA. The new neurometabolic cascade of concussion. Neurosurgery. 2014 Oct 3; 75 Suppl 4:S24–33. doi: 10.1227/NEU.0000000000000505 PMID: 25232881

3. Broglio SP, Cantu RC, Gioia GA, Guskiewicz KM, Kutcher J, Palm M, et al. National Athletic Trainers' Association position statement: management of sport concussion. J Athl Train. 2014 Jan 3; 49(2):245–65. doi: 10.4085/1062-6050-49.1.07 PMID: 24601910

4. McCrea M, Guskiewicz KM, Marshall SW, Barr W, Randolph C, Cantu RC, et al. Acute effects and recovery time following concussion in collegiate football players: the NCAA Concussion Study. JAMA. 2003 Nov 3; 290(19):2556–63. PMID: 14625332

5. Giza CC, Kutcher JS, Ashwal S, Barth J, Getchius TS, Gioia GA, et al. Summary of evidence-based guideline update: evaluation and management of concussion in sports: report of the Guideline Development Subcommittee of the American Academy of Neurology. Neurology. 2013 Jun 2; 80(24):2250–7. doi: 10.1212/WNL.0b013e31828d57dd PMID: 23508730

6. Yuh EL, Hawryluk GW, Manley GT. Imaging concussion: a review. Neurosurgery [Internet]. 2014; 75 Suppl 4:S50. Available from: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00006123-201410001-00005 doi: 10.1227/NEU.0000000000000491 PMID: 25232884

7. Gosselin N, Saluja RS, Chen J-KK, Bottari C, Johnston K, Ptito A. Brain functions after sports-related concussion: insights from event-related potentials and functional MRI. Phys Sportsmed. 2010 Oct 5; 38 (3):27–37. doi: 10.3810/psm.2010.10.1805 PMID: 20959693

8. Murugavel M, Cubon V, Putukian M, Echemendia R, Cabrera J, Osherson D, et al. A longitudinal diffusion tensor imaging study assessing white matter fiber tracts after sports-related concussion. J Neurotrauma. 2014 Nov 6; 31(22):1860–71. doi: 10.1089/neu.2014.3368 PMID: 24786666

9. Barrio JR, Small GW, Wong K-PP, Huang S-CC, Liu J, Merrill DA, et al. In vivo characterization of chronic traumatic encephalopathy using [F-18]FDDNP PET brain imaging. Proc Natl Acad Sci USA. 2015 Apr 2; 112(16):E2039–47. doi: 10.1073/pnas.1409952112 PMID: 25848027

10. Small GW, Kepe V, Siddarth P, Ercoli LM, Merrill DA, Donoghue N, et al. PET scanning of brain tau in retired national football league players: preliminary findings. Am J Geriatr Psychiatry. 2013 Feb 5; 21 (2):138–44. doi: 10.1016/j.jagp.2012.11.019 PMID: 23343487

11. Omalu B, Bailes J, Hamilton RL, Kamboh MI, Hammers J, Case M, et al. Emerging histomorphologic phenotypes of chronic traumatic encephalopathy in American athletes. Neurosurgery. 2011 Jul 5; 69 (1):173–83; discussion 183. doi: 10.1227/NEU.0b013e318212bc7b PMID: 21358359

12. Mendez MF, Paholpak P, Lin A, Zhang JY, Teng E. Prevalence of Traumatic Brain Injury in Early Versus Late-Onset Alzheimer's Disease. J Alzheimers Dis. 2015 Jan 4; 47(4):985–93. doi: 10.3233/JAD-143207 PMID: 26401777

13. Lee Y-KK, Hou S-WW, Lee C-CC, Hsu C-YY, Huang Y-SS, Su Y-CC. Increased risk of dementia in patients with mild traumatic brain injury: a nationwide cohort study. PLoS ONE. 2013 Jan 2; 8(5): e62422. doi: 10.1371/journal.pone.0062422 PMID: 23658727

14. Fakhran S, Yaeger K, Alhilali L. Symptomatic white matter changes in mild traumatic brain injury resemble pathologic features of early Alzheimer dementia. Radiology. 2013 Oct 2; 269(1):249–57. doi: 10.1148/radiol.13122343 PMID: 23781117

