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Journal of Neuroimmunology
Volume 319, 19 March 2018, Pages 1–8
Date of Publication from Ext Publisher: March 2018

Defence Research and Development Canada
External Literature (P)
DRDC-RDDC-2018-P085
June 2018

Canada

IMPORTANT INFORMATIVE STATEMENTS

This document was reviewed for Controlled Goods by Defence Research and Development Canada (DRDC) using the Schedule to the *Defence Production Act*.

Journal of Neuroimmunology 319 (2018) 1–8

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Blood biomarkers are associated with brain function and blood flow following sport concussion

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A B S T R A C T

Background: Secondary injury pathophysiology after sport-related concussion (SRC) is poorly understood. Blood biomarkers may be a useful tool for characterizing these processes, yet there are limitations in their application as a single modality. Combining blood biomarker analysis with advanced neuroimaging may help validate their continued utility in brain injury research by elucidating important secondary injury mechanisms. Hence, the purpose of this study was to evaluate co-modulation between peripheral blood biomarkers and advanced functional brain imaging after SRC.

Methods: Forty-three university level athletes from 7 sports were recruited (16 recently concussed athletes; 15 healthy athletes with no prior history of concussion; 12 healthy athletes with a history of concussion). Seven blood biomarkers were evaluated: s100B, total tau (T-tau), von Willebrand factor (vWF), brain derived neurotrophic factor (BDNF), peroxiredoxin (PRDX)-6, monocyte chemoattractant protein (MCP)-1 and −4. Resting-state functional MRI was employed to assess global neural connectivity (Gconn), and arterial spin labelling was used to evaluate cerebral blood flow (CBF). We tested for concurrent alterations in blood biomarkers and MRI measures of brain function between athlete groups using a non-parametric, bootstrapped resampling framework.

Results: Compared to healthy athletes, recently concussed athletes showed greater concurrent alterations in several peripheral blood biomarker and MRI measures: a decrease in T-Tau and Gconn, a decrease in T-Tau and CBF, a decrease in Gconn with elevated PRDX-6, a decrease in CBF with elevated PRDX-6, and a decrease in Gconn with elevated MCP-4. In addition, compared to healthy athletes with no concussion history, healthy athletes with a history of concussion displayed greater concurrent alterations in blood biomarkers and Gconn; lower GConn covaried with higher blood levels of s100B and MCP-4.

Conclusion: We identified robust relationships between peripheral blood biomarkers and MRI measures in both recently concussed athletes and healthy athletes with a history of concussion. The results from this combinatorial approach further support that human concussion is associated with inflammation, oxidative stress, and cellular damage, and that physiological perturbations may extend chronically beyond recovery. Finally, our results support the continued implementation of blood biomarkers as a tool to investigate brain injury, particularly in a multimodal framework.

1. Introduction

The first week after concussion is a period of peak symptom presentation (McCrory et al., 2013), and represents an important stage for secondary injury processes. Experimental animal studies have found that it is marked by disturbances in neural metabolism and cerebral autoregulation (Giza and Hovda, 2014), and is correlated with the greatest macrophage recruitment to the brain (Mishra et al., 2016). However, our understanding of these processes in humans remains limited.

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https://doi.org/10.1016/j.jneuroim.2018.03.002
Received 13 October 2017; Received in revised form 5 March 2018; Accepted 6 March 2018

Since the identification of the neurometabolic cascade in 2001 (Giza and Hovda, 2001), advanced neuroimaging using Magnetic Resonance Imaging (MRI) has been the most frequently employed modality to evaluate concussion pathophysiology in humans (Giza and Hovda, 2014), and has proven particularly useful for characterizing disturbances in resting brain function. For example, arterial spin labelling (ASL) has provided evidence of altered cerebral blood flow (CBF) in individuals with concussion (Becelewski and Pierzchala, 2002; Churchill et al., 2017b; Ge et al., 2009; Grossman et al., 2013; Meier et al., 2015; Wang et al., 2016), and blood oxygenation level dependent functional MRI (BOLD fMRI) has been used to measure communication between brain regions, with concussed individuals showing patterns of altered connectivity (Churchill et al., 2017b; Johnson et al., 2012; Zhu et al., 2015).

In addition to neuroimaging, blood biomarkers are a convenient and relatively inexpensive tool that can be used to measure analytes linked to pathophysiological processes such as inflammation and neurodegeneration (Di Battista et al., 2013; Sahu et al., 2017), and have been utilized across the severity spectrum of traumatic brain injury (TBI) to help understand the complex interactions between the central nervous system (CNS) and the periphery (Catania et al., 2009; Di Battista et al., 2016a; Jaerve and Muller, 2012). Indeed, recent blood biomarker studies in sport-related concussion (SRC) have identified inflammatory perturbations that correlate with dysregulation of the hypothalamic-pituitary-adrenal axis (Gill et al., 2016; Merchant-Borna et al., 2016). However, blood biomarker analysis in concussion is not without limitations. For example, neuroinjury markers such as s100 calcium binding protein (s100B) (Papa et al., 2014; Savola et al., 2004) and neuron specific enolase (NSE) (Gempp et al., 2014; Isgro et al., 2015; Tolan et al., 2013) are elevated in the peripheral blood after non-head trauma and hemolysis, and increases in s100B have also been observed acutely after exercise (Koh and Lee, 2014). Furthermore, modulation of the neuroendocrine-immune axis can be seen in many forms of stress, acute infection, and chronic health disorders (Chrousos, 1997; Elenkov, 2000; Elenkov and Chrousos, 1999; Jones, 2012; Liezmann et al., 2012; Menard et al., 2017; Webster et al., 1997; Woda et al., 2016). Hence, while athletes are generally regarded as healthy, researchers need to be cognisant of both experimental design and the interpretation of findings when employing blood biomarkers to investigate the pathophysiology of sport-related concussion (SRC).

