Review

Biomarkers to aid the return to play decision following sports-related concussion: a systematic review

Journal of Concussion
Volume 6: 1–17

DOI: 10.1177/20597002211070735
journals.sagepub.com/home/ccn
SAGE

Nipuna Senaratne[1], Alexandra Hunt[2], Eleanor Sotsman[2] and Michael J. Grey[2]

Abstract

Premature return to play (RTP) following sports-related concussion (SRC) is associated with significant morbidity including risk of neurological and non-neurological injury, persistent post-concussion symptoms and chronic neurological deficits. Assessing athletes for RTP is critical but these decisions are currently based on clinical assessments that are subject to bias and symptomatic reporting that rely on compliance. An objective and easily obtained biomarker that can indicate recovery following SRC would aid clinicians to make safer RTP decisions. We performed a systematic review to identify potential biomarkers from saliva, urine and blood sources that could inform the clinical RTP decision. The MEDLINE database was searched. Inclusion criteria were studies focusing on adults diagnosed with SRC, fluid biomarkers from blood, saliva or urine and clinical recovery from SRC or at RTP. We assessed each biomarker for their time course post SRC and relationship to clinical recovery. Secondary outcomes included correlation with symptom scores and predictive value for prolonged RTP. We identified 8 studies all investigating blood-based markers of diffuse axonal injury (tau, NFL, SNTF), neuroglial injury (NSE, VLP-1, UCH-L1, S100B, GFAP), inflammation and hormonal disturbances. Tau, SNTF, UCH-1, GFAP, S100B and the inflammatory cytokine MCP-4 are raised post SRC and return to baseline by RTP. Changes in tau, NFL, SNTF, GFAP and MCP-4 post SRC correlate with severity of concussion as measured by symptom severity or RTP duration. There is only preliminary case-reporting for hormonal biomarkers. The evidence is limited by a lack of highly powered studies, variation in use of athletic and Contact sport controls (CSC) and a lack of consistent sampling and assessment protocols. There is promise for biomarkers to aid RTP decisions following SRC, most notably in use alongside clinical assessment in RTP criteria to allow greater precision in identifying mild and severe concussion.

Keywords

Concussion, biomarker, return to play, sport, mild traumatic brain injury

Date received: 15 March 2021; accepted: 15 December 2021

Introduction

Sports-related concussion (SRC) is a growing public health concern with significant and underestimated morbidity. SRC is a traumatic brain injury that causes transient loss of neurocognitive function and accounts for 25% of mild traumatic brain injuries (mTBI) presenting to the emergency department.[1] Clinical features of SRC are broad and varied, including neurocognitive symptoms (headache, amnesia, impaired concentration, drowsiness), autonomic dysfunction (dizziness, low blood pressure, palpitations, sweating, flushing, gastrointestinal symptoms), sleep disturbance, emotional lability and balance impairment. Any athlete with a suspected SRC must be removed from play. Safely determining when an athlete can return to play (RTP) following SRC diagnosis is a clinical priority as premature RTP puts athletes at increased risk of developing further neurological and non-neurological injuries,[2] persistent post-concussion symptoms[3] and chronic neurological deficits.[4]

[1]Institute of Sport, Exercise & Health, Division of Surgery & Interventional Science, Faculty of Medical Sciences, University College London, London, UK
[2]School of Health Sciences, Faculty of Medicine and Health Sciences, University of East Anglia, Norwich, UK

Corresponding authors:
Nipuna Senaratne, Institute of Sport, Exercise & Health, Division of Surgery & Interventional Science, Faculty of Medical Sciences, University College London, 170 Tottenham Court Road, London, WC1E 6BT, UK.
Email: nipuna.senaratne.19@ucl.ac.uk

Michael J. Grey, School of Health Sciences, Faculty of Medicine and Health Sciences, University of East Anglia, Norwich, UK.
Email: m.grey@uea.ac.uk

However, establishing when an athlete has recovered from an SRC is difficult. Current protocols for managing SRC involve an initial period of rest (24–48 h) followed by a stepwise rehabilitation protocol with gradually increasing physical and cognitive demands,[5] an example of which is shown in Table 1. Each stage takes 24 h with progression dependent on completion of the activity and meeting appropriate criteria (e.g. heart rate, duration of exercise) without recurrence of concussion-related symptoms. If symptoms do recur the athlete should drop back to the previous asymptomatic stage for a further 24 h. Clearance for RTP is determined by completion of the graded rehabilitation protocol and passing clinical assessment at each stage. However, symptom assessments in the recovery phase can be difficult and are subject to bias. They rely on subjective, athlete-dependent assessments of symptoms, whilst it can also be challenging to differentiate concussion-related symptoms from pre-morbid conditions such as chronic sleep dysfunction, migraines, anxiety and attention problems. Standardised neurological and cognitive assessment scales have been developed to assist the sideline diagnosis of SRC and following diagnosis, to aid the RTP decision.[6] However, these are imperfect and no single test can be used for either SRC diagnosis or RTP. Moreover, increasing evidence suggests physiological recovery from concussion may outlast clinical recovery meaning even after a successful graduated RTP protocol, athletes may still be at higher risk of neurological and non-neurological injury.[7] Given the limitations of clinical assessment in determining RTP following SRC, there is a great need for an objective indicator of neurophysiological recovery to enable clinicians to make safer RTP decisions.

A biomarker is an objective physiological indicator of biological disease or an injury state.[8] TBI involves a variety of pathological mechanisms and allowing a range of biomarkers to be detected in blood, saliva, urine and cerebrospinal fluid (CSF) samples following injury. During mTBI shear forces mechanically damage neuronal axons leading to release of intracellular cytoskeleton proteins such as tau, neurofilament light (NFL) and α-II spectrin N-terminal fragment (SNTF)[9,10] and these biomarkers of axonal injury have been shown to accumulate following mild TBI, severe TBI and chronic repetitive head injuries.[11,12] Neuronal cell bodies and astrocytes also undergo mechanical injury during mTBI releasing intracellular proteins such as neuron specific enolase (NSE), visinin-like protein-1 (VLP-1), ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1), S100B and glial fibrillary acidic protein (GFAP).[13–15] Growing evidence suggests secondary pathologies such as neuroinflammation and pituitary dysfunction play a role in TBI. Moderate to severe TBI has been shown to alter peripheral inflammatory cytokine profiles which negatively correlate with outcome[16] whilst repeated concussions have been linked to pituitary dysfunction[17] with cases of secondary growth hormone deficiency[18] and diabetes insipidus[19] raising the possibility that dysfunction of the hypothalamic-pituitary axis may be detectable in the acute post-concussive state.

Fluid biomarkers have potential to aid RTP decisions following SRC. Monitoring a biomarker during the rehabilitation phase post-concussion could provide an objective measure of neurophysiological recovery from injury, which could be used to complement clinical assessment in RTP decision making. Fluid sampling from CSF is invasive and carries significant procedural risks meaning only biomarkers from blood, urine and saliva samples would be feasible for serial monitoring. We performed a systematic review to identify biomarkers from blood, urine or salvia samples that have been assessed after SRC and throughout recovery from SRC. We look at the major pathological mechanisms involved in mTBI and highlight the key biomarkers from each pathway, evaluating their time course post SRC and their relationship to clinical recovery. In this way we identify objective biomarkers of neurophysiological recovery from SRC that could have potential use in RTP decisions.

Table 1. A potential biomarker-informed graduated return to sport strategy following SRC.

