

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 (S100 β),^{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 (UCHL1)^{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 pre-season 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 graduated return to play (GRTP) protocol as set out by World Rugby⁶¹ 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 venopuncture, 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_3 EDTA 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_3 EDTA 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 μ L 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 S100 β , 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 clinicaltrials.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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