One potential strategy to improve the utility of blood biomarkers to brain injury is by analyzing them in parallel with advanced neuroimaging. To date, multimodal analysis using neuroimaging and blood biomarkers has been largely limited to non-sport mild TBI (mTBI), and has most often employed gross computerized tomography (CT) alongside common neuroinjury biomarkers such as s100B and glial fibrillary acidic protein (GFAP) (Akhtar et al., 2003; Muller et al., 2007; Pelinka et al., 2004; Petzold et al., 2003; Unden and Romner, 2010; Wiesmann et al., 2010). In the few studies that have employed advanced neuroimaging techniques, elevations in blood levels of s100B and GFAP have been associated with significant radiologic findings using structural MRI (Ingebrigtsen and Romner, 1996), and altered measures of white matter microstructure using diffusion tensor imaging (DTI) (Ingebrigtsen and Romner, 1996; Kou et al., 2013; Siman et al., 2013). In the sport domain, Marchi and colleagues simultaneously employed peripheral blood analysis and DTI, and found that s100B autoantibody levels were correlated with microstructural abnormalities in football players sustaining a comparatively greater number of subconcussive impacts throughout a competitive season (Marchi et al., 2013). While this provides encouraging preliminary evidence, there are no combinatorial blood biomarker and advanced neuroimaging studies that have evaluated athletes after a physician diagnosed SRC.

Hence, the purpose of this study was to evaluate the relationship between peripheral blood biomarkers related to neuroinjury, and advanced functional brain imaging measures of CBF and functional connectivity, after SRC. We hypothesized that concussed athletes, compared to non-concussed athletes, would display greater covariance between blood biomarker concentrations and advanced MRI measures.

2. Methods

2.1. Study participants

Forty-three (43) athletes were recruited from interuniversity teams at a single institution (including volleyball, hockey, soccer, football, rugby, basketball and lacrosse). Sixteen (16) athletes were recruited within 1–7 days (blood draw, median = 3.6 days; MRI scans, median = 4 days) of a physician diagnosed concussion (AcuConc). Fifteen (15) healthy athletes were recruited without any prior history of concussion (NoConc), along with twelve (12) healthy athletes with a history of concussion diagnosed > 6 months prior to brain imaging (HxConc). For NoConc and HxConc groups, scanning and blood draws were conducted a median of 7 days apart; AcuConc participants underwent scanning and blood draws at a median of 4 days apart. Subjects were excluded if they presented with seasonal allergies, cold, or any known acute infection at the time of blood sampling. The study procedures were approved by research ethics boards (REBs) at the University of Toronto and St. Michael's Hospital, and carried out in accordance with REB guidelines. All patients provided written informed consent prior to study participation.

2.2. Magnetic resonance imaging

Participants were imaged at St. Michael's Hospital using an MRI system operating at 3 Tesla (Magnetom Skyra, Siemens, Erlangen, Germany) with a 20-channel head receiver coil. The imaging sequences and data processing are briefly summarized below; see Supplementary methods for further details. Anatomical imaging included three-dimensional T1-weighted magnetization prepared rapid acquisition gradient echo imaging (3D MPRAGE), along with 3D fluid attenuated inversion recovery imaging (FLAIR) and 3D susceptibility-weighted imaging (SWI) to screen for structural abnormalities including lesions and micro-hemorrhage, respectively. No structural abnormalities were found for either concussed or control athletes in this study.

All functional imaging data were acquired for participants at rest, after they were instructed to lie still with their eyes closed and not focus on anything. 2D pulsed Arterial Spin Labelling (ASL) was acquired, consisting of a single calibration scan and 45 tag-control pairs. The ASL data were processed and analyzed using a combination of the ASLtbx package (cfn.upenn.edu/~zewang/ASLtbx.php) and in-house software, producing a brain map of absolute CBF for each subject in units of mL/100 g/min. Functional MRI (fMRI) data were acquired via multi-slice T2*-weighted echo planar imaging, producing a time-series of 195 images. Processing was performed using the Analysis of Functional Neuroimages package (AFNI; afni.nimh.nih.gov) and in-house software. For each brain voxel, global functional connectivity (Gconn) was then measured by computing the Pearson correlation between its BOLD time-series and all other brain voxels, and taking the mean of all (positive) connectivity values. This approach produced a brain map reflecting the total integrative function of each voxel (Cole et al., 2012; Rubinov and Sporns, 2010), which has been used to identify functional alterations after concussion for both symptomatic and asymptomatic individuals (Churchill et al., 2017b, 2017c). To perform group-level analyses, ASL and fMRI data were co-registered to a common MNI152 template space using the FMRIB Software Library package (FSL; https://fsl.fmrib.ox.ac.uk) and resampled to 2x2x2 mm resolution. Subsequent analyses were restricted to grey matter by retaining only voxels that intersected with the MNI152 brain mask and a grey matter mask, generated using FSL utilities.

2.3. Blood biomarkers

Venous blood samples were drawn from athletes after consent was obtained. Healthy athletes with and without concussion history were sampled prior to the beginning of the competitive season, and concussed athletes were sampled within 7 days of injury (median = 3.6 days). Blood was drawn into a 10-mL K_2EDTA (with 4 mM sodium metabisulfite [Na2S2O5]) or 4-mL non-additive (Vacutainer, Becton Dickinson, NJ, USA) tube. Within one hour of sampling, specimens were centrifuged for 2 min using the PlasmaPrep 12™ centrifuge (Separation Technology Inc., FL, USA). Plasma and serum supernatants were then aliquoted and frozen at −70 °C until analysis.

