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A blood biomarker and clinical correlation cohort study protocol to diagnose sports-related concussion and monitor recovery in elite rugby

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ABSTRACT

Introduction In professional rugby, sports-related concussion (SRC) remains the most frequent time loss injury. Therefore, accurately diagnosing SRC and monitoring player recovery, through a multi-modal assessment process, is critical to SRC management. In this protocol study, we aim to assess SRC over multiple time points post-injury to determine the value of multi-modal assessments to monitor player recovery. This is of significance to minimise premature return-to-play and, ultimately, to reduce the long-term effects associated with SRC. The study will also establish the logistics of implementing such a study in a professional setting to monitor a player's SRC recovery.

Methods and analysis All players from the participating professional rugby club within the Irish Rugby Football Union are invited to participate in the current study. Player assessment includes head injury assessment (HIA), neuropsychometric assessment (ImPACT), targeted biomarker analysis and untargeted biomarker analysis. Baseline HIA, ImPACT, and blood draws are performed prior to the start of playing season. During the baseline tests, player's complete consent forms and an SRC history questionnaire. Subsequently, any participant that enters the HIA process over the playing season due to a suspected SRC will be clinically assessed (HIA and ImPACT) and their blood will be drawn within 3 days of injury, 6 days post-injury, and 13 days post-injury.

Ethics and dissemination Ethical approval was attained from the Science and Engineering Research Ethics Committee, University of Limerick (Approval Code: 2018_06_11_S&E). On completion of the study, further manuscripts will be published to present the results of the tests and their ability to measure player recovery from SRC.

Trial registration number NCT04485494.

INTRODUCTION

Sports-related concussion (SRC) remains the most frequent match-related time loss injury in professional rugby.[1–3] Although professional athletes report low levels of SRC-associated disability and fast recovery times compared to concussions in non-athletes, the prevalence of SRC exposes players to repetitive traumatic events in addition to higher levels of physical activity during the recovery period.[4] This places the players at risk despite apparently moderate acute injuries.[5 6] Thus, accurately diagnosing SRC as well as monitoring player recovery is critical to injury management. Recent concussion consensus statements recommend screening for SRC using a multi-modal set of tests.[7] In professional rugby, a modified sports concussion assessment tool 5 (SCAT 5), known as the head injury assessment (HIA) is implemented to diagnose SRC and monitor player recovery.[8] However, recent assessment of the HIA in match settings has shown only a moderate level of sensitivity (76.8%).[8]

Numerous novel areas of diagnosis are currently under investigation including protein-based biomarkers.[9] In the context of SRC, these protein biomarkers typically consist of neurological proteins normally confined to the central nervous system or associated with neurological cell damage.[4 10] Following SRC, these proteins could be detected in systemic circulation or in the cerebrospinal fluid (CSF).[10 11] Here, the presence of these neurological proteins can be quantified, and it is suggested that the levels could reflect injury to the central nervous system caused by moderate traumatic brain injury (mTBI). However, due to the invasive nature of lumbar punctures, required to obtain a CSF sample, methods of CSF-based quantification are not considered a viable, practical option for routine diagnosis and prognosis of SRC.[10] Instead, the analysis of blood samples for biomarkers is more practical and acceptable to the participant.

Research regarding the use of blood-based biomarkers for concussion first began in 2000 and since then, approximately 50 potential biomarkers have been investigated with varying success for TBI diagnosis and prognosis.[4 11–24] Amongst these biomarkers, S100 calcium-binding protein β (S100β),[15–19 22–60] glial fibrillar acid protein (GFAP),[15–17 19 21 24–26 28 31 38–40 45 49 51] brain-derived neurotrophic factor (BDNF),[18 19 24] and ubiquitin carboxy-terminal hydrolase L1 (UCH-L1)[21 25 26 28 38–40 42 45 49] have been investigated. These proteins have been found to be capable of identifying concussive injuries and estimating SRC injury severity and prognosis. Recent studies have compared biomarker levels at different time points post-diagnosis or investigated the relationship between early biomarker levels and return to play (RTP) times.[19 37 46] However, the use of these biomarkers for actively evaluating player recovery following SRC has yet to be fully investigated. In this study, biomarker levels will be measured across multiple time points post-SRC to assess the value of these biomarkers to monitor player recovery compared to the clinical assessments at each of these time points. This is of significance for minimising premature RTP and, ultimately, aiming to reduce the chronic long-term effects associated with SRC.