Stage	Activity	Goal	Biomarker	Neurophysiology Assessment(s)	Clinical Assessment	Decision
0	On-field assessment	Determine fitness for continued play	Negative AND	Negative AND	Negative	Clear to play
			Positive OR	Positive OR	Positive	Stage 1 GRTP
1	Daily activities that do not cause symptoms	Gradual introduction of normal activities				
2	Light aerobic exercise	Increase heart rate				
3	Sport specific aerobic exercise of moderate-vigorous intensity	Increase intensity	Negative AND	Negative AND	Positive	Next Stage of GRTP
4	Non-contact training drills Re-start resistance training	Increase cognitive and co-ordination demands	Positive OR	Positive OR	Positive	Remain at current GTRP stage
5	Full training	Further increase in cognitive demands Assess functional performance Restore confidence				
6	Medical clearance & return to play	Gradual introduction of normal activities	Gradual introduction of normal activities			

Methods

A systematic review of the literature was conducted in accordance with PRISMA guidelines.[20] A sensitive MEDLINE search strategy was comprised to identify relevant studies up to August 2021 using 3 groups of MESH terms: 'concussion', 'human' and 'fluid biomarker – blood, saliva, urine': (("Brain Concussion"[Mesh] OR "mild traumatic brain injury"[All Fields] OR mTBI[All Fields]) AND "humans"[MeSH Terms]) AND (("serum"[MeSH Terms] OR "serum"[All Fields]) OR ("blood"[Subheading] OR "blood"[All Fields] OR "blood"[MeSH Terms]) OR ("urine"[Subheading] OR "urine"[All Fields] OR "urine"[MeSH Terms]) OR ("saliva"[MeSH Terms] OR "saliva"[All Fields])) AND English[lang]. Reference lists of included papers were also searched for relevant studies.

Eligibility criteria

Inclusion criteria

1. Study population consists of adults (age 18 or over) diagnosed with an SRC
2. Studies examining fluid biomarkers in blood, saliva and urine
3. Studies examining biomarkers during recovery from concussion or at RTP

Exclusion criteria

1. Studies assessing chronic neurological symptoms following repeated head injuries.
2. Studies focusing on subconcussive head injuries
3. Non-fluid biomarkers
4. Conference abstracts

Study selection

All studies identified were screened independently by 3 authors (NS, ES and AH). This involved an initial title and abstract screening followed by full text screening to assess if eligibility criteria were met. Any disagreements regarding inclusion or exclusion were resolved by consensus.

Biomarker assessment

Biomarker studies were assessed to identify time courses post-SRC and relationship with clinical recovery. Secondary outcomes included correlation with symptom severity and predictive value for athletes with prolonged RTP.

Risk of bias assessment

The risk of bias of included studies was assessed independently by three authors (NS, ES and AH) using the ROBINS-I tool[21] which explores seven bias domains: bias due to confounding, bias in selection of participants into the study, bias in classification of interventions, bias due to deviations from intended intervention, bias due to missing data, bias in measurement of outcomes, bias in selection of the reported result. Each of the seven domains was assessed for low, moderate, serious or critical risk of bias. An overall rating for risk of bias for each study based on the scores of these seven domains: low risk of bias was determined if all domains were rated low risk, moderate risk if all domains rated low or moderate risk, serious risk if at least one domain rated serious but not critical and critical risk if at least one domain was rated critical.

Results

Search results

The literature search revealed 572 potentially relevant papers (Figure 1). Title and abstract screening identified 12 potentially relevant papers. Upon review of full texts, 5 of these were excluded as they did not evaluate the biomarker in the recovery period or involved duplicate data sets leaving 8 key studies. We present the results by biomarker in Tables 2–6. Risk of bias assessments are shown in Table 7. All 7 studies investigated biomarkers from blood samples. No studies evaluating the correlation of biomarkers from urine or saliva with RTP were identified.

Biomarkers of axonal injury

Tau. McCrea and colleagues[22] evaluated a range of biomarkers including tau in a prospective cohort study of 1760 US collegiate athletes. 264 athletes were diagnosed with SRC in line with US Department of Defense guideline[22] using the Sport Concussion Assessment Tool 3 (SCAT-3)[23] and underwent biomarker sampling were taken at 1 h and 24–48 h post SRC, at RTP and at 7d post RTP. These were compared with preseason baseline levels, contact sport controls (CSC - matched for demographics as well as sport, position, years of participation and concussion history) and non-contact sport controls (NCSC - matched for exertional requirement and demographics). Tau was higher than preseason, CSC and NCSC at 1 h, lower than preseason or controls at 24–48 h and was no different to preseason or either control at RTP (Table 2). It showed no difference in athletes with loss of consciousness and post-traumatic amnesia (LOC-PTA), used as symptomatic markers of severe concussions.

Shahim et al. also assessed total tau[24] and tau fragments A and C[25] in a prospective cohort study of 288 professional Swedish ice hockey players between 2012–2015. They took total tau samples from 87 players at 1 h, 12 h, 36 h and 144 h following SRC and at RTP and compared biomarker levels to preseason baseline (n = 288), athletic controls

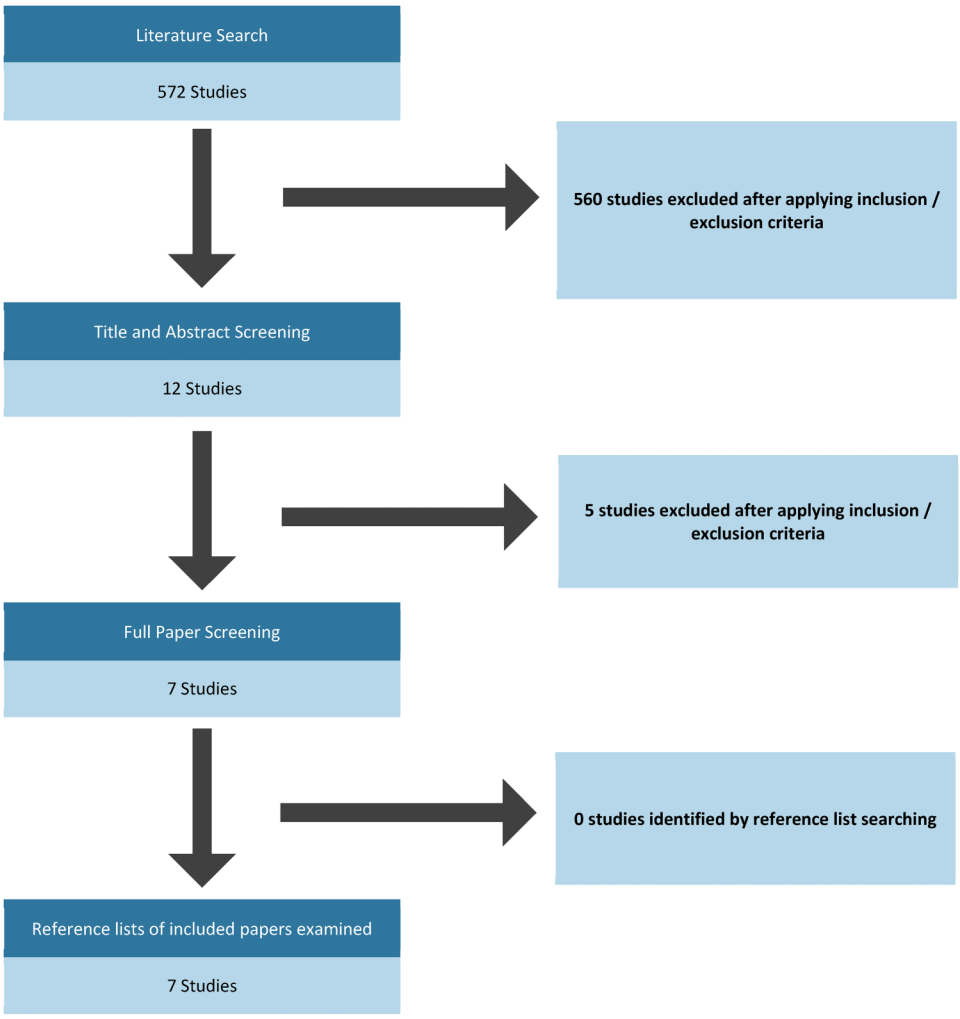

Figure 1. Search results.