15. Anthony DC, Couch Y. The systemic response to CNS injury. Exp Neurol. 2014 Aug 5; 258:105–11. doi: 10.1016/j.expneurol.2014.03.013 PMID: 25017891

16. Balu R. Inflammation and immune system activation after traumatic brain injury. Current Neurology and Neuroscience Reports [Internet]. 2014; 14(10):484. Available from: http://link.springer.com/article/10.1007/s11910-014-0484-2 doi: 10.1007/s11910-014-0484-2 PMID: 25138025

17. Jaerve A, Müller HW. Chemokines in CNS injury and repair. Cell Tissue Res. 2012 Jul; 349(1):229–48. doi: 10.1007/s00441-012-1427-3 PMID: 22700007

18. Blaylock RL, Maroon J. Immunoexcitotoxicity as a central mechanism in chronic traumatic encephalopathy-A unifying hypothesis. Surgical neurology international [Internet]. 2011; 2:107. Available from: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=21886880&retmode=ref&cmd=prlinks doi: 10.4103/2152-7806.83391 PMID: 21886880

19. Loane DJ, Kumar A. Microglia in the TBI brain: The good, the bad, and the dysregulated. Exp Neurol. 2016 Jan 5; 275 Pt 3:316–27. doi: 10.1016/j.expneurol.2015.08.018 PMID: 26342753

20. Loane DJ, Byrnes KR. Role of microglia in neurotrauma. Neurotherapeutics. 2010 Oct 5; 7(4):366–77. doi: 10.1016/j.nurt.2010.07.002 PMID: 20880501

21. Gentleman SM, Leclercq PD, Moyes L, Graham DI, Smith C, Griffin WS, et al. Long-term intracerebral inflammatory response after traumatic brain injury. Forensic Sci Int. 2004 Dec 4; 146(2–3):97–104. PMID: 15542269

22. Johnson VE, Stewart JE, Begbie FD, Trojanowski JQ, Smith DH, Stewart W. Inflammation and white matter degeneration persist for years after a single traumatic brain injury. Brain. 2013 Jan 2; 136(Pt 1):28–42. doi: 10.1093/brain/aws322 PMID: 23365092

23. Smith C, Gentleman SM, Leclercq PD, Murray LS, Griffin WS, Graham DI, et al. The neuroinflammatory response in humans after traumatic brain injury. Neuropathol Appl Neurobiol. 2013 Oct 2; 39(6):654–66. doi: 10.1111/nan.12008 PMID: 23231074

24. Ramlackhansingh AF, Brooks DJ, Greenwood RJ, Bose SK, Turkheimer FE, Kinnunen KM, et al. Inflammation after trauma: microglial activation and traumatic brain injury. Ann Neurol. 2011 Sep 4; 70 (3):374–83. doi: 10.1002/ana.22455 PMID: 21710619

25. Takeuchi H, Jin S, Wang J, Zhang G, Kawanokuchi J, Kuno R, et al. Tumor necrosis factor-alpha induces neurotoxicity via glutamate release from hemichannels of activated microglia in an autocrine manner. J Biol Chem. 2006 Jul 5; 281(30):21362–8. PMID: 16720574

26. Yawata I, Takeuchi H, Doi Y, Liang J, Mizuno T, Suzumura A. Macrophage-induced neurotoxicity is mediated by glutamate and attenuated by glutaminase inhibitors and gap junction inhibitors. Life Sci. 2008 May 5; 82(21–22):1111–6. doi: 10.1016/j.lfs.2008.03.010 PMID: 18452953

27. Shijie J, Takeuchi H, Yawata I, Harada Y, Sonobe Y, Doi Y, et al. Blockade of glutamate release from microglia attenuates experimental autoimmune encephalomyelitis in mice. Tohoku J Exp Med. 2009 Feb; 217(2):87–92. PMID: 19212100

28. Stellwagen D, Beattie EC, Seo JY, Malenka RC. Differential regulation of AMPA receptor and GABA receptor trafficking by tumor necrosis factor-alpha. J Neurosci. 2005 Mar 3; 25(12):3219–28. PMID: 15788779