Biomarkers were evaluated using Meso Scale Diagnostics (MSD) 96-well MULTI-ARRAY® technology. The platform uses an array-based multiplex format with electrochemiluminescence detection, employing a sandwich immunoassay comprised of 1) capture antibodies coated on arrays within plate wells, and 2) detection antibodies conjugated with an electrochemiluminescent SULFO-Tag™. We employed a prototype neuroinjury-related biomarker assay developed at MSD, in part through work supported by US Army Medical Research and Materiel Command (Contract No. W81XWH-13-C-0196), and as reported previously by our group (Di Battista et al., 2016b). Seven markers were chosen from this panel due to 1) their theorized involvement in secondary injury pathophysiology, and 2) previous findings from our group (Di Battista et al., 2016b). The final assessment included s100B, total tau (T-tau), von Willebrand factor (vWF), brain derived neurotrophic factor (BDNF), peroxiredoxin (PRDX)-6, and monocyte chemoattractant protein (MCP)-1 and −4. MCP-4 was assayed in-house as part of a chemokine 10-plex kit and run on an MSD® Sector Imager™ 6000 with Discovery Workbench software (version 3.0.18). Biomarker values were employed for statistical analyses only if they fell within the lower and upper limits of detection. For statistical analysis, each marker was only evaluated if detectable in ≥80% of analyzed samples. Please see Supplementary Table 1 for biomarker concentrations across groups, Supplementary Table 2 for biomarker detectability information, and Supplementary Table 3 for assay sensitivity information.

2.4. Analysis of MRI and blood biomarkers

This study examined whether concussion was associated with simultaneous within-subject variations in both MRI measures and blood biomarkers. Prior to analysis, the imaging and biomarker data were tested for significant deviations from normality, as heavy distribution tails may lead to biased or unstable parameter estimates; co-expression analyses are particularly sensitive to these effects if both neuroimaging and biomarkers are heavy-tailed. While mean CBF showed no significant evidence of deviation from normality (kurtosis: 2.41, $p = 0.767$), mean Gconn did (kurtosis: 6.49; $p < 0.001$). Moreover, biomarker kurtosis ranged from 3.10 ($p = 0.271$) to 29.50 ($p < 0.001$). To ensure comparability of results, both biomarker and neuroimaging data were rank-transformed prior to subsequent analyses.

This study tested for "co-modulation", or simultaneous differences in expression of both MRI values x and biomarker values y, between each pair of athlete groups (HxConc vs. NoConc, AcuConc vs. NoConc, AcuConc vs. HxConc). For each comparison, the set of MRI values x_s and biomarker values y_s (for subjects $s = 1…S$), were z-scored over the subjects in both groups to obtain x_s^* and y_s^*. The sum of z-scores $z_s^* = x_s^* + y_s^*$ reflects co-expression of these markers; if x_s^* and y_s^* are both positive (or negative) then the magnitude of z_s^* will be large and positive (or negative). The mean difference in z_s^* values was then measured between the two athlete groups. Effect size was estimated by bootstrap resampling on subjects (1000 iterations) and by computing the bootstrap ratio (i.e., mean/standard error). This study also measured "anti-modulation" of MRI and blood biomarkers by analyzing the difference of z-scores $z_s^* = x_s^* - y_s^*$; in this case, if x_s^* is positive and y_s^* negative (or vice-versa) then the magnitude of z_s^* will be large. The mean difference in z_s^* values was evaluated between groups using the same bootstrapping approach described above. For both of CBF and Gconn, these analyses were performed per brain voxel, for each blood biomarker. Multiple comparison correction was then conducted over all brain voxels at a False-Discovery Rate (FDR) of 0.05. This represents a novel approach for directly quantifying concurrent alterations in biomarker datasets within individual subjects. This procedure is based on the statistical framework established by Strother and colleagues (Strother et al., 2002), for quantifying reproducible activations in neuroimaging data. However, to our knowledge it is the first application of these statistical methods to combined neuroimaging and blood data.

Table 1
Athlete characteristics.

	NoConc (n = 15)	HxConc (n = 12)	AcuConc (n = 16)
Age	19.0 (18.7–20.5)	21.0 (20.5–22.0)	18.5 (18.0–19.75)
Sex – n (%) male	8 (53.3)	6 (50.0)	7 (43.7)
Concussion Hx	–	2 (1.0–3.0)	1 (0.7–2.0)
Sport – n (%)			
Basketball	2 (13.3)	0 (0.0)	1(6.2)
Football	1 (6.7)	1 (8.3)	2 (12.5)
Hockey	0 (0.0)	3 (25.0)	2 (12.5)
Lacrosse	1 (6.7)	0 (0.0)	3 (18.7)
Rugby	2 (13.3)	0 (0.0)	6 (37.5)
Soccer	3 (20.0)	2 (16.7)	0 (0.0)
Volleyball	6 (40.0)	6 (50.0)	2 (12.5)
SCAT3			
Total Symptoms	3.0 (0.0–6.5)	2.0 (1.0–5.0)	5.0 (4.0–11.7)
Symptom Severity	3.0 (0.0–10.0)	5.0 (3.0–6.0)	7.0 (4.0–14.0)

NoConc, no history of concussion; HxConc, history of concussion; AcuConc, acute concussion.
Unless otherwise stated, all characteristics are reported as the median and interquartile range.