Aims & objectives

1) Measure the levels of blood biomarkers in rugby players that have experienced a concussion, confirmed via HIA and neuropsychometric assessment, to assess the utility of these biomarkers for concussion diagnosis.
2) Track the levels of the blood biomarkers over time, post-injury, to determine if biomarker levels correlate with clinical recovery.
3) Use an untargeted approach to identify potential novel biomarkers to diagnose SRC and monitor player recovery.
4) Ultimately, determine the feasibility of integrating blood draws to the clinical assessment protocols within a professional sports setting.

METHODS AND ANALYSIS

Study design

A prospective cohort study of SRC with uninjured baselines (participants) and age-matched, exercise controls (healthy, non-athletes).

Population

All participants were drawn from the list of professional rugby players from one professional rugby club within the Irish Rugby Football Union (IRFU). Randomisation was not conducted as there was no intervention arm in the study. During biochemical analysis only the participant number will be known and there is no difference in technique used between participants. Initial contact was made via the IRFU chief medical officer for permission to run the study over an initial three-year period, which has been granted. Following this, permission was sought and granted from the head coach and medical staff of the identified professional rugby team. Each year, all professional rugby players on the team are invited to participate in the study. Sample size is dependent on the number of players per team per year.

Age-matched controls will be from a consenting cohort of healthy volunteers. The inclusion criteria for these controls are that they are male and match the ages of the participant from the professional sports player cohort. The exclusion criteria for the control cohort is if they play the sport of rugby either at a professional or amateur level within the previous 12 months. Further, the age-matched control participants must not have had a head injury or central nervous system illness in the previous 12 months to the blood draw.

Inclusion criteria

Participants are eligible to participate if they (1) are part of the professional rugby team; (2) aged 18 or over; (3) consent to take part in the study over the whole year.

Exclusion criteria

Participants are excluded if they (1) are unable to attend the preseason baseline draw; (2) unable to give informed consent.

Data collection/investigations

Informed consent is obtained from each participant before commencing testing and assessments (see online supplemental material). Baseline testing is conducted for each consenting player (ie, participant) during the preseason period and further testing is carried out during the season in the event of a SRC. Participants adhere to the normal clinical assessment for a SRC carried out by the team's medical officer. This clinical assessment is in line with the World Rugby's head injury assessment (HIA) protocol and a validated computer-based neuropsychometric assessment (ImPACT) for concussion. The participants also adhere to the gradated return to play (GRTP) protocol as set out by World Rugby[61] and the IRFU.[62]

Baseline questionnaire

Participants complete a baseline questionnaire (see online supplemental material) which includes questions regarding their concussion history including symptoms associated with, length of, and outcome of previous SRC injuries. Players are not included or excluded based on their SRC history. The questionnaire was developed in line with previous sports-based concussion studies.[63–66]

Time frames

The study has been designed to span a minimum of 3 years to capture three playing seasons (1 playing season per year). Each year, during the preseason period, a baseline blood sample is acquired following exercise and a HIA and neuropsychometric assessment is completed. The exercise routine is part of the pre-season training programme within the first week of training. All

participants carry out the same cardio training routine prior to the blood draw. Subsequently, over the course of the season, if any participant is suspected of suffering a SRC during a professional match or training session, that participant enters the HIA process, in line with current regulations. As part of this study, any participant that enters the standard HIA process will undergo a HIA, a neuropsychometric assessment, and blood sampling at the following time points:

1) Within 72 hours of injury,
2) 6 days post-injury (±1 day); this aligns with the minimum GRTP protocol for a professional rugby player, no player will return to play before 6 days,[1]
3) 13 days post-injury (±1 day) to examine if biomarkers are still present despite the results of the HIA and the neuropsychometric assessment. This time point is to capture if the blood biomarkers are still present after a period of recovery of SRC which has been suggested to be approximately 10–13 days.[67 68]

The final time point for sample collection at 13 days post-injury was chosen as it was most common timepoint for players to have returned to play after the initial 7-day GRTP, based on clinical assessment. This allows for the clinical and biochemical assessment of players who returned prior to 14 days and to compare any alterations in blood biomarkers or if biochemical recovery had occurred in conjunction with clinical recovery. Further, the players that enter HIA process in year 1 are followed up in the baseline blood sample in year 2 (similar in year 3). Furthermore, the majority of players who do not enter the HIA process will have baseline blood samples taken over the course of the 3 pre-season blood draws that can be measured and compared over the three seasons.

Serial head injury assessment

Participants that suffer a suspected SRC will complete the HIA protocol (see online supplemental material) at the time points outlined above. This is a form of the SCAT5 which has been modified for professional rugby and GRTP.[61] This assessment will be carried out by the medical officer of the professional rugby club.

Neuropsychometric assessment

During preseason, all players in the club complete a baseline computer-based neuropsychometric assessment—ImPACT [https://impacttest.com/]. This assessment measures different cognitive domains including visual memory, visual processing speed, reaction times, working memory and attention.[69 70] Post-injury, participants are re-assessed once symptom free to determine recovery of these domains and to assess any persistent patterns of deficit. This assessment forms part of the overall concussion assessment and management plan.

Blood draw and storage

The medical officer and/or clinical research nurse take blood samples through venepuncture, according to local policy guidelines, at the time points outlined above. A total of five vials of blood are collected: three 7.5 mL plasma vials (K_3EDTA collection tubes; Sarstedt 01.1605.004) and two 4.9 mL serum vials (serum gel with clotting activator collection tubes; Sarstedt 04.1935).

Following blood sample acquisition, the sample is anonymised with a unique participant identifier, which ensures participant confidentiality. Furthermore, study team members carrying out blood sample analysis will not be involved in the consenting process of study participants and, thus, are blind to their identity, thereby minimising potential bias. The blood samples are transported in a sealed transport box on ice to a biochemistry lab located near the blood draw location. Approximately 1.5 mL aliquots of whole blood are prepared immediately from one of the two K_3EDTA tubes. The second K_3 EDTA tube and the three serum tubes are allowed to stand at room temperature for 30 min to facilitate separation of the blood components. The vials are then centrifuged at 2000 xg for 10 min at 4°C. The serum and plasma samples are aliquoted into cryovials with a minimum volume of 400 µL per cryovial.

All aliquoted cryovials are placed within a −80°C freezer for long-term storage. Each aliquot can be removed to probe for different biomarkers without multiple freeze-thaw cycles of a core sample if the samples were not aliquoted into multiple vials.

Blood biomarkers: targeted assessment

Serum or plasma samples are analysed, at different SRC time points, using commercial immunosorbent assays, to determine the levels of different blood-based biomarkers. The targeted biomarkers to be investigated are S100β, GFAP, UCH-L1, BDNF.

Blood biomarkers: untargeted assessment

Serum or plasma samples will be analysed, at different SRC time points, using mass spectrometry analysis, in a discovery-based approach to identify any new candidate blood-based biomarkers for further evaluation. Here, samples undergo plasma immunoaffinity fractionation to deplete the most abundant plasma proteins, due to their dominating concentration, thereby increasing the overall coverage for detection of proteins present at lower concentrations. Biomarkers will be considered for further assessment if they are detected post-SRC or during recovery, and they were not detected in the blood of control samples or the concentration has deviated significantly from baseline levels. A sub-panel of suitable candidate biomarkers will be further evaluated through direct assessment of the associated participants' blood via immunoblotting and/or ELISA analysis for discovery and verification purposes towards investigation in a larger cohort.