29. Perry HV, Holmes C. Microglial priming in neurodegenerative disease. Nature Publishing Group [Internet]. 2014; 10(4):217. Available from: http://www.nature.com/doifinder/10.1038/nrneurol.2014.38

30. Di Battista AP, Rhind SG, Baker AJ. Application of blood-based biomarkers in human mild traumatic brain injury. Front Neurol. 2013 Jan 2; 4:44. doi: 10.3389/fneur.2013.00044 PMID: 23641234

31. Muccigrosso MM, Ford J, Benner B, Moussa D, Burnsides C, Fenn AM, et al. Cognitive deficits develop 1month after diffuse brain injury and are exaggerated by microglia-associated reactivity to peripheral immune challenge. Brain Behav Immun. 2016 May; 54:95–109. doi: 10.1016/j.bbi.2016.01.009 PMID: 26774527

32. Su S-HH, Xu W, Li M, Zhang L, Wu Y-FF, Yu F, et al. Elevated C-reactive protein levels may be a predictor of persistent unfavourable symptoms in patients with mild traumatic brain injury: a preliminary study. Brain Behav Immun. 2014 May 4; 38:111–7. doi: 10.1016/j.bbi.2014.01.009 PMID: 24456846

33. Prodan CI, Vincent AS, Dale GL. Coated-platelet levels are persistently elevated in patients with mild traumatic brain injury. J Head Trauma Rehabil. 2014 Jan 3; 29(6):522–6. doi: 10.1097/HTR.0000000000000010 PMID: 24336148

34. Comper P, Hutchison M, Magrys S, Mainwaring L, Richards D. Evaluating the methodological quality of sports neuropsychology concussion research: a systematic review. Brain Inj. 2010 Jan 5; 24(11):1257–71. doi: 10.3109/02699052.2010.506854 PMID: 20828229

35. Dick RW. Is there a gender difference in concussion incidence and outcomes? Br J Sports Med. 2009 May 5; 43 Suppl 1:i46–50. doi: 10.1136/bjsm.2009.058172 PMID: 19433425

36. Hootman JM, Dick R, Agel J. Epidemiology of collegiate injuries for 15 sports: summary and recommendations for injury prevention initiatives. J Athl Train. 2007 Jan 1; 42(2):311–9. PMID: 17710181

37. Brown DA, Elsass JA, Miller AJ, Reed LE, Reneker JC. Differences in Symptom Reporting Between Males and Females at Baseline and After a Sports-Related Concussion: A Systematic Review and Meta-Analysis. Sports Med. 2015 Jul 3; 45(7):1027–40. doi: 10.1007/s40279-015-0335-6 PMID: 25971368

38. Baker JG, Leddy JJ, Darling SR, Shucard J, Makdissi M, Willer BS. Gender Differences in Recovery From Sports-Related Concussion in Adolescents. Clin Pediatr (Phila). 2015 Sep 2;

39. Fish EN. The X-files in immunity: sex-based differences predispose immune responses. Nat Rev Immunol. 2008 Sep 1; 8(9):737–44. doi: 10.1038/nri2394 PMID: 18728636

40. Bouman A, Heineman MJ, Faas MM. Sex hormones and the immune response in humans. Hum Reprod Update. 2005 Jan 6; 11(4):411–23. PMID: 15817524

41. Guskiewicz KM, Register-Mihalik J, McCrory P, McCrea M, Johnston K, Makdissi M, et al. Evidence-based approach to revising the SCAT2: introducing the SCAT3. Br J Sports Med. 2013 Apr 1; 47 (5):289–93. doi: 10.1136/bjsports-2013-092225 PMID: 23479486

42.   Galetta MS, Galetta KM, McCrossin J, Wilson JA, Moster S, Galetta SL, et al. Saccades and memory: baseline associations of the King-Devick and SCAT2 SAC tests in professional ice hockey players. J Neurol Sci. 2013 May 3; 328(1–2):28–31. doi: 10.1016/j.jns.2013.02.008 PMID: 23499425

43.   Debad J, Campbell C, D'Costa J, Tsionsky M, Plisova T, Glezer EN, etal. Multiplexed immunoassays for Brain Injury Markers. Poster presented at: Keystone Symposium—Axons: From Celll Biology to Pathology (J4), Santa Fe, NM, January 24–27, 2016.

