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# BMJ Open

## An investigation into Repetitive Concussion in Sport (RECOS): A study protocol of a prospective, observational cohort study

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## Title

An investigation into Repetitive Concussion in Sport (RECOS): A study protocol of a prospective, observational cohort study

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## Keywords

Concussion; Traumatic brain injury; Sport concussion; Biomarkers; Imaging; Post-concussion syndrome

## Abstract

### Introduction

Sport-related concussion (SRC) management remains a diagnostic dilemma to clinicians in all strata of care, coaching staff and players alike. The lack of objective diagnostic and prognostic biomarkers and over-reliance on subjective clinical assessments carries a significant health risk of undiagnosed concussive episodes and early return to play before full recovery increasing the risk of sustaining additional concussion, and leading to long-term sequelae and/or unfavorable outcome.

### Objective

To identify a set of parameters (neuroimaging with neurophysiological, biological and neuropsychological tests) that may support pitch-side and outpatient clinical decision making in order to objectively diagnose concussion, determine the severity of injury, guide a safe return to play and identify the potential predictors of the long-term sequelae of concussion.

### Methods and analysis

An observational, prospective, cohort study recruiting between 2017 and 2020. The participants will have a baseline pre-season screening (brain imaging, neuropsychological assessments, serum, urine and saliva sampling). If a screened player later suffers a concussion and/or multiple concussions then he/she will be assessed again with the same protocol within 72hours, and their baseline data will be used as internal control as well as normative data. Inferential statistical analysis will be performed to determine correlations between biological, imaging techniques and neuropsychological assessments.

## Ethics and dissemination

Ethics approval has been obtained. The results of this study will be presented at national and international conferences and submitted for publication in peer reviewed journals.

## Article summary

### Strengths and limitations of this study

- This study will contribute to better understanding of the extent and nature of the metabolic and physiological window of vulnerability present in cerebral tissue following mild traumatic brain injury (mTBI) sustained during sport.
- The study will help to determine the relationship between a single concussion and subsequent/consecutive episodes in terms of their effect on that window of brain vulnerability with a view to guiding return to play following single and repetitive concussions.
- To inter-correlate neuroimaging with neurophysiological, biological and neuropsychological tests for the prospective development of potential pitch-side and ambulatory technologies.
- This study will recruit from a population of adult athletes and therefore applications of study findings to other populations, such as non-athletes, will require further validation.
- Neuroimaging may not be suitable for all the participants, such as pregnant, claustrophobic athletes, and those with metallic implants.

## Introduction

Sport-related concussion (SRC) is defined as a brief period of loss of consciousness (LOC), memory loss or feeling dazed or confused following trauma to the head, face or neck, and is a common cause of mild traumatic brain injury (mTBI).<sup>1,2</sup> In the US, the Centers for Disease Control and Prevention estimate that 1.6 to 3.8 million concussions occur in sports and recreational activities annually, but there is fear that the number may be much larger, as the majority of incidents are unrecognized or unreported.<sup>3</sup>

The majority of patients improve rapidly following a single concussion, but 10-20% of individuals have persistent symptoms (e.g. headache, dizziness, fogginess, imbalance, and anxiety) at three months. In a minority (2-4%) the symptoms may become permanent.<sup>4,5</sup> There is a higher risk of sustaining further concussion if they returned to play before full recovery which prolongs recovery time after each incident.<sup>4,5</sup>

Certain groups of patients, such as athletes, soldiers and children are at greater risk of repetitive mild traumatic brain injury (mTBI); potentially leading to a catastrophic form of brain injury known as second impact syndrome (SIS), thought to be due to the second insult occurring during a window of metabolic vulnerability of the brain.<sup>6-8</sup>

In susceptible individuals, repetitive head trauma has been linked to early neurodegenerative conditions such as Parkinson's, amyotrophic lateral sclerosis and Alzheimer's disease. The risk of neurodegenerative conditions has been demonstrated to treble in retired professional American football players<sup>9</sup> and following the media attention dedicated to this issue, the link between mTBI and chronic traumatic encephalopathy (CTE) has become a major public concern.<sup>10,11</sup>