3. Results

3.1. Demographics and clinical data

A summary of athlete characteristics can be found in Table 1. All groups spanned similar age ranges and male-to-female ratio (Table 1): AcuConc (9 female (56%), median age: 18.5 yrs), HxConc (5 female (42%), median age: 21.0 yrs), NoConc (8 female (53%), median age: 19.0 yrs). The highest represented sport in the AcuConc group was rugby (n = 6, 37.5%) while the highest represented sport for both the NoConc and HxConc groups was volleyball (n = 6 (40%); n = 6 (50%), respectively). In concussed athletes, at the time of blood draw, the median total number of symptoms was 5.0 (IQR 4.0–11.7), while the median symptom severity score was 7.0 (IQR 4.0–14.0). The median days to return-to-play was 36 (IQR 14.2–84.5), only one athlete suffered loss of consciousness (6%), and three athletes reported amnesia (18.7%) (data not shown).

3.2. Analysis of MRI and blood biomarkers

Associations between CBF and blood biomarkers are depicted in Fig. 1. The comparison of recently concussed athletes (AcuConc vs. NoConc) showed greater differences in MRI and biomarker expression than the HxConc vs. NoConc comparison. Extensive negative co-modulation of CBF and T-tau was seen in the AcuConc vs. NoConc comparison, with similar but more spatially sparse effects observed for AcuConc vs. HxConc, indicating that concurrently reduced CBF and T-tau is more pronounced in recently concussed individuals compared to healthy individuals with or without a history of concussion. Negative anti-modulation of CBF with PRDX-6 was also observed in the AcuConc vs. NoConc comparison, indicating that the inverse relationship

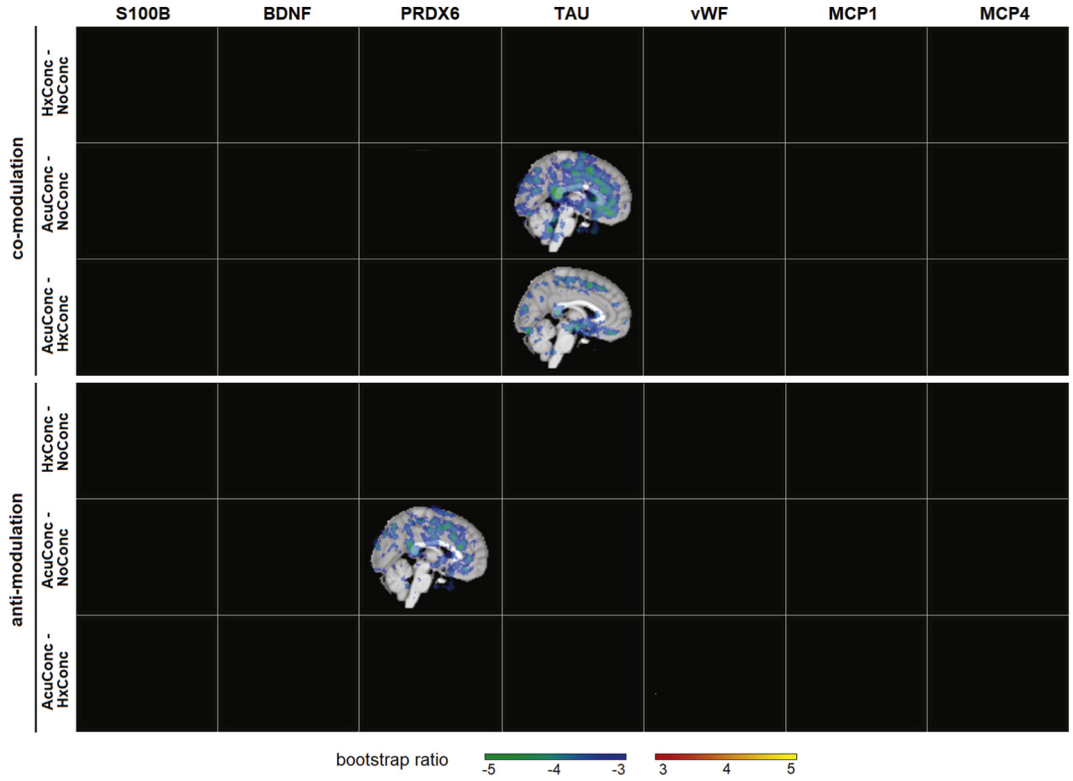

Fig. 1. Coupling of blood biomarkers and cerebral blood flow (CBF) associated with concussion. Brain maps are shown for all biomarkers that have significant coupling with regional CBF values (FDR = 0.05 threshold). Plots depict bootstrap ratio values as a maximum intensity projection (MIP) in the sagittal plane, overlaid on MNI152 atlas (x = 0). The top panel shows "co-modulation": both biomarker and CBF values increase with concussion history (positive), or both are decreased (negative). The bottom panel shows "anti-modulation": CBF values increase while biomarker levels decrease (positive), or the opposite response (negative). Each row shows pairwise between-group differences for no prior concussion (NoConc), history of concussion (HxConc) or subacute concussion (AcuConc).

between CBF and PRDX-6 was stronger in recently concussed individuals compared to those without a history of concussion.