(AC) (n = 12) and non-AC (n = 19). Athletes cleared for RTP had to be symptom free and complete a graduated RTP protocol with no symptoms at any stage in accordance with the latest Concussion in Sport Group guidelines.[5] 49 players returned to play within 10d, 38 took longer than 10d and 7 had symptoms persisting for more than 1 year. Total tau at 1 h post SRC was higher than preseason ($p = 0.05$) and Healthy controls (HC) ($p < 0.001$) but not AC ($p = 0.80$) and correlated with scores on the Rivermead Post-Concussion Symptoms Questionnaire (RPQ). Tau levels then normalised by 12 h and was at baseline levels by RTP. Tau at 1 h could predict players who took longer than 10d to RTP (OR = 1.9, 95% CI 1.1–1.36, $p = 0.032$, AUC 0.67). A similar protocol was used for tau fragments using 28 cases of SRC. Tau C was increased in all time points vs preseason ($p = 0.03$) with tau A showing no change. Both normalised by RTP. Tau A at 1 h and 12 h could discriminate players with prolonged RTP > 10d (1 h: AUC 0.87, 95% CI 0.71–1, $p = 0.01$; 12 h: AUC 0.91, 95% CI 0.78–1, $p = 0.005$).

Gil et al. also investigated tau in 623 American university athletes between 2009 and 2014.[26] Samples were collected from 46 athletes at 6 h, 24 h, 72 h and 168 h post SRC diagnosed by the Sport Concussion Assessment Tool 2 (SCAT-2)[27] and compared with preseason baseline (n = 623), AC (n = 37) matched for sport, prior concussion history and demographics, and non-AC (n = 21) matched for demographics. Balance Error Scoring System (BESS)[28] + Immediate Post Concussion Assessment and Cognitive Testing (ImPACT)[29] were measured 7d post-concussion. RTP decisions were made by individual universities following national guidelines with athletes being asymptomatic at rest and at each point during a graduated RTP protocol. After SRC tau was higher at 6 h, 24 h, 72 h and 168 h vs non-AC ($p < 0.01$) but was lower vs AC at 24 h and 72 h (Table 2). Tau was higher in athletes with prolonged RTP (> 10d) compared with short RTP (< 10d) at 6 h ($p < 0.01$), 24 h ($p < 0.01$) and 72 h ($p = 0.022$) and could accurately predict athletes with

Table 2. Biomarkers of axonal injury.

Study Details	Patient Group	Methods	Risk of Bias	Results	Comments
Tau					
McCrea et al. 2020[22] JAMA Network Open Prospective cohort study	1760 university athletes from USA SRC: n = 264 Contact sport controls (CSC) matched for sport, position, years of participation, concussion history, institution, sex, ethnicity, intellectual function: n = 138 Non-contact sport controls (NCSC) matched for exertional requirement, institution, sex, ethnicity, intellectual function: n = 102	**SRC Diagnosis and RTP** SRC diagnosis and RTP decisions made according to Department of Defence Guidelines using SCAT-3 assessment. **Severity of SRC** Athletes with loss of consciousness and post-traumatic amnesia (LOC-PTA) were used as symptomatic markers of severe concussions **Blood Sampling** 1) Preseason baseline 2) Post SRC: 1 h, 24–48 h 3) At RTP 4) 7d post RTP	Low	**Post SRC** Tau at 1 h higher vs preseason, CSC and NCSC (preseason: mean difference 0.221 pg/mL, 95% CI 0.046–0.396 pg/mL, p = 0.004; CSC: mean difference, 0.230 pg/mL, 95% CI 0.020–0.439 pg/mL, p = 0.03; NCSC: mean difference 0.266 pg/mL, 95% CI 0.038–0.493 pg/mL, p = 0.02) Tau lower at 24–48 hrs vs preseason, CSC and NCSC (preseason: mean difference – 0.320 pg/mL, 95% CI, – 0.461 to – 0.178 pg/mL, p < 0.001; CSC: mean difference – 0.285 pg/mL; 95% CI – 0.475 to – 0.094 pg/mL, p = 0.001; NCSC: mean difference – 0.217 pg/mL, 95% CI – 0.425 to – 0.009 pg/mL, p = 0.04) **At RTP** Tau no different at RTP or 7d post RTP vs preseason, CSC or NCSC Tau at RTP or 7d post RTP no different in LOC-PTA vs no LOC-PTA.	Tau is raised at 1 h post SRC compared to preseason, contact and non-contact sport controls. Tau then declines and returns to baseline by clinical recovery. Tau shows no difference at RTP in athletes who suffered loss of consciousness or post-traumatic amnesia **Study Strengths** - sample size - use of contact and non-contact sport controls - extensive matching of contact sport controls to account for previous cumulative head injury exposure
Shahim et al. 2018[24] Neurology Prospective cohort study	288 professional ice hockey players from the Swedish Hockey League between Sept 2012 – March 2015 SRC: n = 87 Noncontact sport athletic controls (AC): n = 12 Healthy controls (HC): n = 19	**SRC Diagnosis and RTP** SRC diagnosis and RTP decisions made according to latest Concussion in Sport guidelines.[5] To RTP athletes had to be symptom free and complete a graduated RTP protocol with no symptoms at any stage with aim of RTP within 6d **Severity of SRC**	Serious	**Post SRC** Tau at 1 h higher than preseason (p = 0.05) and HC (p < 0.001) but not AC (p = 0.80). Tau at 1 h correlates with RPQ scores (ρ = 0.32, p = 0.056). Tau at 1 h higher in and could predict players with RTP >10d vs RTP <10d (OR = 1.9, 95% CI 1.1–	Tau is raised at 1 h post SRC compared to preseason levels but not AC and returns to baseline by RTP. Tau at 1 h correlates with symptom severity and can predict athletes with long RTP. **Study Strengths** - sample size

(continued)

Table 2. Continued.

Study Details	Patient Group	Methods	Risk of Bias	Results	Comments
		Rivermead Post-Concussion Symptoms Questionnaire (RPQ) measured at 1 h post SRC **Blood Sampling** 1) Preseason baseline levels from 4 teams 2) Post SRC: 1 h, 12 h, 36 h, 144 h 3) At RTP - RTP < 10d n = 49 - RTP > 10d n = 38 - 7 players had persistent symptoms for >1 yr and had to retire from the game		1.36, p = 0.032, AUC 0.67) Tau could not separate players who had to retire due to the SRC from those who didn't. **At RTP** Tau normalised at RTP	- use of athletic controls **Study Limitations** - lack of direct comparisons between individual baseline and post-SRC levels (due to lack of available data) - athletic controls not matched and not sampled at 36 h or 144 h after training game
Gill et al. 2017[26] Neurology Prospective cohort study	623 University Athletes between 2009–2014 SRC: n = 46 Athletic controls (AC) matched for sport, hx of SRC, demographics: n = 37 Non-athletic healthy controls (HC) matched for demographics: n = 21	**SRC Diagnosis and RTP** SRC diagnosed according to Sport Concussion Assessment Tool2[27] RTP determined by each university following national guidelines: asymptomatic at rest and at each point during a graduated RTP protocol **Blood Sampling** 1) Preseason baseline 2) Post SRC: 6 h, 24 h, 72 h, 168 h AC sampled at same time points Single unrelated sampling time point for HC Balance Error Scoring System (BESS) + Immediate Postconucssion Assessment and Cognitive Testing (ImPACT) were carried out 7 days post-concussion	Low	**Post SRC** Tau higher at 6 h, 24 h, 72 h and 168 h post SRC and in AC vs HC (p < 0.01) Tau lower post SRC vs athletic controls at 24 h (6.06 vs 7.89 pg/mL, p = 0.030) and 72 h (5.19 vs 6.94 pg/mL, p = 0.041) Tau at 6 h and 72 h higher and could predict players with longer RTP (>10d) - 6 h: 10.98 vs 7.02 pg/mL, p < 0.01; AUC 0.81; 95% CI 0.62–0.97, p = 0.01 - 72 h: 6.29 vs 3.94 pg/mL, p = 0.022; AUC 0.82; 95% CI 0.68–0.96, p < 0.01 BESS and ImPACT scores no different between SRC and controls and no different between long RTP and short RTP	Tau is elevated up to 168 h post SRC compared with HC but not AC and returns to baseline by RTP. Tau at 6 h and 72 h can significantly predict athletes with prolonged RTP which could not be matched by the BESS and ImPACT tools. **Study Strengths** - sample size - use of paired baseline samples - use of well-matched athletic controls **Study Limitations** - SRC and RTP decisions made by different medical teams - no tau measurement at RTP - 2 SRC did not have RTP data
Shahim et al. 2016[25] Journal of	288 professional ice hockey players in Sweden during 2012–13	**SRC Diagnosis and RTP** SRC diagnosis and RTP decisions made according to latest Concussion	Serious	**Post SRC** Tau A no different post SRC vs preseason	Tau C but not tau A raised post SRC and both are at baseline by RTP.