Outcomes

Each participant that is believed to have an SRC enters the HIA process. These participants have clinical assessments

conducted at pre-defined time points, in line with the World Rugby guidelines. Blood samples are also acquired at these time points to quantify the levels of blood-based biomarkers. Biomarker levels are correlated to the results of the HIA and neuropsychometric assessments. This facilitates preliminary investigation of the correlation between clinical assessments and biomarker levels to determine if the biomarkers can be used to objectively assess SRC recovery, in a professional sports club setting.

Statistical analysis

This pilot study has been developed to determine the logistics of including blood draws in the current clinical process for HIA within a professional sports setting and to assist with the calculation of sample size needed for an expanded study in a larger cohort study. To determine the power size from the data obtained in year 3 of the study, a Cohen's f^2 test with an f value of 0.25 (medium effect size) will be used to determine the effect size of participants needed, assuming a power of 80% and a significance level of 5% for comparing the different groups.

ETHICS AND DISSEMINATION

Ethical approval

Ethical approval was sought from the University of Limerick's Faculty of Science and Engineering Research Ethics Committee. The study was granted full approval (2018_06_11_S&E). Each year, an updated letter and renewed ethics application are required by the Ethics Committee confirming that the professional rugby club are willing to continue to participate in the study.

Quality management and confidentiality

A collaborative relationship exists between the professional rugby team, the study coordinators at the University of Limerick and the University of Limerick's (UL) Clinical Research Support Unit (CRSU). The CRSU is an integral part of the Health Research Institute (HRI) at the University of Limerick. The CRSU provides advice regarding clinical research and how to conduct it to the highest standard of research and clinical governance, in accordance with the requirements of ICH Good Clinical Practice (ICH-GCP) Guidelines and all applicable regulatory requirements. The entire study team have access to and use of the CRSU's Quality Management System (QMS) in relation to areas such as consenting participants, documentation of study activities and phlebotomy. All staff involved in study-related procedures have received training for standardisation of procedures, including phlebotomy, administration of questionnaires, physical measurements and laboratory testing.

Data management

Good quality research depends on data integrity and participant protection. The Principal Investigator (PI) is responsible for the data and results of clinical investigations in this study as well as ensuring that all the data are credible and accurate. The PI will protect the rights, safety and confidentiality of participants of this study. This starts with the informed consent process and all players who agree to participate are fully aware of how their data will be used and stored. Participants will be assigned a unique, anonymous study number by the CRSU study co-ordinator and a 'key' linking the participants to these study numbers is maintained by the CRSU study co-ordinator in a locked, restricted access environment. The signed consent forms and paper files (questionnaires) are also stored in this manner. All the information gathered from this study are stored on a secure, password-protected computer. Paper files (baseline questionnaires) will be treated confidentially in line with good clinical practice. In any future publications relating to this study, the participants' identity will not be disclosed. In the event that a participant wishes to withdraw from the study or access their individual data, a 'gatekeeper' system will be followed. All participant samples and information are stored in a restricted access facility. For updated information on the status of this study please go to clinicaltrial.gov, using study number NCT04485494.

Dissemination

When data collection is complete, further papers will be written, presenting the preliminary results. These will highlight temporal changes across the study period, from baseline and between the time points throughout recovery. Following the publication of the preliminary results of this study, a more detailed sample size calculation can be performed to achieve adequate statistical power in future interventional studies to determine the usefulness of each individual test platform used individually or in combination. The participants within the study are reported to on the study updates at least twice a year.