The associations between Gconn and blood biomarkers are depicted in Fig. 2. Consistent with CBF, co-modulation effects were only seen with T-tau; Gconn showed negative co-modulation in the AcuConc vs. NoConc comparison, with similar but spatially sparser effects for AcuConc vs. HxConc, indicating that lower GConn is related to elevated T-tau levels to a greater degree in acutely concussed athletes compared to healthy athletes with or without a history of concussion. Furthermore, consistent with CBF, negative anti-modulation of Gconn with PRDX-6 was observed in the AcuConc vs. NoConc comparison, indicating that the association between lower Gconn and elevated PRDX-6 is more extensive in recently concussed individuals compared to individuals without a history of concussion. However, anti-modulation of Gconn was seen across a more extensive suite of biomarkers. In particular, s100B showed the greatest effects for HxConc vs. NoConc, with weaker effects for AcuConc vs. NoConc, indicating that co-modulatory effects are greatest for individuals with a history of concussion. MCP4 also showed extensive negative anti-modulation for both AcuConc vs. NoConc and HxConc vs. NoConc, while MCP1 showed highly sparse effects for AcuConc only. Finally, only T-tau showed significant anti-modulation in AcuConc vs. HxConc.

In order to better interpret the spatial pattern of concussion effects seen for CBF (Fig. 1) and Gconn (Fig. 2), Fig. 3 depicts voxel-wise maps counting the number of brain-biomarker analyses that detected significant concussion effects. For CBF, significant coupling with biomarkers is mainly seen fronto-temporally. Areas overlapping across multiple analyses include the bilateral orbitofrontal regions (axial slices $-14, -4$), bilateral insula (slice $+6$), anterior cingulate (slices $+16$, $+26, +36$), midcingulate (slices $+36, +46$) and middle frontal lobes (slices $+46, +56$). There is also a tendency towards right lateralization, with overlap in the right middle temporal (slices $-14, -4$), inferior occipital (slice -14), superior temporal (slice $-4, +6, +16, +26$), postcentral (slice $+26$) and angular gyri (slice $+36, +46$). For Gconn, effects appear to be primarily in bilateral occipital and parietal regions, with the most reliable brain-biomarker coupling effects seen in the inferior occipital lobes (slices $-4, +6$) middle occipital lobes, cuneus (slice $+26$) and precuneus (slices $+46, +56$), and midcingulate cortex (slices $+36$, $+46$). Consistent with CBF, some fronto-temporal peaks are also seen in right middle temporal (slice $+6$), right superior temporal (slice 16), right inferior frontal (slice $+26$) and bilateral middle frontal brain regions (slice $+46$).

4. Discussion

The principal finding of this study was the robust associations observed between advanced functional MRI and peripheral blood biomarkers in athletes after SRC. We observed simultaneous alterations of multiple neuroinjury-related blood biomarkers and neuroimaging measures (Gconn and CBF) in acutely concussed athletes versus healthy athletes without prior concussion. Furthermore, healthy athletes with a history of concussion exhibited a more limited set of concurrent perturbations in peripheral blood and neuroimaging markers relative to healthy athletes with no prior concussion history. These findings were identified based on a novel co/anti-modulation analytic approach. This provided a robust, non-parametric framework for measuring concurrent variations in both brain imaging and blood biomarkers within an individual, giving greater insight into how these factors are inter-related following a concussion.

Decreases in CBF and Gconn were associated with lower T-tau levels in concussed versus healthy athletes. While the specific underlying mechanisms cannot be ascertained in the present study, our results are potentially supportive of concussion-related abhorrent waste clearance from the brain (Iliff et al., 2014; Iliff et al., 2012). Indeed, the recently