44.   Meehan W, Taylor A, Berkner P, Sandstrom N, Peluso M, Kurtz M, et al. Division III Collision Sports Are Not Associated with Neurobehavioral Quality of Life. J Neurotraum. liebert; 2016; 33(2):254–9.

45.   Wold S, Sjöström M, Eriksson L. PLS-regression: a basic tool of chemometrics. Chemometrics and intelligent laboratory systems [Internet]. Elsevier; 2001; 58(2):109–30. Available from: http://www.sciencedirect.com/science/article/pii/S0169743901001551

46.   Ballabio D, Consonni V. Classification tools in chemistry. Part 1: linear models. PLS-DA. Analytical Methods [Internet]. 2013; Available from: http://pubs.rsc.org/en/content/articlehtml/2013/ay/c3ay40582f

47.   Armitage E, Godzien J, Alonso-Herranz V, López-Gonzálvez Á, Barbas C. Missing value imputation strategies for metabolomics data. Electrophoresis [Internet]. 2015; 36(24):3050. Available from: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=26376450&retmode=ref&cmd=prlinks doi: 10.1002/elps.201500352 PMID: 26376450

48.   Chodobski A, Zink BJ, Szmydynger-Chodobska J. Blood–Brain Barrier Pathophysiology in Traumatic Brain Injury. Translational Stroke Research [Internet]. 2011; 2(4):492. Available from: http://link.springer.com/10.1007/s12975-011-0125-x doi: 10.1007/s12975-011-0125-x PMID: 22299022

49.   Webster KM, Wright DK, Sun M, Semple BD, Ozturk E, Stein DG, et al. Progesterone treatment reduces neuroinflammation, oxidative stress and brain damage and improves long-term outcomes in a rat model of repeated mild traumatic brain injury. J Neuroinflammation. 2015 Jan 4; 12:238. doi: 10.1186/s12974-015-0457-7 PMID: 26683475

50.   Bao F, Shultz SR, Hepburn JD, Omana V, Weaver LC, Cain DP, et al. A CD11d monoclonal antibody treatment reduces tissue injury and improves neurological outcome after fluid percussion brain injury in rats. J Neurotrauma. 2012 Sep 4; 29(14):2375–92. doi: 10.1089/neu.2012.2408 PMID: 22676851

51.   Buonora JE, Yarnell AM, Lazarus RC, Mousseau M, Latour LL, Rizoli SB, et al. Multivariate analysis of traumatic brain injury: development of an assessment score. Front Neurol. 2015 Jan 4; 6:68. doi: 10.3389/fneur.2015.00068 PMID: 25870583

52.   Di Battista AP, Rhind SG, Hutchison MG, Hassan S, Shiu MY, Inaba K, et al. Inflammatory cytokine and chemokine profiles are associated with patient outcome and the hyperadrenergic state following acute brain injury. J Neuroinflammation. 2016 Jan 5; 13:40. doi: 10.1186/s12974-016-0500-3 PMID: 26883121

53.   Shultz SR, Bao F, Omana V, Chiu C, Brown A, Cain DP. Repeated mild lateral fluid percussion brain injury in the rat causes cumulative long-term behavioral impairments, neuroinflammation, and cortical loss in an animal model of repeated concussion. J Neurotrauma. 2012 Jan 5; 29(2):281–94. doi: 10.1089/neu.2011.2123 PMID: 21933013

54.   Jaerve A, Schiwy N, Schmitz C, Mueller HW. Differential effect of aging on axon sprouting and regenerative growth in spinal cord injury. Exp Neurol. 2011 Oct 6; 231(2):284–94. doi: 10.1016/j.expneurol.2011.07.002 PMID: 21806987