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Contributors The project and study design were conceived by AR, JK, IK, TC, and JM, the project coordination is led by JM. The funding to carry out the study was procured by AR, DW, IK, TC, and JM. The participant recruitment was carried out by JM, JK, RH, and EC. The baseline tests and HIA were carried out by JK. The logistics of the blood draw and consenting was developed by JK, RH, EC and JM. The Quality Management and confidentiality protocol and implementation was led by MR. The biomarker testing protocol and analysis was carried out by AR, RC and DW. The untargeted assessment process and analysis was developed and implemented by KMcG. JK, AR, JM, RH, and MR all contributed to the writing of the manuscript. All authors provided feedback and critically reviewed on all drafts and approved the final manuscript.

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REFERENCES

1 England Professional Rugby Injury Surveillance Project Steering Group. England professional rugby injury surveillance project: 2017–2018 report.
2 Schwellnus MP, Jordaan E, van Rensburg CJ, *et al.* Match injury incidence during the super rugby tournament is high: a prospective cohort study over five seasons involving 93 641 player-hours. *Br J Sports Med* 2019;53:620–7.
3 Fuller CW, Taylor A, Kemp SP, *et al.* Rugby world cup 2015: world rugby injury surveillance study. *Br J Sports Med* 2017;51:51–7.
4 Papa L. Potential blood-based biomarkers for concussion. *Sports Med Arthrosc* 2016;24:108.
5 Rabinowitz AR, Li X, Levin HS. Sport and nonsport etiologies of mild traumatic brain injury: similarities and differences. *Ann Rev Psychol* 2014;65:301–31.
6 Bey T, Ostick B. Second impact syndrome. *Western J Emerg Med* 2009;10:6.
7 McCrory P, Meeuwisse W, Dvorak J, *et al.* Consensus statement on concussion in sport: the 5th international conference on concussion in sport held in Berlin, October 2016. *Br J Sports Med* 2017;51:838–47.
8 Fuller GW, Kemp SP, Decq P. The International Rugby Board (IRB) pitch side concussion assessment trial: a pilot test accuracy study. *Br J Sports Med* 2015;49:529–35.
9 McCrea M, Meier T, Huber D, *et al.* Role of advanced neuroimaging, fluid biomarkers and genetic testing in the assessment of sport-related concussion: a systematic review. *Br J Sports Med* 2017;51:919–29.
10 O'Connell B, ÁM K, Mockler D, *et al.* Use of blood biomarkers in the assessment of sports-related concussion: a systematic review in the context of their biological significance. *Clin J Sport Med* 2018;28:561–71.
11 Rogatzki MJ, Baker J. Traumatic brain injury in sport with special focus on biomarkers of concussion injury. *J Neurol Neurophysiol* 2016;7:2.
12 Jeter CB, Hergenroeder GW, Hylin MJ, *et al.* Biomarkers for the diagnosis and prognosis of mild traumatic brain injury/concussion. *J Neurotrauma* 2013;30:657–70.
13 Zetterberg H, Smith DH, Blennow K. Biomarkers of mild traumatic brain injury in cerebrospinal fluid and blood. *Nat Rev Neurol* 2013;9:201.
14 Shan R, Szmydynger-Chodobska J, Warren OU, *et al.* A new panel of blood biomarkers for the diagnosis of mild traumatic brain injury/concussion in adults. *J Neurotrauma* 2016;33:49–57.
15 Meier TB, Huber DL, Bohorquez-Montoya L, *et al.* A prospective study of acute blood-based biomarkers for sport-related concussion. *Ann Neurol* 2020;87:907–20.
16 Çevik S, Özgenç MM, Güneyk A, *et al.* NRGN, S100B and GFAP levels are significantly increased in patients with structural lesions resulting from mild traumatic brain injuries. *Clin Neurol Neurosurg* 2019;183:105380.
17 Lagerstedt L, Egea-Guerrero JJ, Bustamante A, *et al.* Combining H-FABP and GFAP increases the capacity to differentiate between CT-positive and CT-negative patients with mild traumatic brain injury. *PloS One* 2018;13:e0200394.
18 Di Battista AP, Churchill N, Schweizer TA, *et al.* Blood biomarkers are associated with brain function and blood flow following sport concussion. *J Neuroimmunol* 2018;319:1–8.
19 Di Battista AP, Rhind SG, Baker AJ, *et al.* An investigation of neuroinjury biomarkers after sport-related concussion: from the subacute phase to clinical recovery. *Brain Injury* 2018;32:575–82.