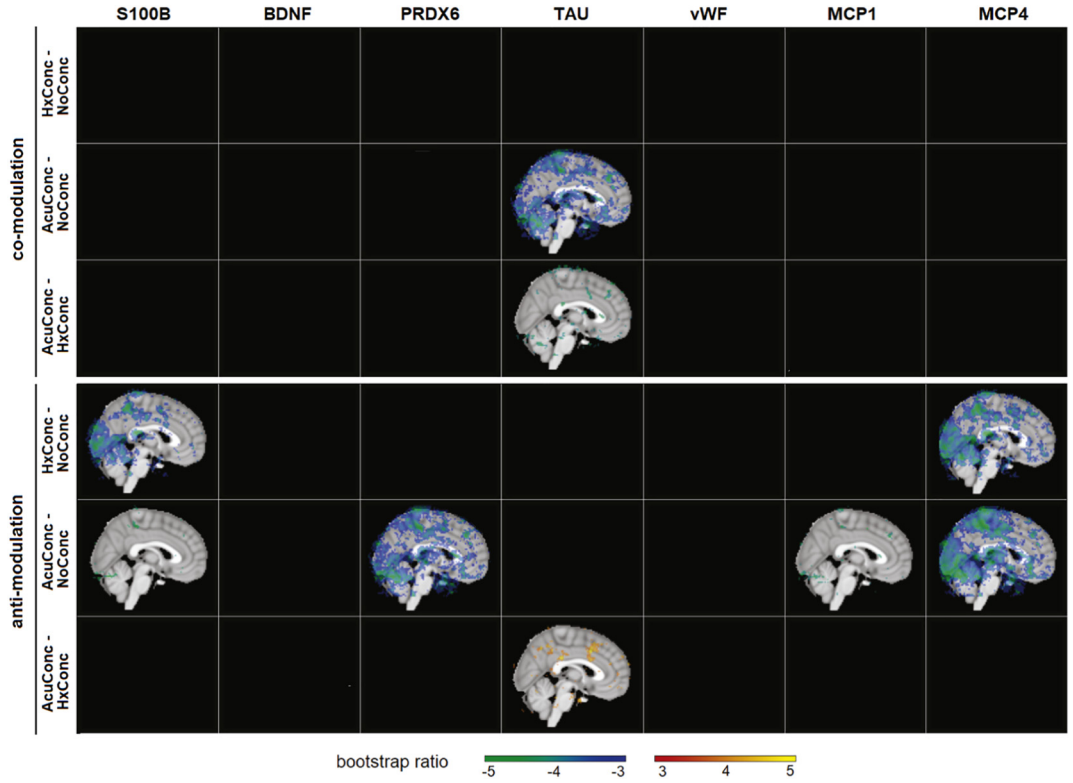

Fig. 2. Coupling of Blood biomarkers and global connectivity (Gconn) associated with concussion. Brain maps are shown for all biomarkers that have significant coupling with regional Gconn values (FDR = 0.05 threshold). Plots depict bootstrap ratio values as a maximum intensity projection (MIP) in the sagittal plane, overlaid on MNI152 atlas (x = 0). The top panel shows "co- modulation": both biomarker and Gconn values increase with concussion history (positive), or both are decreased (negative). The bottom panel shows "anti-modulation": Gconn values increase while biomarker levels decrease (positive), or the opposite response (negative). Each row shows pairwise between-group differences for no prior concussion (NoConc), history of concussion (HxConc) or subacute concussion (AcuConc).

discovered glymphatic system is perturbed after experimental TBI, possibly impeding Tau clearance from the brain into the peripheral circulation (Iliff et al., 2014; Iliff et al., 2012; Plog et al., 2015). In addition, glymphatic clearance is dependent on arterial blood flow (Iliff et al., 2013), which may be affected by impaired autonomic regulation, a common sequela of concussion (Len et al., 2011). While not yet evaluated in humans, the covariant alterations we observed in peripheral T-tau, blood flow and function are supportive of this theory. Our findings are also supported by a recent study by Gill and colleagues, who identified lower plasma T-tau levels in athletes at approximately 3 days post-concussion (Gill et al., 2017). Interestingly, Gill and colleagues noted that measures of T-tau are potentially confounded by exercise in athletes (Gill et al., 2017; Shahim et al., 2015). However, in a study by Shahim and colleagues, it was found that while some brain injury-related biomarkers were elevated in the peripheral blood at one hour after a pre-season training session in hockey players, T-tau was not altered (Shahim et al., 2015). Nonetheless, future studies should attempt to control for extracranial sources of Tau and may be improved by the evaluation of its more CNS-centric isoforms, such as its phosphorylated conformation.

In the current study, decreases in CBF and Gconn were associated with elevated blood PRDX-6 levels to a greater degree in recently concussed versus healthy athletes. These findings are consistent with the hypothesis that elevated PRDX-6 is an indicator of concussion-related oxidative stress (Buonora et al., 2015a; Buonora et al., 2015b; Singh et al., 2016), which is expected to be most pronounced early after injury. The causal mechanisms remain to be fully elucidated, however, this may signify that oxidative stress during the acute metabolic cascade is associated with impairments in neural functioning and metabolic activity, the latter corresponding to our observed reduction in CBF. Alternatively, post-concussion impairments in cerebral autoregulation may lead to reduced CBF (Len et al., 2011), with subtle

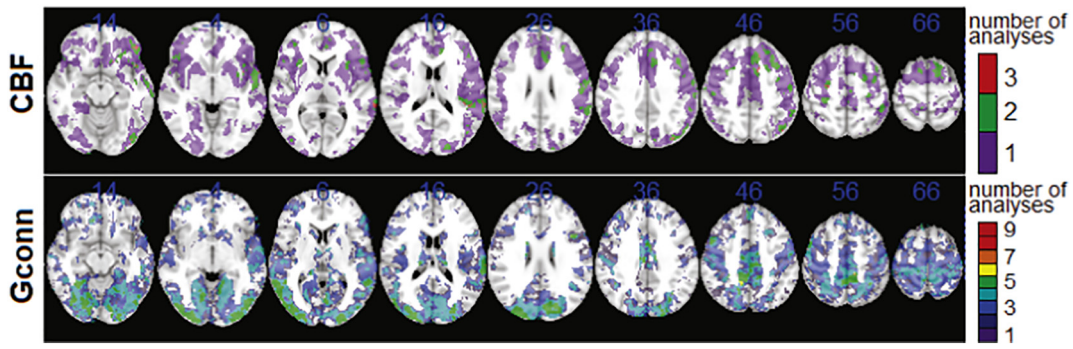

Fig. 3. Brain regions where coupling with blood biomarkers is most reliably associated with effects of concussion. Colour maps depict the number of analyses in which voxels show a significant concussion effect for cerebral blood flow (CBF) based on results in Fig. 1, and for global functional connectivity (Gconn) based on results in Fig. 2.

ischemic effects contributing to oxidative stress and reduced neurometabolic activity. However, further research is required to support these hypotheses.

We found a greater inverse association between Gconn and s100B levels in concussed versus healthy athletes. These results are in general agreement with Marchi et al., who found a relationship between s100B autoantibodies and DTI in football players sustaining comparatively more subconcussive impacts over the course of a season (Marchi et al., 2013), and are also supported by the works of Ingebrigtsen and colleagues, who noted a relationship between s100B and cerebral contusion (MRI) in a case report study of 3 non-sport mTBI patients (Ingebrigtsen and Romner, 1996). Yet, it is difficult to consolidate these findings, as the imaging modalities, patient cohorts, and injuries differed between studies. Interpretation is further complicated by the potential non-specificity of s100B to concussion; s100B may be elevated after exercise and non-head trauma (Falcone et al., 2015; Savola et al., 2004). Yet, beyond s100B, perturbations in peripheral blood levels of calpain cleaved aII spectrin N-terminal fragment (SNTF) and GFAP have also been correlated with changes in advanced neuroimaging metrics in mTBI patients (Kou et al., 2013; Siman et al., 2013). Hence, our results contribute to a growing body of literature which has shown that changes in peripheral biomarkers associated with neuroinjury, correlate with changes in advanced neuroimaging measures after both mTBI and SRC.