(continued)

Table 2. Continued.

Study Details	Patient Group	Methods	Risk of Bias	Results	Comments
Neurotrauma Prospective cohort study	season SRC: n = 28	in Sport guidelines.[5] To RTP athletes had to be symptom free and complete a graduated RTP protocol with no symptoms at any stage with aim of RTP within 6d **Blood Sampling** 1) Preseason baseline 2) Post SRC: 1 h, 12 h, 36 h, 144 h 3) At RTP - RTP < 6d n = 13 - RTP > 6d n = 15)		Tau C post SRC higher vs preseason at all time points (p = 0.03) Tau A at 1 h and 12 h could discriminate between RTP < 10d and RTP > 10d - 1 h: AUC 0.87, 95% CI 0.71–1, p = 0.01 - 12 hh: AUC 0.91, 95% CI 0.78–1, p = 0.005 Tau C did not differ between short and long RTP **At RTP** Tau A and C normalised at RTP	Only tau A at 1 h or 12 h can predict athletes with prolonged RTP **Study Limitations** - lack of direct comparisons between individual baseline and post-SRC levels (due to lack of available data) - small sample size - lack of athletic controls
NFL					
McCrea et al. 2020[22] JAMA Network Open Prospective cohort study	1760 university athletes from USA SRC: n = 264 Contact sport controls (CSC) matched for sport, position, years of participation, concussion history, institution, sex, ethnicity, intellectual function: n = 138 Non-contact sport controls (NCSC) matched for exertional requirement, institution, sex, ethnicity, intellectual function: n = 102	**SRC Diagnosis and RTP** SRC diagnosis and RTP decisions made according to Department of Defence Guidelines using SCAT-3 assessment. **Severity of SRC** Athletes with loss of consciousness and post-traumatic amnesia (LOC-PTA) were used as symptomatic markers of severe concussions **Blood Sampling** 1) Preseason baseline 2) Post SRC: 1 h, 24–48 h 3) At RTP 4) 7d post RTP	Low	**Post SRC** NFL no different vs preseason, CSC or NCSC at any timepoint post SRC **At RTP** NFL at RTP and 7d post RTP higher in LOC-PTA vs no LOC-PTA (mean difference 0.498 pg/mL, 95% CI 0.295–0.701 pg/mL, p < 0.001), CSC (mean difference 0.481 pg/mL, 95% CI 0.271–0.692 pg/mL, p < 0.001) and NCSC (mean difference 0.448 pg/mL 95% CI 0.228–0.668 pg/mL, p < 0.001).	In SRC with loss of consciousness or post-traumatic amnesia NFL remains high at and beyond clinical recovery. **Study Strengths** - sample size - use of contact and non-contact sport controls - extensive matching of contact sport controls to account for previous cumulative head injury exposure
Shahim et al. 2018[24] Neurology Prospective cohort study	288 professional ice hockey players from the Swedish Hockey League between Sept 2012 – March 2015 SRC: n = 87 Noncontact sport athletic	**SRC Diagnosis and RTP** SRC diagnosis and RTP decisions made according to latest Concussion in Sport guidelines.[5] To RTP athletes had to be symptom free and complete a graduated RTP protocol with no symptoms at any stage with aim of	Serious	**Post SRC** NFL at 1 h higher than preseason (p = 0.02), HC (p = 0.03) and AC (p = 0.01). NFL at 12 h, 36 h, 144 h higher than preseason levels (p < 0.05) NFL at 1 h correlates with	Serum NFL at 1 h is raised post SRC compared with preseason, HC and AC. NFL remains raised at RTP NFL correlates with symptom severity and can predict athletes with long RTP at all

(continued)

Table 2. Continued.

Study Details	Patient Group	Methods	Risk of Bias	Results	Comments
	controls (AC): n = 12 Healthy controls (HC): n = 19	RTP within 6d **Severity of SRC** Rivermead Post-Concussion Symptoms Questionnaire (RPQ) measured at 1 h post SRC **Blood Sampling** 1) Preseason baseline levels from 4 teams 2) Post SRC: 1 h, 12 h, 36 h, 144 h 3) At RTP - RTP < 10d n = 49 - RTP > 10d n = 38 - 7 players had persistent symptoms for >1 yr and had to retire from the game		symptom severity RPQ scores ($\rho = 0.41$, $p = 0.011$) NFL at all time points could separate players with RTP >10d vs RTP <10d: - 1 h: OR = 8.8, 95% CI 3.0–36.0, $p < 0.001$, AUC 0.82 - 12 h: OR = 2.8 95% CI 1.3–7.3, $p = 0.021$, AUC 0.72 - 36 h: OR = 3.0, 95% CI 1.4–7.8 $p = 0.011$, AUC 0.73 - 144 h: OR = 3.30 95% CI 1.40–11.5, $p = 0.025$, AUC 0.73 NFL at 144 h could separate players who resigned from the game vs those who didn't (AUC 0.89, $p = 0.005$) **At RTP** NFL levels highest at RTP vs all other time points post SRC and preseason ($p < 0.001$)	time-points. NFL at 144 h could predict players who had to retire from the game due to PCS symptoms lasting > 1 year **Study Strengths** - sample size - use of athletic controls **Study Limitations** - lack of direct comparisons between individual baseline and post-SRC levels (due to lack of available data) - athletic controls not matched and not sampled at 36 h or 144 h after training game
SNTF					
Siman et al. 2015[30] Journal of Neurotrauma Prospective cohort study	288 professional ice hockey players in Sweden during 2012–13 season SRC: n = 28	**SRC Diagnosis and RTP** SRC diagnosis and RTP decisions made according to latest Concussion in Sport guidelines.[5] To RTP athletes had to be symptom free and complete a graduated RTP protocol with no symptoms at any stage with aim of RTP within 6d **Blood Sampling** 1) Baseline Preseason 2) Post SRC: 1, 12, 36, 144 h 3) At RTP - RTP < 6d n = 13 - RTP > 6d n = 15)	Serious	**Post SRC** SNTF higher at 12, 36, 144 h post SRC vs preseason ($p < 0.05$) SNTF at 36 h could predict RTP > 6d (AUC = 0.85; 95% CI 0.73–0.97) Multivariate measure of tau and SNTF combined correlates with RTP better than tau alone, but worse than SNTF alone **AT RTP** SNTF returned to baseline by RTP	SNTF is elevated up to 144 h post SRC and returns to baseline by RTP. SNTF at 36 h can predict athletes with long RTP. **Study Limitations** - lack of direct comparisons between individual baseline and post-SRC levels (due to lack of available data) - small sample size - lack of athletic controls

Key: SRC = sports-related concussion, RTP = return to play, d = days, SEM = sports exercise medicine PLSDA = Partial Least Squares Discriminant Analysis, PLS = partial least squares, IQR = interquartile range

Table 3. Biomarkers of neuronal injury.