55.   Breland UM, Michelsen AE, Skjelland M, Folkersen L, Krohg-Sørensen K, Russell D, et al. Raised MCP-4 levels in symptomatic carotid atherosclerosis: an inflammatory link between platelet and monocyte activation. Cardiovasc Res. 2010 May 6; 86(2):265–73. doi: 10.1093/cvr/cvq044 PMID: 20139115

56.   Neselius S, Zetterberg H, Blennow K, Randall J, Wilson D, Marcusson J, et al. Olympic boxing is associated with elevated levels of the neuronal protein tau in plasma. Brain Inj. 2013 Jan 2; 27(4):425–33. doi: 10.3109/02699052.2012.750752 PMID: 23473386

57.   Neselius S, Brisby H, Theodorsson A, Blennow K, Zetterberg H, Marcusson J. CSF-biomarkers in Olympic boxing: diagnosis and effects of repetitive head trauma. PLoS ONE. 2012 Jan; 7(4):e33606. doi: 10.1371/journal.pone.0033606 PMID: 22496755

58.   Olivera A, Lejbman N, Jeromin A, French LM, Kim H- SS, Cashion A, et al. Peripheral Total Tau in Military Personnel Who Sustain Traumatic Brain Injuries During Deployment. JAMA Neurol. 2015 Oct 4; 72 (10):1109–16. doi: 10.1001/jamaneurol.2015.1383 PMID: 26237304

59.   Stern RA, Tripodis Y, Baugh CM, Fritts NG, Martin BM, Chaisson C, et al. Preliminary Study of Plasma Exosomal Tau as a Potential Biomarker for Chronic Traumatic Encephalopathy. J Alzheimers Dis. 2016 Feb 3; 51(4):1099–109. doi: 10.3233/JAD-151028 PMID: 26890775

60.   Blennow K, Hampel H, Weiner M, Zetterberg H. Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. Nat Rev Neurol. 2010 Mar 1; 6(3):131–44. doi: 10.1038/nrneurol.2010.4 PMID: 20157306

61. Zetterberg H, Wilson D, Andreasson U, Minthon L, Blennow K, Randall J, et al. Plasma tau levels in Alzheimer's disease. Alzheimers Res Ther. 2013 Jan 2; 5(2):9. doi: 10.1186/alzrt163 PMID: 23551972

62. Bulut M, Koksal O, Dogan S, Bolca N, Ozguc H, Korfali E, et al. Tau protein as a serum marker of brain damage in mild traumatic brain injury: preliminary results. Adv Ther. 2006 Jan; 23(1):12–22. PMID: 16644603

63. Alves J. Blood–brain barrier and traumatic brain injury. J Neurosci Res. wiley; 2014; 92(2):141–7. doi: 10.1002/jnr.23300 PMID: 24327344

64. Iliff JJ, Wang M, Liao Y, Plogg BA, Peng W, Gundersen GA, et al. A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. Sci Transl Med. 2012 Aug 3; 4(147):147ra111. doi: 10.1126/scitranslmed.3003748 PMID: 22896675

65. Plog BA, Dashnaw ML, Hitomi E, Peng W, Liao Y, Lou N, et al. Biomarkers of traumatic injury are transported from brain to blood via the glymphatic system. J Neurosci. 2015 Jan 3; 35(2):518–26. doi: 10.1523/JNEUROSCI.3742-14.2015 PMID: 25589747

66. Marchi N, Bazarian JJ, Puvenna V, Janigro M, Ghosh C, Zhong J, et al. Consequences of repeated blood-brain barrier disruption in football players. PLoS ONE. 2013 Jan 2; 8(3):e56805. doi: 10.1371/journal.pone.0056805 PMID: 23483891

67. Zetterberg H, Smith DH, Blennow K. Biomarkers of mild traumatic brain injury in cerebrospinal fluid and blood. Nat Rev Neurol. 2013 Apr 1; 9(4):201–10. doi: 10.1038/nrneurol.2013.9 PMID: 23399646

68. Erlanger DM. Exposure to sub-concussive head injury in boxing and other sports. Brain Inj. 2015 Jan 4; 29(2):171–4. doi: 10.3109/02699052.2014.965211 PMID: 25313457