We observed concurrent decreased functional activity and higher blood levels of MCP-1 and MCP-4 in acutely concussed athletes compared to athletes with no history of concussion. Both MCP-1 and -4 are chemoattractant proteins experimentally implicated in leukocyte trafficking to the brain after TBI (Catania et al., 2009; Jaerve and Muller, 2012; Semple et al., 2010a; Semple et al., 2010b). Indeed, we recently identified elevated levels of both chemokines in the acute phase after moderate-to-severe TBI in humans, and found they were related to poor patient outcome and trauma-induced hyperadrenergic surging (Di Battista et al., 2016a). While inflammation is an important component of secondary brain injury throughout all phases of recovery, its biological pleiotropy makes the interpretation of the current results difficult. For example, MCP-4 is a candidate biomarker for several inflammatory-related health conditions such as arthritis, obesity, asthma and respiratory infection (Baumann et al., 2013; Breland et al., 2010; Gentili et al., 2016; Okugawa et al., 2016; Pradeep et al., 2013; Relster et al., 2017). Furthermore, both MCP-1 and -4 have been associated with disturbances in the neuroendocrine-immune axis in posttraumatic stress disorder (Dalgard et al., 2017). However, in the present study, the covariant perturbations identified between peripheral chemokines and neuroimaging, alongside the exclusion of all participants with seasonal allergies, cold, or any other infection at the time of blood sampling, helped address a number of these potential confounds. Hence, while not definitive, these findings provide supportive evidence of the role of chemokines in secondary injury pathophysiology after concussion.

Compared to athletes with no history of concussion, we found that healthy athletes with a history of concussion displayed significantly higher covariance between biomarkers and functional connectivity. Specifically, lower Gconn was associated with higher levels of s100B and MCP-4. Interestingly, the relationship between s100B and Gconn was more spatially extensive than that seen in the acutely concussed versus healthy athlete comparison. Furthermore, the extensive anti-modulation observed with MCP-4 is aligned with our previous finding of elevated MCP-4 in male athletes with a history of multiple concussions (Di Battista et al., 2016b). Taken together, these results support a growing body of literature suggesting that physiological perturbations observed acutely after concussion may continue beyond clinical symptom resolution (Kamins et al., 2017). Interestingly, in a recent systematic review on this topic by Kamin and colleagues, the authors suggest that while physiological changes have been observed at clinical recovery across several modalities, fMRI and fluid biomarkers are understudied (Kamins et al., 2017). Hence, the results of the current study help address this gap in the literature, adding to our knowledge of biological recovery after sport concussion.

The different functional imaging modalities used in this study, including ASL and resting-state fMRI, provide complementary information about possible neurophysiological responses related to blood biomarkers following SRC. Based on the reported spatial distributions (Fig. 3), the results indicate that SRC is primarily associated with covariation of blood biomarkers and frontotemporal CBF. The findings are consistent with these grey matter regions being vulnerable to primary injury from head impacts (Viano et al., 2005), with prior neuroimaging studies showing fronto-temporal effects associated with both acute injury and a history of concussion (Churchill et al., 2017a; Churchill et al., 2017b; Meier et al., 2015; Wang et al., 2016). Conversely, analyses of Gconn showed the greatest brain-biomarker coupling in occipito-parietal regions. Hence, the effects of neuroinjury on functional connectivity are primarily in areas associated with visual function and visuospatial orientation. Given the absence of effects on fronto-temporal Gconn, functional connectivity disturbances may be a consequence of the delayed effects of impact, e.g., shear/strain forces disrupting white matter pathways associated with visual function. These results are also consistent with prior studies that have reported effects of concussion on resting state functional connectivity in occipital and parietal regions (Johnson et al., 2012; Slobounov et al., 2011; Zhang et al., 2010).

The imaging results in this study provide novel information about the neurobiological outcomes of concussion, and potential brain areas showing altered function. The present focus on grey matter measures of CBF and Gconn, rather than more conventional DTI measures of white matter (Kou et al., 2013; Marchi et al., 2013), allowed for the localization of affected brain areas, and aided in the interpretation of the vulnerable aspects of physical function and cognition. For example, while peripheral blood indices of brain injury may be more directly linked to DTI markers of white matter integrity (Marchi et al., 2013; Siman et al., 2013), our ASL results suggest that any effects of white matter injury are compounded by concomitant decreases in cortical perfusion, while our fMRI results suggest that affected white matter pathways tend to involve occipito-parietal domains. This information is complementary to prior studies with DTI and biomarkers, and helps develop a more complete picture of the neurophysiological alterations that are most consistently associated with specific blood biomarker responses to concussion. A critical area of future research will be to develop more comprehensive multi-modal analyses which combine both functional and structural MRI with peripheral blood markers.