Study Details	Patient Group	Methods	Risk of Bias	Results	Comments
NSE					
Shahim et al. 2018[24] Neurology Prospective cohort study	288 professional ice hockey players from the Swedish Hockey League between Sept 2012 – March 2015 SRC: n = 87 Noncontact sport athletic controls (AC): n = 12 Healthy controls (HC): n = 19	**SRC Diagnosis and RTP** SRC diagnosis and RTP decisions made according to latest Concussion in Sport guidelines.[5] To RTP athletes had to be symptom free and complete a graduated RTP protocol with no symptoms at any stage with aim of RTP within 6d **Severity of SRC** Rivermead Post-Concussion Symptoms Questionnaire (RPQ) measured at 1 h post SRC **Blood Sampling** 1) Preseason baseline levels from 4 teams 2) Post SRC: 1 h, 12 h, 36 h, 144 h 3) At RTP - RTP < 10d n = 49 - RTP > 10d n = 38 - 7 players had persistent symptoms for >1 yr and had to retire from the game	Serious	**Post SRC** NSE at 1 h higher vs HC ($p = 0.002$) but not preseason ($p = 0.8$) or AC ($p = 0.55$) No correlation between NSE and RPQ ($\rho = -0.017$, $p = 0.92$) NSE could not separate players with prolonged RTP (>10d) or players who had to retire due to the SRC. **At RTP** NSE normalised at RTP	NSE not raised post SRC compared to preseason or AC or correlate with clinical recovery. **Study Strengths** - sample size - use of athletic controls **Study Limitations** - lack of direct comparisons between individual baseline and post-SRC levels (due to lack of available data) - athletic controls not matched and not sampled at 36 h or 144 h after training game
VLP-1					
Shahim et al. 2015[31] Brain Injury Prospective cohort study	288 professional ice hockey players in Sweden during 2012–13 season SRC: n = 28	**SRC Diagnosis and RTP** SRC diagnosis and RTP decisions made according to latest Concussion in Sport guidelines.[5] To RTP athletes had to be symptom free and complete a graduated RTP protocol with no symptoms at any stage with aim of RTP within 6d **Blood Sampling** 1) Preseason baseline 2) Post SRC: 1, 12, 36, 144 h 3) At RTP - RTP < 6d n = 13 - RTP > 6d n = 15	Serious	**Post SRC** No significant change in VLP-1 at 1, 12 and 144 h post SRC No correlation between VLP-1 levels at any time point and RTP duration	VLP-1 does not change post SRC or correlate with clinical recovery **Study Limitations** - lack of direct comparisons between individual baseline and post-SRC levels (due to lack of available data) - small sample size - lack of athletic controls

(continued)

Table 3. Continued.

Study Details	Patient Group	Methods	Risk of Bias	Results	Comments
UCH-L1					
McCrea et al. 2020[22] JAMA Network Open Prospective cohort study	1760 university athletes from USA SRC: n = 264 Contact sport controls (CSC) matched for sport, position, years of participation, concussion history, institution, sex, ethnicity, intellectual function: n = 138 Non-contact sport controls (NCSC) matched for exertional requirement, institution, sex, ethnicity, intellectual function: n = 102	**SRC Diagnosis and RTP** SRC diagnosis and RTP decisions made according to Department of Defence Guidelines using SCAT-3 assessment. **Severity of SRC** Athletes with loss of consciousness and post-traumatic amnesia (LOC-PTA) were used as symptomatic markers of severe concussions **Blood Sampling** 1) Preseason baseline 2) Post SRC: 1 h, 24–48 h 3) At RTP 4) 7d post RTP	Low	**Post SRC** UCH-L1 at 1 h higher vs preseason, CSC and NCSC at 1 h (preseason: mean difference 0.449 pg/mL, 95% CI 0.167–0.732 pg/mL, $p < 0.001$; CSC: mean difference 0.577 pg/mL, 95% CI 0.236–0.919 pg/mL, $p < 0.001$; NCSC: mean difference 0.463 pg/mL, 95% CI 0.088–0.839 pg/mL, $p = 0.01$) UCH-L1 at 24–48 h no different vs preseason, CSC or NCSC. **At RTP** UCH-L1 at RTP lower vs preseason (mean difference – 0.321 pg/mL, 95% CI, – 0.546 to – 0.095 pg/mL, $p < 0.001$) and NCSC (mean difference – 0.373 pg/mL, 95% CI – 0.714 to – 0.032 pg/mL, $p = 0.03$) but no different to CSC UCH-L1 at 7d post RTP hrs no different vs preseason, CSC or NCSC. UCH-L1 at RTP or 7d post RTP no different in LOC-PTA vs no LOC-PTA.	UCH-L1 is raised at 1 h post SRC compared to preseason, contact and non-contact sport controls. UCH-L1 then declines and returns to baseline by clinical recovery. UCH-L1 shows no difference at RTP in athletes who suffered loss of consciousness or post-traumatic amnesia **Study Strengths** - sample size - use of contact and non-contact sport controls - extensive matching of contact sport controls to account for previous cumulative head injury exposure

Key: SRC = sports-related concussion, RTP = return to play, d = days, SEM = sports exercise medicine PLSDA = Partial Least Squares Discriminant Analysis, PLS = partial least squares, IQR = interquartile range

Table 4. Biomarkers of astroglial injury.

Study Details	Patient Group	Methods	Risk of Bias	Results	Comments
GFAP					
McCrea et al. 2020[22] JAMA Network Open Prospective cohort study	1760 university athletes from USA SRC: n = 264 Contact sport controls (CSC) matched for sport, position, years of participation, concussion history, institution, sex, ethnicity, intellectual function: n = 138 Non-contact sport controls (NCSC) matched for exertional requirement, institution, sex, ethnicity, intellectual function: n = 102	**SRC Diagnosis and RTP** SRC diagnosis and RTP decisions made according to Department of Defence Guidelines using SCAT-3 assessment. **Severity of SRC** Athletes with loss of consciousness and post-traumatic amnesia (LOC-PTA) were used as symptomatic markers of severe concussions **Blood Sampling** 1) Preseason baseline 2) Post SRC: 1 h, 24–48 h 3) At RTP 4) 7d post RTP	Low	**Post SRC** GFAP at 1 h higher vs preseason, CSC and NCSC (preseason: mean difference 0.430 pg/mL, 95% CI 0.339–0.521 pg/mL, $p < 0.001$; CSC: mean difference 0.419 pg/mL, 95% CI 0.295–0.543 pg/mL, $p < 0.001$; NCSC: mean difference 0.378 pg/mL, 95% CI 0.242–0.514 pg/mL, $p < 0.001$) GFAP at 24–48 h higher vs preseason, CSC and NCSC (preseason: mean difference 0.255 pg/mL, 95% CI 0.183–0.328 pg/mL, $p < 0.001$; CSC: mean difference 0.191 pg/mL; 95% CI 0.076–0.306 pg/mL, $p < 0.001$; NCSC: mean difference 0.177 pg/mL, 95% CI 0.050–0.304 pg/mL, $p = 0.003$) **At RTP** GFAP higher at RTP and 7d post RTP vs baseline (RTP: mean difference 0.124 pg/mL, 95% CI 0.056–0.191 pg/mL, $p < 0.001$; 7d post RTP: mean difference 0.092 pg/mL, 95% CI 0.022–0.162 pg/mL, $p = 0.002$) GFAP at RTP and 7d post RTP no different to CSC and NCSC GFAP at RTP higher in LOC-PTA vs no LOC-PTA (mean difference 0.196 pg/mL, 95% CI 0.022–0.371 pg/mL, $p = 0.02$), CSC (mean difference 0.253 pg/mL, 95% CI 0.070–0.435 pg/mL, $p = 0.002$) and NCSC (mean difference 0.193 pg/mL, 95% CI 0.001–0.385 pg/mL, $p = 0.048$)	GFAP is raised up to 48 h after SRC compared to preseason, contact and non-contact sport controls. At clinical recovery GFAP is higher than preseason levels but no different to athletic controls. GFAP at RTP in athletes who suffered loss of consciousness or post-traumatic amnesia is higher than those who didn't and higher than athletic controls. **Study Strengths** - sample size - use of contact and non-contact sport controls - extensive matching of contact sport controls to account for previous cumulative head injury exposure

(continued)

Table 4. Continued.