69. Broglio SP, Eckner JT, Martini D, Sosnoff JJ, Kutcher JS, Randolph C. Cumulative head impact burden in high school football. J Neurotrauma. 2011 Oct 6; 28(10):2069–78. doi: 10.1089/neu.2011.1825 PMID: 21787201

70. Peake JM, Suzuki K, Hordern M, Wilson G, Nosaka K, Coombes JS. Plasma cytokine changes in relation to exercise intensity and muscle damage. Eur J Appl Physiol. 2005 Dec 4; 95(5–6):514–21. PMID: 16151834

71. Deyhle MR, Gier AM, Evans KC, Eggett DL, Nelson WB, Parcell AC, et al. Skeletal Muscle Inflammation Following Repeated Bouts of Lengthening Contractions in Humans. Front Physiol. 2015 Jan 4; 6:424. doi: 10.3389/fphys.2015.00424 PMID: 26793125

72. Gu Y, Oyama F, Ihara Y. Tau is widely expressed in rat tissues. J Neurochem. 1996 Sep; 67(3):1235–44. PMID: 8752131

73. Maurage C- AA, Bussière T, Sergeant N, Ghesteem A, Figarella-Branger D, Ruchoux M- MM, et al. Tau aggregates are abnormally phosphorylated in inclusion body myositis and have an immunoelectrophoretic profile distinct from other tauopathies. Neuropathol Appl Neurobiol. 2004 Dec 3; 30(6):624–34. PMID: 15541003

74. Nogalska A, D'Agostino C, Engel WK, Askanas V. Novel demonstration of conformationally modified tau in sporadic inclusion-body myositis muscle fibers. Neurosci Lett. 2011 Oct 1; 503(3):229–33. doi: 10.1016/j.neulet.2011.08.042 PMID: 21896314

75. Avila J, Simón D, Díaz-Hernández M, Pintor J, Hernández F. Sources of extracellular tau and its signaling. J Alzheimers Dis. 2014 Jan 3; 40 Suppl 1:S7–S15. doi: 10.3233/JAD-131832 PMID: 24531154

76. Schneider Soares FMM, Menezes de Souza N, Libório Schwarzbold M, Paim Diaz A, Costa Nunes J, Hohl A, et al. Interleukin-10 is an independent biomarker of severe traumatic brain injury prognosis. Neuroimmunomodulation. 2012 Jan; 19(6):377–85. doi: 10.1159/000342141 PMID: 23075771

77. Ferreira LC, Regner A, Miotto KD, Moura S d, Ikuta N, Vargas AEE, et al. Increased levels of interleukin-6, -8 and -10 are associated with fatal outcome following severe traumatic brain injury. Brain Inj. 2014 Jan 3; 28(10):1311–6. doi: 10.3109/02699052.2014.916818 PMID: 24830571

78. Taşçi A, Okay O, Gezici A, Ergün R, Ergüngör F. Prognostic value of interleukin-1 beta levels after acute brain injury. Neurological Research [Internet]. 2003; 25(8):871. Available from: http://www.maneyonline.com/doi/abs/10.1179/016164103771953998 PMID: 14669533

79. Kumar RG, Boles JA, Wagner AK. Chronic Inflammation After Severe Traumatic Brain Injury: Characterization and Associations With Outcome at 6 and 12 Months Postinjury. J Head Trauma Rehabil. 2015 Jan 4; 30(6):369–81. doi: 10.1097/HTR.0000000000000067 PMID: 24901329

80. Prodan CI, Vincent AS, Dale GL. Coated-Platelet Levels Increase with Number of Injuries in Patients with Mild Traumatic Brain Injury. J Neurotrauma. 2016 May; 33(9):818–24. doi: 10.1089/neu.2014.3846 PMID: 26414016

81. Ramien C, Taenzer A, Lupu A, Heckmann N, Engler JB, Patas K, et al. Sex effects on inflammatory and neurodegenerative processes in multiple sclerosis. Neurosci Biobehav Rev. 2016 Jan 5;

82. Cahill L. Why sex matters for neuroscience. Nat Rev Neurosci. 2006 Jun 4; 7(6):477–84. PMID: 16688123