20 Lagerstedt L, Egea-Guerrero JJ, Rodríguez-Rodríguez A, *et al.* Early measurement of interleukin-10 predicts the absence of CT scan lesions in mild traumatic brain injury. *PloS One* 2018;13:e0193278.
21 Asken BM, Bauer RM, DeKosky ST, *et al.* Concussion BASICS III: serum biomarker changes following sport-related concussion. *Neurology* 2018;91:e2133–e43.
22 Lagerstedt L, Egea-Guerrero JJ, Bustamante A, *et al.* H-FABP: a new biomarker to differentiate between CT-positive and CT-negative patients with mild traumatic brain injury. *PloS One* 2017;12:e0175572.
23 Halstrom A, MacDonald E, Neil C, *et al.* Elevation of oxidative stress indicators in a pilot study of plasma following traumatic brain injury. *J Clin Neurosci* 2017;35:104–8.
24 Di Battista AP, Rhind SG, Richards D, *et al.* Altered blood biomarker profiles in athletes with a history of repetitive head impacts. *PloS One* 2016;11:e0159929.
25 Czeiter E, Amrein K, Gravesteijn BY, *et al.* Blood biomarkers on admission in acute traumatic brain injury: relations to severity, CT findings and care path in the CENTER-TBI study. *EBio Med* 2020;56:102785.
26 Thelin E, Al Nimer F, Frostell A, *et al.* A serum protein biomarker panel improves outcome prediction in human traumatic brain injury. *J Neurotrauma* 2019;36:2850–62.
27 Le Sage N, Tardif P-A, Frenette J, *et al.* Detection of S-100β protein in plasma and urine after a mild traumatic brain injury. *Can J Neurol Sci* 2019;46:599–602.
28 Mahan MY, Thorpe M, Ahmadi A, *et al.* Glial fibrillary acidic protein (GFAP) outperforms S100 calcium-binding protein B (S100B) and ubiquitin C-terminal hydrolase L1 (UCH-L1) as predictor for positive computed tomography of the head in trauma subjects. *World Neurosurg* 2019;128:e434–e44.
29 Bouvier D, Balayssac D, Durif J, *et al.* Assessment of the advantage of the serum S100B protein biomonitoring in the management of paediatric mild traumatic brain injury: PROS100B: protocol of a multicentre unblinded stepped wedge cluster randomised trial. *BMJ Open* 2019;9:e027365.
30 Park D-W, Park S-H, Hwang S-K. Serial measurement of S100B and NSE in pediatric traumatic brain injury. *Child's Nerv Syst* 2019;35:343–8.
31 Kaneko T, Era T, Karino K, *et al.* Serum glial fibrillary acidic protein is a highly specific biomarker for traumatic brain injury in humans compared with S-100B and neuron-specific enolase. *Crit Care* 2019;22:307–12.
32 Allouchery G, Moustafa F, Roubin J, *et al.* Clinical validation of S100B in the management of a mild traumatic brain injury: issues from an interventional cohort of 1449 adult patients. *CCLM* 2018;56:1897–904.
33 Park S-H, Hwang S-K. Prognostic value of serum levels of S100 calcium-binding protein B, neuron-specific enolase, and interleukin-6 in pediatric patients with traumatic brain injury. *World Neurosurg* 2018;118:e534–e42.
34 Oris C, Pereira B, Durif J, *et al.* The biomarker S100B and mild traumatic brain injury: a meta-analysis. *Pediatrics* 2018;141:6.
35 Kelmendi FM, Morina AA, Mekaj AY, *et al.* Serum s100b levels can predict computed tomography findings in paediatric patients with mild head injury. *Biomed Res Int* 2018;2018.
36 Egea-Guerrero JJ, Rodríguez-Rodríguez A, Quintana-Díaz M, *et al.* Validation of S100B use in a cohort of Spanish patients with mild traumatic brain injury: a multicentre study. *Brain Injury* 2018;32:459–63.
37 Shahim P, Tegner Y, Marklund N, *et al.* Neurofilament light and tau as blood biomarkers for sports-related concussion. *Neurology* 2018;90:e1780–e88.
38 Meier TB, Nelson LD, Huber DL, *et al.* Prospective assessment of acute blood markers of brain injury in sport-related concussion. *J Neurotrauma* 2017;34:3134–42.
39 Welch RD, Ellis M, Lewis LM, *et al.* Modeling the kinetics of serum glial fibrillary acidic protein, ubiquitin carboxyl-terminal hydrolase-L1, and S100B concentrations in patients with traumatic brain injury. *J Neurotrauma* 2017;34:1957–71.
40 Lewis LM, Schloemann DT, Papa L, *et al.* Utility of serum biomarkers in the diagnosis and stratification of mild traumatic brain injury. *Acad Emerg Med* 2017;24:710–20.
41 Bouvier D, Duret T, Abbot M, *et al.* Utility of S100B serum level for the determination of concussion in male rugby players. *Sports Med* 2017;47:781–9.