Study Details	Patient Group	Methods	Risk of Bias	Results	Comments
S100B					
Shahim et al. 2018[24] Neurology Prospective cohort study	288 professional ice hockey players from the Swedish Hockey League between Sept 2012 – March 2015 SRC: n = 87 Noncontact sport athletic controls (AC): n = 12 Healthy controls (HC): n = 19	**SRC Diagnosis and RTP** SRC diagnosis and RTP decisions made according to latest Concussion in Sport guidelines.[5] To RTP athletes had to be symptom free and complete a graduated RTP protocol with no symptoms at any stage with aim of RTP within 6d **Severity of SRC** Rivermead Post-Concussion Symptoms Questionnaire (RPQ) measured at 1 h post SRC **Blood Sampling** 1) Preseason baseline levels from 4 teams 2) Post SRC: 1 h, 12 h, 36 h, 144 h 3) At RTP - RTP < 10d n = 49 - RTP > 10d n = 38 - 7 players had persistent symptoms for >1 yr and had to retire from the game	Serious	**Post SRC** S100B at 1 h higher than preseason ($p = 0.002$), HC ($p < 0.0001$) and AC ($p = 0.014$) No correlation between S100B and RPQ ($\rho = 0.12$, $p = 0.51$) S100B could not separate players with prolonged RTP (>10d) or players who had to retire due to the SRC. **At RTP** S100B normalised at RTP	S100B at 1 h is raised compared to preseason, HC and AC. S100B does not correlate with symptom severity and cannot predict athletes with long RTP. **Study Strengths** - sample size - use of athletic controls **Study Limitations** - lack of direct comparisons between individual baseline and post-SRC levels (due to lack of available data) - athletic controls not matched and not sampled at 36 h or 144 h after training game

Key: SRC = sports-related concussion, RTP = return to play, d = days, SEM = sports exercise medicine PLSDA = Partial Least Squares Discriminant Analysis, PLS = partial least squares, IQR = interquartile range

Table 5. Biomarkers of pituitary dysfunction.

Study Details	Patient Group	Methods	Risk of Bias	Results	Comments
PRL					
La Fountaine et al. 2016[32] Journal of Neurotrauma Observational case study	4 male intercollegiate athletes	**Blood Sampling** 1)??< 48 h post SRC 2) 7d post SRC 3) 14d post SRC	Serious	**Post SRC** PRL increased in all 4 athletes across the 3 visits The athlete with the lowest PRL at visit 1 was associated with the longest RTP	Observational evidence that PRL after SRC is increased and correlates with clinical recovery **Study Limitations** - small sample size - descriptive statistics - lack of controls - no comparisons vs baseline levels - no discussion on standardisation of SRC dx / RTP decisions

Key: SRC = sports-related concussion, RTP = return to play, d = days

Table 6. Biomarkers of neuroinflammation.

Study Details	Patient Group	Methods	Risk of Bias	Results	Comments
Neuroinflammatory cytokines - IFN, TNF- α, MPO, IL-8, IP-10, MCP-1, MCP-4, MIP-1α, IP-1β, TARC					
Di Battista et al. 2019[33] Journal of Neuroinflammation Prospective cohort study	175 interuniversity male and female athletes from 12 sports (basketball, field hockey, american football, ice hockey, lacrosse, mountain biking, rugby, football, swimming, athletics, volleyball, water polo) SRC: n = 43 Healthy controls (HC): n = 102	**SRC Diagnosis and RTP** SRC diagnosis and RTP decisions made by SEM clinic in accordance with Concussion in Sport Group guidelines.[5] To RTP athletes had to have resolution of symptoms, balance and cognitive deficits and complete a graded exercise protocol **Blood Sampling** 1) Preseason baseline 2) Subacute Phase Post Injury: median = 4d post SRC 3) At RTP: median = 25d post SRC, IQR = 15–55d	Moderate	**Post SRC** MCP-4 and MIP-1β higher vs HC ($p < 0.05$). MCP-4 and MIP-1β could discriminate SRC vs control (PLSDA analyses) - MCP-4: bootstrap ratio = 3.5, $p < 0.001$) - MIP-1β: bootstrap ratio = 3.3, $p = 0.001$) MCP-4 and MCP-1 positively correlated with days to recovery (PLS analysis) - MCP-4: bootstrap ratio = 2.7, $p = 0.007$ - MCP-1: bootstrap ratio = 3.5, $p < 0.001$ **At RTP** No significant differences in any biomarker levels vs preseason	MCP-4 is elevated post SRC and returns to baseline by clinical recovery. Elevation in MCP-4 level correlates with severity of SRC. **Study Limitations** - Only one sample post SRC limits interpretation of the markers' temporal profile - no athletic controls

Key: SRC = sports-related concussion, RTP = return to play, d = days, SEM = sports exercise medicine PLSDA = Partial Least Squares Discriminant Analysis, IQR = interquartile range

prolonged recovery at 6 h and 72 h (Table 2). BESS and ImPACT scores showed no difference between long RTP and short RTP.

Overall, tau is raised acutely after SRC compared to pre-season and well-matched AC, remains raised for 24–72 h and returns to baseline by RTP. Within this acute period

Table 7. Risk of bias assessments.

Study	Confounding	Selection of Participants	Classification of interventions	Deviations of intended interventions	Missing data	Measurement of outcomes	Selection of reported result	Overall Risk of Bias
McCrea et al. 2020[22]	Low	Low	Low	Low	Low	Low	Low	**Low**
Di Battista et al. 2019[33]	Moderate	Low	Low	Low	Moderate	Low	Low	**Moderate**
Shahim et al. 2018[24]	Serious	Low	Low	Low	Low	Low	Low	**Serious**
Gill et al. 2017[26]	Moderate	Low	Low	Low	Low	Low	Low	**Moderate**
Shahim et al. 2016	Serious	Low	Low	Low	Moderate	Low	Low	**Serious**
La Fountaine et al. 2016[32]	Serious	Low	Moderate	Low	Low	Low	Low	**Serious**
Siman et al. 2015[30]	Serious	Low	Low	Low	Moderate	Low	Low	**Serious**
Shahim et al. 2015[31]	Serious	Low	Low	Low	Moderate	Low	Low	**Serious**

tau shows good correlation with athletes with more severe concussions.

Neurofilament light (NFL). In addition to tau, McCrea et al. also assessed NFL in their study of US collegiate athletes. They found that NFL was no different at any time point post SRC compared to preseason, CSC or NCSC. However, in athletes who suffered loss of consciousness or post-traumatic amnesia NFL increased over time and was higher at RTP and 7d compared to preseason and both controls (preseason: mean difference 0.498 pg/mL, 95% CI 0.295–0.701 pg/mL, $p < 0.001$; CSC: mean difference 0.481 pg/mL, 95% CI 0.271–0.692 pg/mL, $p < 0.001$; NCSC:mean difference 0.448 pg/mL 95% CI 0.228–0.668 pg/mL, $p < 0.001$).

Shahim et al. also investigated NFL,[24] finding that NFL was higher than preseason levels at 1 h, 12 h, 36 h and 144 h post SRC and higher than healthy and AC at 1 h. At 1 h NFL levels correlated with symptom severity scores on the RPQ ($\rho = 0.41$, $p = 0.011$). At RTP NFL levels remained raised vs preseason levels ($p < 0.001$). At all times post-SRC, NFL could separate players with RTP > 10d vs RTP < 10d (1 hr: OR = 8.8, 95% CI 3.0–36.0, $p = 0.0006$, AUC 0.82; 12 h: OR = 2.8 95% CI 1.3–7.3, $p = 0.021$, AUC 0.72; 36 h: OR = 3.0, 95% CI 1.4–7.8 $p = 0.011$, AUC 0.73; 144 h: OR = 3.30 95% CI 1.40–11.5, $p = 0.025$, AUC 0.73). NFL at 144 h post SRC could separate athletes with persistent PCS symptoms lasting over a year who had to retire from the game (AUC 0.89, $p = 0.005$).

These studies show NFL is raised after severe concussions, correlating with symptom severity scores and RTP duration. In these patients NFL does not return to baseline by clinical recovery - as measured consistently throughout being symptom and sign free on concussion assessment scales such as the SCAT 5.

α-II spectrin breakdown products. Siman et al.[30] investigated the potential of SNTF as a concussion biomarker in part of their cohort of professional ice hockey players. Serum SNTF levels at 12 h, 36 h and 144 h post SRC (n = 28) were higher than preseason, returning to baseline at RTP. SNTF at 36 h was a good predictor of prolonged RTP > 6d (AUC = 0.85; 95% CI 0.73–0.97). SNTF changes at 12–36 h post SRC correlated with tau changes at similar time points ($R^2 = 0.84$, n = 24) and multivariate measures of tau and SNTF better correlated with RTP than tau alone but worse than SNTF alone. This study demonstrates SNTF is raised post SRC vs preseason levels, correlates with longer RTP at 36 h and returns to baseline by RTP.