Although we identified highly robust associations between blood biomarkers and MRI, the results of this study must be interpreted within the context of its limitations. A limited sample size precluded further group stratifications to examine potential differences according to sex, sport participation, and detailed concussion history (i.e., one vs. multiple previous concussions). Furthermore, it is still unclear if the relationships we identified are confounded by biological disturbances unrelated to concussion, such as academic and competitive stress, exercise, the proximity between imaging and blood draw (median = 4 days), or between the time of injury and time of assessment (median = 4 days). Furthermore, we did not evaluate concussed and healthy athletes at the same time of the athletic season, and it is possible that this may have influenced our findings. Future studies must be cognizant of such potential confounds and may want to specifically address how various psychological and physical stress-related mechanisms potentially augment combinatorial blood and brain biomarker signatures. Furthermore, future studies may want to include biomarkers beyond those included in the current study. For example, evaluating autoantibodies to biomarkers such as s100B and GFAP in the subacute phase of injury may be effective, as these molecules display a longer half-life in the blood compared to their antigen counterparts (Marchi et al., 2013; Zhang et al., 2014).

We identified robust relationships between peripheral blood

biomarker concentrations and advanced MRI measures that were significantly greater in concussed versus healthy athletes, and in healthy athletes with a history of concussion compared to those with no prior concussion history. These findings contribute to the growing body of human evidence suggesting that secondary injury after concussion includes cellular damage, oxidative stress and inflammation. Our results also support the continued combinatorial use of advanced neuroimaging and peripheral blood biomarkers to help elucidate secondary injury pathophysiology after SRC, both in the subacute phase and chronically after clinical recovery.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jneuroim.2018.03.002.

Competing interests

None.

Acknowledgements

The authors would like to acknowledge Maria Shiu and Sarah Watling for their help with data collection during the study period. This study was funded by the Canadian Institute of Military and Veteran Health Research (W7714-145967) (CIMVHR) – Task 7.

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5. DATE OF PUBLICATION (Month and year of publication of document.)	6a. NO. OF PAGES (Total pages, including Annexes, excluding DCD, covering and verso pages.)	6b. NO. OF REFS (Total references cited.)
March 2018	8	74

7. DOCUMENT CATEGORY (e.g., Scientific Report, Contract Report, Scientific Letter.)

External Literature (P)

8. SPONSORING CENTRE (The name and address of the department project office or laboratory sponsoring the research and development.)

DRDC – Toronto Research Centre
Defence Research and Development Canada
1133 Sheppard Avenue West
Toronto, Ontario M3K 2C9
Canada

9a. PROJECT OR GRANT NO. (If appropriate, the applicable research and development project or grant number under which the document was written. Please specify whether project or grant.)	9b. CONTRACT NO. (If appropriate, the applicable number under which the document was written.)
	W7714-145967/001/SV

10a. DRDC PUBLICATION NUMBER (The official document number by which the document is identified by the originating activity. This number must be unique to this document.)	10b. OTHER DOCUMENT NO(s). (Any other numbers which may be assigned this document either by the originator or by the sponsor.)
DRDC-RDDC-2018-P085	04KJ09

11a. FUTURE DISTRIBUTION WITHIN CANADA (Approval for further dissemination of the document. Security classification must also be considered.)

Public release

11b. FUTURE DISTRIBUTION OUTSIDE CANADA (Approval for further dissemination of the document. Security classification must also be considered.)

12. KEYWORDS, DESCRIPTORS or IDENTIFIERS (Use semi-colon as a delimiter.)

brain injury; concussion; biomarker; s100B; GFAP; T-Tau; PRDX-6; MCP-1; MCP-4; athletes

13. ABSTRACT/RÉSUMÉ (When available in the document, the French version of the abstract must be included here.)

Background: Secondary injury pathophysiology after sport-related concussion (SRC) is poorly understood. Blood biomarkers may be a useful tool for characterizing these processes, yet there are limitations in their application as a single modality. Combining blood biomarker analysis with advanced neuroimaging may help validate their continued utility in brain injury research by elucidating important secondary injury mechanisms. Hence, the purpose of this study was to evaluate co-modulation between peripheral blood biomarkers and advanced functional brain imaging after SRC. Methods: Forty-three university level athletes from 7 sports were recruited (16 recently concussed athletes; 15 healthy athletes with no prior history of concussion; 12 healthy athletes with a history of concussion). Seven blood biomarkers were evaluated: s100B, total tau (T-tau), von Willebrand factor (vWF), brain derived neurotrophic factor (BDNF), peroxiredoxin (PRDX)-6, monocyte chemoattractant protein (MCP)-1 and −4. Restingstate functional MRI was employed to assess global neural connectivity (Gconn), and arterial spin labelling was used to evaluate cerebral blood flow (CBF). We tested for concurrent alterations in blood biomarkers and MRI measures of brain function between athlete groups using a non-parametric, bootstrapped resampling framework. Results: Compared to healthy athletes, recently concussed athletes showed greater concurrent alterations in several peripheral blood biomarker and MRI measures: a decrease in T-Tau and Gconn, a decrease in T-Tau and CBF, a decrease in Gconn with elevated PRDX-6, a decrease in CBF with elevated PRDX-6, and a decrease in Gconn with elevated MCP-4. In addition, compared to healthy athletes with no concussion history, healthy athletes with a history of concussion displayed greater concurrent alterations in blood biomarkers and Gconn; lower GConn covaried with higher blood levels of s100B and MCP-4. Conclusion: We identified robust relationships between peripheral blood biomarkers and MRI measures in both recently concussed athletes and healthy athletes with a history of concussion. The results from this combinatorial approach further support that human concussion is associated with inflammation, oxidative stress, and cellular damage, and that physiological perturbations may extend chronically beyond recovery. Finally, our results support the continued implementation of blood biomarkers as a tool to investigate brain injury, particularly in a multimodal framework.