42 Dey S, Gangadharan J, Deepika A, *et al.* Correlation of ubiquitin C terminal hydrolase and S100β with cognitive deficits in young adults with mild traumatic brain injury. *Neurol India* 2017;65:761.
43 Calcagnile O, Anell A, Undén J. The addition of S100B to guidelines for management of mild head injury is potentially cost saving. *BMC Neurol* 2016;16:200.
44 Thelin EP, Jeppsson E, Frostell A, *et al.* Utility of neuron-specific enolase in traumatic brain injury; relations to S100B levels, outcome, and extracranial injury severity. *Critical Care* 2016;20:285.
45 Mondello S, Kobeissy F, Vestri A, *et al.* Serum concentrations of ubiquitin C-terminal hydrolase-L1 and glial fibrillary acidic protein after pediatric traumatic brain injury. *Sci Rep* 2016;6:1–8.
46 Ercole A, Thelin E, Holst A, *et al.* Kinetic modelling of serum S100b after traumatic brain injury. *BMC Neurol* 2016;16:1–8.
47 Kellermann I, Kleindienst A, Hore N, *et al.* Early CSF and serum S100B concentrations for outcome prediction in traumatic brain injury and subarachnoid hemorrhage. *Clin Neurol Neurosurg* 2016;145:79–83.
48 Asadollahi S, Heidari K, Taghizadeh M, *et al.* Reducing head computed tomography after mild traumatic brain injury: screening value of clinical findings and S100B protein levels. *Brain Injury* 2016;30:172–8.
49 Welch RD, Ayaz SI, Lewis LM, *et al.* Ability of serum glial fibrillary acidic protein, ubiquitin C-terminal hydrolase-L1, and S100B to differentiate normal and abnormal head computed tomography findings in patients with suspected mild or moderate traumatic brain injury. *J Neurotrauma* 2016;33:203–14.
50 Manzano S, Holzinger IB, Kellenberger CJ, *et al.* Diagnostic performance of S100B protein serum measurement in detecting intracranial injury in children with mild head trauma. *Emerg Med J* 2016;33:42–6.
51 Papa L, Mittal MK, Ramirez J, *et al.* In children and youth with mild and moderate traumatic brain injury, glial fibrillary acidic protein out-performs S100β in detecting traumatic intracranial lesions on computed tomography. *J Neurotrauma* 2016;33:58–64.
52 Undén L, Calcagnile O, Undén J, *et al.* Validation of the Scandinavian guidelines for initial management of minimal, mild and moderate traumatic brain injury in adults. *BMC Med* 2015;13:292.
53 Studer M, Goeggel Simonetti B, Heinks T, *et al.* Acute S100B in serum is associated with cognitive symptoms and memory performance 4 months after paediatric mild traumatic brain injury. *Brain Injury* 2015;29:1667–73.
54 Thompson WH, Thelin EP, Lilja A, *et al.* Functional resting-state fMRI connectivity correlates with serum levels of the S100B protein in the acute phase of traumatic brain injury. *NeuroImage Clin* 2016;12:1004–12.