Biomarkers of neuronal injury

Neuron specific enolase (NSE). Shahim et al.[25] assessed NSE in their ice hockey study and found no change in NSE levels post SRC vs preseason baseline and no correlation with number of days taken to RTP.

Visinin-like protein-1 (VLP-1). Shahim et al.[31] assessed serum VLP-1 in their ice hockey study and found no changes in VLP-1 post-concussion at 1 h, 12 h and 144 h compared with preseason levels, with a reduction at 36 h. There was no correlation between VLP-1 levels and RTP duration. Overall, these markers of neuronal injury do not show any change post SRC.

Ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1). McCrea et al. also assessed UCH-L1 in their US collegiate athlete study and found that UCH-L1 increased 1 h post SRC compared to preseason baselines and CSC and NCSC (preseason: mean difference 0.449 pg/mL, 95% CI 0.167–0.732 pg/mL, $p < 0.001$; CSC: mean difference 0.577 pg/mL, 95% CI 0.236–0.919 pg/mL, $p < 0.001$; NCSC: mean difference 0.463 pg/mL, 95% CI 0.088–0.839 pg/mL, $p = 0.01$). At the 24–48 h time point UCH-L1 was no different vs preseason or controls. At RTP UCH-L1 was lower vs preseason (mean difference − 0.321 pg/mL, 95% CI, − 0.546 to − 0.095 pg/mL, $p < 0.001$) and NCSC (mean difference − 0.373 pg/mL, 95% CI − 0.714 to − 0.032 pg/mL, $p = 0.03$) but no different to CSC. By 7d post RTP there

were no differences. At neither points post RTP did UCH-L1 show any difference in athletes who experienced loss of consciousness or post-traumatic amnesia).

Biomarkers of astroglial injury

S100b. Shahim et al. also looked at S100B in their ice hockey study.[24]They found that S100B at 1 h post SRC was higher vs preseason samples ($p < 0.001$), as well as healthy ($p < 0.001$) and AC ($p = 0.014$). S100B at 1 h did not correlate with RPQ scores and could not discriminate between players with long or short RTP. At 12 h, 36 h and 144 h and RTP there was no difference between S100B post SRC and preseason samples.

Shahim and colleagues show S100B increases post SRC compared to athletic and non-AC and returns to baseline by RTP. S100B does not correlate with symptom severity or athletes with prolonged RTP.

Glial fibrillary acidic protein (GFAP). Amongst their other biomarkers, McCrea and colleagues also investigated GFAP. They showed that GFAP is increased at 1 h post SRC and 24–48 h post SRC compared to preseason, CSC and NCSC (Table 4). At RTP and 7d post RTP GFAP was higher than preseason but no different to either athletic control group (Table 4). At RTP athletes who suffered loss of consciousness or post-traumatic amnesia had higher GFAP levels compared to those who didn't and compared to both groups of AC (Table 4).

Biomarkers of pituitary dysfunction

Prolactin (PRL). La Fountaine et al.[32] investigated PRL in 4 intercollegiate athletes who suffered SRC taking samples at 3 time-points post SRC (within 48 h, after 7d and after 14d). PRL levels increased across the 3 visits in all 4 athletes but the athlete with the lowest initial PRL level was associated with the longest RTP.

This study provides preliminary observational evidence that PRL levels may correlate with clinical recovery.

Biomarkers of neuroinflammation

Di Battista et al. assessed a range of inflammatory biomarkers following SRC in interuniversity athletes.[33] In athletes who suffered SRCs ($n = 43$) or MSK injuries ($n = 30$) they assessed a panel of cytokines (IFN-γ, TNF-α, MPO, IL-8, eotaxin, IP-10, MCP-1, MCP-4, MIP-1α, MIP-1β and TARC) at preseason, within 8d of injury and within 14d of RTP and compared these with HC ($n = 102$). SRC diagnosis and RTP decisions were made at a single sports medicine clinic in accordance with the most recent Concussion in Sport Group Guidelines.[5] Two biomarkers (MCP-4 and MIP-1β) were raised post SRC and could discriminate between SRC and healthy athletes ($p < 0.001$ and $p = 0.001$ respectively). These differences resolved by RTP. MCP-4 and MCP-1 correlated with days to RTP ($p = 0.007$ and $p < 0.001$). These changes were not seen in the MSK-injury patients. The inflammatory cytokine MCP-4 is elevated post SRC compared to HC, returns to baseline by RTP and correlates with length of recovery.

Discussion

A variety of concussion biomarkers taken from blood samples have been evaluated following SRC. Markers of axonal injury including tau, NFL and SNTF are consistently raised after SRC and show varying time courses of recovery post-concussion. In addition, they show a correlation with severity of concussion measured either by symptom severity or duration for RTP. Amongst other classes of biomarkers, the neuronal marker UCH-L1, astroglial proteins GFAP and S100B and the neuroinflammatory cytokine MCP-4 are also raised post SRC and return to baseline at RTP with GFAP and MCP-4 also showing a correlation with severity of injury. Other markers of neuronal injury such as NSE and VLP-1 are not affected by SRC and markers of pituitary dysfunction such as PRL have only been described in case studies. Our review identified no studies evaluating the utility of urine and saliva biomarkers for RTP following SRC which reflects their more relatively recent recognition as potentially useful concussion biomarkers. Identifying changes in microRNA expression in saliva has recently been recognised as a potential diagnostic marker for concussed athletes[34,35] and similar changes are also seen in athletes following recurrent concussive episodes.[36] Exploration of the diagnostic and prognostic utility of microRNAs in saliva and urine for SRC is ongoing[37] and given the ease of access and non-invasiveness of these tests in comparison to blood sampling, the results are highly anticipated.

The current evidence base has a number of limitations. Only a small number of studies have correlated concussion biomarkers with clinical recovery each with relatively poor sample sizes. Study protocols varied in their sampling time-points, use of controls, clinical assessments of SRC and had a range of severities of concussion. In particular, the lack of widespread use of AC, particularly matched for contact history, limits inferences given these markers are influenced by physical activity and previous history of head trauma. The body of evidence is strongest for tau and NFL which are the only markers to be assessed in multiple cohorts with good sample sizes and consistent use of AC some with extensive matching for prior history of head injury. NSE, UCH-L1, GFAP and S100B have only been evaluated in single cohorts but on each occasion this did utilise a large cohort and AC. The evidence is weakest for SNTF, VLP-1 and inflammatory cytokines, coming from a single small sample with no AC whilst PRL has only been investigated in a small case series. Overall, these do reflect significant limitations in the evidence base and

further higher-powered and standardised studies are needed to strengthen our knowledge.

Despite these limitations there is evidence to support further research into the potential use of biomarkers to assist clinicians to make RTP decisions. The present review identifies several biomarkers including tau, SNTF, UCH-L1, S100B, GFAP and MCP-4 that show deviations post SRC and return to baseline by clinical recovery from injury. Sampling these markers post-concussion and regularly throughout rehabilitation could provide valuable information on neuropathological recovery. In the future, alongside clinical recovery and successful completion of a graduated rehabilitation programme, improvement of a biomarker to baseline level could be included in criteria for RTP. Given neurophysiological and clinical recoveries may be distinct, this could mean an athlete who has clinically recovered from SRC could be precluded from RTP due to persisting biomarker abnormalities (eg Table 1). However, use of these markers in this way is not without its own risks. Currently the safety implications of these biomarkers are poorly understood. For example, in severe concussions NFL is raised at clearance for RTP suggesting some neurophysiological markers lag clinical recovery. It is unclear currently if returning to sport with incomplete neurophysiological recovery from SRC carries with it further neurological or non-neurological risks. Unnecessarily precluding athletes from returning to sport can be harmful for the individual athlete and team perspective and many sports currently operate mandatory lock out periods preventing return to training or match play for a period after injury. In athletes with normal clinical examinations biomarker data supporting full neurophysiological recovery could support a more individualised decision-making process that could prevent players being kept out of play longer than required (eg Table 1). Future work to establish the clinical significance and risk profiles of biomarkers is needed to allow their safe and effective use.