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3 Compounding this problem is the fact that athletes, like soldiers, are highly trained,  
4 young, fit, motivated individuals proven to under-report concussive symptoms.<sup>12, 13</sup>  
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8 Athletes are, therefore, at risk of SIS and CTE. Currently, there is no validated  
9 clinical biomarker available to assess mTBI, SIS and cumulative brain damage,  
10 leading to an over-reliance on self-reporting of symptoms in the management of  
11 mTBI.<sup>14</sup>  
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17 At present, the mainstream assessment of mTBI both in sports and in the  
18 primary/secondary healthcare setting involves the functional and symptomatic  
19 assessment of an individual using neurocognitive tests.<sup>15</sup> These tests have  
20 significant limitations, particularly the lack of baseline/premorbid measurement in  
21 terms of sensitivity and specificity<sup>16, 17</sup> and the susceptibility to multiple  
22 confounding factors<sup>18</sup> such as musculoskeletal injury and/or pre-morbid disability.  
23 This together with their requirement for a high level of engagement and  
24 concentration limits their application in outpatient clinic or pitch-side.  
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## 37 **Methods and analysis**

### 38 **Study design**

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40 A cohort of contact sport athletes (e.g. rugby, football, American football, etc.) will  
41 be recruited throughout the West Midlands region and through referrals from  
42 sports clubs anywhere in the Great Britain.  
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49 The assessments are carried out in two settings, (1) the sports clubs with clinical  
50 areas dedicated to sample taking and data collection and (2) Birmingham  
51 concussion clinic at the Queen Elizabeth Hospital Birmingham (QEHB) or at the  
52 University of Birmingham (UoB).  
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3 Eligible individuals will be identified by key members of their coaching staff who  
4 have been trained regarding the inclusion specifications and the procedures  
5 involved in recruitment (Table 1). Athletes who sustain a head injury during  
6 competition or training will be advised to attend an assessment session between  
7 48h and 72h after their concussion at the concussion clinic at QEHB, although  
8 attending directly or as soon as is practically possible will be advised. Should the  
9 injury be sufficiently serious that the attending staff believes immediate medical  
10 assistance or transfer to the nearest Emergency Department is required, then  
11 standard clinical procedures should be followed in these cases.  
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22 Consent to participate in the study will be obtained from the participants in a  
23 standardised written consent form. Details of the consent will be maintained as per  
24 the UoB and University Hospitals Birmingham NHS Foundation Trust (UHBNFT)  
25 guidelines, and maintained for the study period and archived thereafter.  
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31 Consented non-concussed athletes will participate in a baseline screening  
32 consisting of same assessments as for the concussed players, although these may  
33 not include Magnetic Resonance Imaging (MRI) screening in all subjects due to  
34 resources constrains. The uninjured cohort provides normative data, as well as  
35 internal control data if a screened player later suffers a concussion during the  
36 season. Pre-season screening assessments will be conducted at either the  
37 University of Birmingham site or the QEHB site or at the club facilities.  
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**Table 1. Summary of eligibility criteria**

| Inclusion criteria   | Exclusion criteria   |
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| Male or female athletes participating in contact sports, aged 16-65 years, with fluent English speaking. | Individuals who require hospital admission after initial assessment for their TBI.   |
| Single or repetitive mTBI sustained in contact sport less than 72 hours prior to assessment.             | Intracranial haemorrhage, brain tissue injury, or non-TBI related pathologies on initial CT/MRI scan.  |
| Normal neurological objective examination at the time of enrolment.                                      | Pregnancy (urine pregnancy test will be performed for confirmation).   |
|  | Any history of neurodegenerative pathology or any recent or ongoing illness affecting the central nervous system (e.g. Parkinson's, multiple sclerosis, meningitis, epilepsy, neoplasm). |
|  | History of chronic alcoholism or drug abuse.   |
|  | Any other sustained injury that requires hospital admission.   |

Up to 400 singly or multiply concussed athletes (where the second concussion has occurred within 21 days of the first event) will be recruited, consented and assessed by:

- Sampling of 25ml venous blood, 2ml saliva and 5ml urine for metabolomics and genomic analysis (Table 2).
- The Immediate post-concussion assessment and cognitive testing (ImPACT) is a computerised neurocognitive test for evaluating sport-related concussion. It measures multiple aspects of cognitive functioning, and