55 Al Nimer F, Thelin E, Nyström H, *et al.* Comparative assessment of the prognostic value of biomarkers in traumatic brain injury reveals an independent role for serum levels of neurofilament light. *PloS One* 2015;10:e0132177.
56 Pinelis V, Sorokina E, Semenova J, *et al.* Biomarkers in children with traumatic brain injury. *Zhurnal Nevrologii I Psikhiatrii Imeni SS Korsakova* 2015;115:66–72.
57 Heidari K, Asadollahi S, Jamshidian M, *et al.* Prediction of neuropsychological outcome after mild traumatic brain injury using clinical parameters, serum S100B protein and findings on computed tomography. *Brain Injury* 2015;29:33–40.
58 Ryb GE, Dischinger PC, Auman KM, *et al.* S-100 β does not predict outcome after mild traumatic brain injury. *Brain Injury* 2014;28:1430–5.
59 Kiechle K, Bazarian JJ, Merchant-Borna K, *et al.* Subject-specific increases in serum S-100B distinguish sports-related concussion from sports-related exertion. *PLoS One* 2014;9:e84977.
60 Abbasi M, Sajjadi M, Fathi M, *et al.* Serum S100B protein as an outcome prediction tool in emergency department patients with traumatic brain injury. *Turkish J Emerg Med* 2014;14:147–52.
61 World Rugby. *World rugby concussion guidance*. 2017. World Rugby https://playerwelfare.worldrugby.org/content/getfile.php?h=d66f98b9815023fbf00e8ef28b20cdb6&p=pdfs/World_Rugby_Concussion_Guidance_EN.pdf
62 Union IRF. *Concussion guidelines for the domestic game*. 2018.
63 Ackery A, Provvidenza C, Tutor CH. Concussion in hockey: compliance with return to play advice and follow-up status. *Can J Neurol Sci* 2009;36:207–12.
64 Meehan WP, Mannix R, Monuteaux MC, *et al.* Early symptom burden predicts recovery after sport-related concussion. *Neurology* 2014;83:2204.
65 Tegner Y, Lorentzon R. Concussion among Swedish elite ice hockey players. *Br J Sports Med* 1996;30:251–5.
66 Perel PWJ, Ravi R, Shakur H. Prognosis following head injury: a survey of doctors from developing and developed countries. *JECP*.
67 Benson BW, Meeuwisse WH, Rizos J, *et al.* A prospective study of concussions among national hockey league players during regular season games: the NHL-NHLPA concussion program. *CMAJ* 2011;183:905–11.
68 Iverson GL, Brooks BL, Collins MW, *et al.* Tracking neuropsychological recovery following concussion in sport. *Brain Injury* 2006;20:245–52.
69 Echemendia RJ, Meeuwisse W, McCrory P, *et al.* The sport concussion assessment tool 5th edition (SCAT5): background and rationale. *Br J Sports Med* 2017;51:848–50.
70 Schatz P, Pardini JE, Lovell MR, *et al.* Sensitivity and specificity of the ImPACT test battery for concussion in athletes. *Arch Clin Neuropsychol* 2006;21:91–9.