Several markers including tau, NFL, SNTF, GFAP and MCP-4 show correlations with severe concussions with more pronounced symptoms or longer RTP duration and tau was shown to outperform the commonly used clinical assessments (the BESS and ImPACT tools) in severe cases.[24] As such a secondary use for these biomarkers may be in the early detection of severe SRCs. This would help to highlight athletes whose injury may be more severe than indicated by clinical assessment alone and who need a longer and more closely observed rehabilitation period. To facilitate this further elucidation of the temporal and prognostic profiles of these biomarkers is needed to identify the most clinically useful and practical sampling time points post SRC.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship and/or publication of this article.

ORCID iD

Nipuna Senaratne https://orcid.org/0000-0001-7920-3465

References

1. Kelly KD, Lissel HL, Rowe BH, et al. Sport and recreation-related head injuries treated in the emergency department. *Clin J Sport Med* 2001; 11: 77–81.
2. Chmielewski TL, Tatman J, Suzuki S, et al. Impaired motor control after sport-related concussion could increase risk for musculoskeletal injury: implications for clinical management and rehabilitation. *J Sport Health Sci* 2020; 10 (2): 154–161. 10.1016/j.jshs.2020.11.005
3. Cantu RC and Register-Mihalik JK. Considerations for return-to-play and retirement decisions after concussion. *PM R* 2011; 3: S440–S444. 2011/11/09.
4. McKee AC, Cantu RC, Nowinski CJ, et al. Chronic traumatic encephalopathy in athletes: progressive tauopathy after repetitive head injury. *J Neuropathol Exp Neurol* 2009; 68: 709–735. 2009/06/19.
5. McCrory P, Meeuwisse W, Aubry M, et al. Consensus statement on concussion in sport—the 4th international conference on concussion in sport held in zurich, November 2012. *Clin J Sport Med* 2013; 23: 89–117.
6. Dessy AM, Yuk FJ, Maniya AY, et al. Review of assessment scales for diagnosing and monitoring sports-related concussion. *Cureus* 2017; 9: e1922. 2018/02/20.
7. McPherson AL, Nagai T, Webster KE, et al. Musculoskeletal injury risk after sport-related concussion: a systematic review and meta-analysis. *Am J Sports Med* 2019; 47: 1754–1762. 2018/08/04.
8. Jeter CB, Hergenroeder GW, Hylin MJ, et al. Biomarkers for the diagnosis and prognosis of mild traumatic brain injury/concussion. *J Neurotrauma* 2013; 30: 657–670.
9. Ahmadzadeh H, Smith DH and Shenoy VB. Viscoelasticity of tau proteins leads to strain rate-dependent breaking of microtubules during axonal stretch injury: predictions from a mathematical model. *Biophys J* 2014; 106: 1123–1133.
10. Roberts-Lewis JM, Savage MJ, Marcy VR, et al. Immunolocalization of calpain I-mediated spectrin degradation to vulnerable neurons in the ischemic gerbil brain. *J Neurosci Official JSoc Neurosci* 1994; 14: 3934–3944.
11. Siman R, Giovannone N, Hanten G, et al. Evidence that the blood biomarker SNTF predicts brain imaging changes and persistent cognitive dysfunction in mild TBI patients. *Front Neurol* 2013; 4: 190–190.
12. Shahim P, Gren M, Liman V, et al. Serum neurofilament light protein predicts clinical outcome in traumatic brain injury. *Sci Rep* 2016; 6: 36791–36791.
13. Skogseid IM, Nordby HK, Urdal P, et al. Increased serum creatine kinase BB and neuron specific enolase following head injury indicates brain damage. *Acta Neurochir* 1992; 115: 106–111.
14. Lee J-M, Blennow K, Andreasen N, et al. The brain injury biomarker VLP-1 is increased in the cerebrospinal fluid of

Alzheimer disease patients. *Clin Chem* 2008; 54: 1617–1623. 2008/08/14.
15. Kleindienst A and Bullock MR A critical analysis of the role of the neurotrophic protein S100B in acute brain injury. *J Neurotrauma* 2006; 23: 1185–1200.
16. Di Battista AP, Rhind SG, Hutchison MG, et al. Inflammatory cytokine and chemokine profiles are associated with patient outcome and the hyperadrenergic state following acute brain injury. *J Neuroinflammation* 2016; 13: 40–40.
17. Fernandez-Rodriguez E, Bernabeu I, Castro AI, et al. Hypopituitarism after traumatic brain injury. *Endocrinol Metab Clin N Am* 2015; 44: 151–159.
18. Ives JC, Alderman M and Stred SE. Hypopituitarism after multiple concussions: a retrospective case study in an adolescent male. *J Athl Train* 2007; 42: 431–439. 2007/12/07.
19. Foley CM and Wang DH. Central diabetes insipidus following a sports-related concussion: a case report. *Sports Health* 2012; 4: 139–141.
20. Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* 2009; 6: e1000097.
21. Sterne JA, Hernán MA, Reeves BC, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. *BMJ (Clinical research ed)* 2016; 355: i4919–i4919.
22. McCrea M, Broglio SP, McAllister TW, et al. Association of blood biomarkers with acute sport-related concussion in collegiate athletes: findings From the NCAA and department of defense CARE consortium. *JAMA Netw Open* 2020; 3: e1919771. 2020/01/25.
23. Guskiewicz K, Register-Mihalik J and McCrory P, et al. SCAT3. Br J Sports Med 2013; 47: 259. 2013/03/13.
24. Shahim P, Tegner Y, Marklund N, et al. Neurofilament light and tau as blood biomarkers for sports-related concussion. *Neurology* 2018; 90: e1780–e1788. 2018/04/13.
25. Shahim P, Linemann T, Inekci D, et al. Serum Tau fragments predict return to play in concussed professional Ice hockey players. *J Neurotrauma* 2016; 33: 1995–1999.
26. Gill J, Merchant-Borna K, Jeromin A, et al. Acute plasma tau relates to prolonged return to play after concussion. *Neurology* 2017; 88: 595–602. 2017/01/06.
27. McCrory P, Meeuwisse W, Johnston K, et al. Consensus statement on concussion in sport: the 3rd international conference on concussion in sport held in zurich, November 2008. *J Athl Train* 2009; 44: 434–448.
28. Riemann BL and Guskiewicz KM. Effects of mild head injury on postural stability as measured through clinical balance testing. *J Athl Train* 2000; 35: 19–25. 2006/03/25.
29. Maroon JC, Lovell MR, Norwig J, et al. Cerebral concussion in athletes: evaluation and neuropsychological testing. *Neurosurgery* 2000; 47: 659–669. discussion 669–672. 2000/09/12.
30. Siman R, Shahim P, Tegner Y, et al. Serum SNTF increases in concussed professional ice hockey players and relates to the severity of postconcussion symptoms. *J Neurotrauma* 2015; 32: 1294–1300.
31. Shahim P, Mattsson N, Macy EM, et al. Serum visinin-like protein-1 in concussed professional ice hockey players. *Brain Inj* 2015; 29: 872–876.
32. La Fountaine MF, Toda M, Testa A, et al. Suppression of Serum prolactin levels after sports concussion with prompt resolution Upon independent clinical assessment To permit return-to-play. *J Neurotrauma* 2016; 33: 904–906.
33. Di Battista AP, Churchill N, Rhind SG, et al. Evidence of a distinct peripheral inflammatory profile in sport-related concussion. *J Neuroinflammation* 2019; 16: 17–17.
34. Di Pietro V, Porto E, Ragusa M, et al. Salivary MicroRNAs: diagnostic markers of mild traumatic brain injury in contact-sport. *Front Mol Neurosci* 2018; 11: 290. 2018/09/05.
35. LaRocca D, Barns S, Hicks SD, et al. Comparison of serum and saliva miRNAs for identification and characterization of mTBI in adult mixed martial arts fighters. *PloS one* 2019; 14: e0207785–e0207785.
36. Hicks S, Loeffert J, Loeffert A, et al. 4043 Saliva microRNA for pediatric concussion assessment. *Journal of Clinical and Translational Science* 2020; 4: 112–112.
37. Yakoub KM, O'Halloran P, Davies DJ, et al. Study of concussion in rugby union through MicroRNAs (SCRUM): a study protocol of a prospective, observational cohort study. *BMJ open* 2018; 8: e024245–e024245.