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3 consists of five testing composites, i.e. verbal memory, visual memory,  
4 processing speed, reaction time, and impulse control.<sup>19</sup>  
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- 7 - The Screening Module of Neuropsychological Assessment Battery (NAB)  
8 (Digit Span).<sup>18</sup>  
9
- 10 - Wechsler Adult Intelligence Scale Version 4 (WAIS-IV) (Digit Symbol  
11 Coding and Symbol Search).<sup>20</sup>  
12
- 13 - Nine Hole Peg Test (NHPT) to assess fine motor skill; the individual will be  
14 asked to place and remove nine pegs at one time, as quickly as possible,  
15 from nine holes in a peg board. This test evaluates coordination of upper  
16 limbs and hand dexterity.<sup>21</sup>  
17
- 18 - The Medical Symptom Validity Test (MSVT) is a validated and  
19 computerized performance validity test. It is designed to assess the degree to  
20 which the participant has engaged appropriately in the testing process.<sup>22</sup>  
21
- 22 - Balance assessments (Virtual Reality-Wii Balance Board system, gait  
23 analysis, Modified Balance Error Scoring System (mBESS)).  
24 Individuals stand on a Wii Balance Board to measure centre of pressure  
25 changes whilst being perturbed in a virtual reality environment. Sufficient  
26 safety measures will be put in place to ensure participants do not fall from  
27 the raised platform. Gait analysis will be also undertaken.<sup>20-27</sup>  
28  
29 A mBESS test will also be performed whereby a trained member of the  
30 RECOS team will assess the number of errors participants make whilst  
31 undertaking eyes closed dual, single leg and a tandem stances for 30 seconds  
32 respectively.<sup>27</sup>  
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- 34 - An Immersion questionnaire to complete after the virtual reality system to  
35 investigate their level of immersion and sense of presence in the virtual  
36 reality environment and questionnaire using the Metabolic Equivalent of  
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3 Tasks (MET) will be used to assess participants' physical activity levels prior  
4 to the sustaining of a concussion.<sup>28</sup>  
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- 6 - MRI to assess neuronal energy metabolism, functional brain network and  
7 tissue mechanical parameters.  
8
- 9 - Functional Near-Infrared Spectroscopy (fNIRS) to assess brain activation  
10 patterns during neurocognitive tasks.  
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16 The Sport Concussion Assessment Tool – 5<sup>th</sup> Edition (SCAT5)<sup>27</sup> may be taken  
17 within the sport club facilities immediately after concussion. Any other data will be  
18 collected during the pitch-side clinical assessment on concussed athletes within the  
19 Rugby Football Union guidelines, i.e. the Injury Surveillance Programme, may be  
20 used in the study. After the match the concussed participants will visit QEHB or  
21 the UoB or dedicated sport club facilities where all the assessments will be  
22 completed with short breaks in-between when necessary (figure 1).  
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31 Magnetic Resonance (MR) scanning (approximately 1h) will be performed using a  
32 3T MR scanner at the QEHB. First of all, a standard structural MR scan will be  
33 acquired (T1-, T2-, and T2\* -weighted and FLAIR images), followed by MR  
34 Spectroscopy (1H-MRS) (NAA/Choline, NAA/Creatine and Choline/Creatine  
35 ratios.)<sup>29</sup>, resting-state functional MRI (fMRI) (subjects are awake, eyes closed,  
36 motionless), Diffusion tensor imaging (DTI), and Magnetic Resonance  
37 Elastography (MRE) on brain will be performed to obtain quantitative values for  
38 tissue mechanical parameters.<sup>30, 31</sup>  
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48 Prior to any female participant undergoing MR imaging, a pregnancy test will be  
49 performed after the expressed consent of the individual.  
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52 These assessments will be repeated in the QEHB or sports facilities during the  
53 follow up and when the individual is deemed fit to return to play, until  
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normalisation of the MR Spectroscopy (1H-MRS) or self-reported symptoms are observed.

The Near-Infrared Spectroscopy (NIRS) is a safe non-invasive brain imaging technique that utilises Near-Infrared (NIR) light absorption to assess brain oxygen saturation. The NIR light is currently widely used in clinical practice (e.g. pulse oximeter and brain monitoring during cardiac surgery). fNIRS measurements are performed once within 48-72 hours from the injury using a frequency domain NIRS device (ISS™ IMAGENT; Champaign, Illinois, USA) and a digitizer (POLHEMUS FASSTRAK®; Colchester, Vermont, USA).<sup>32,33</sup>

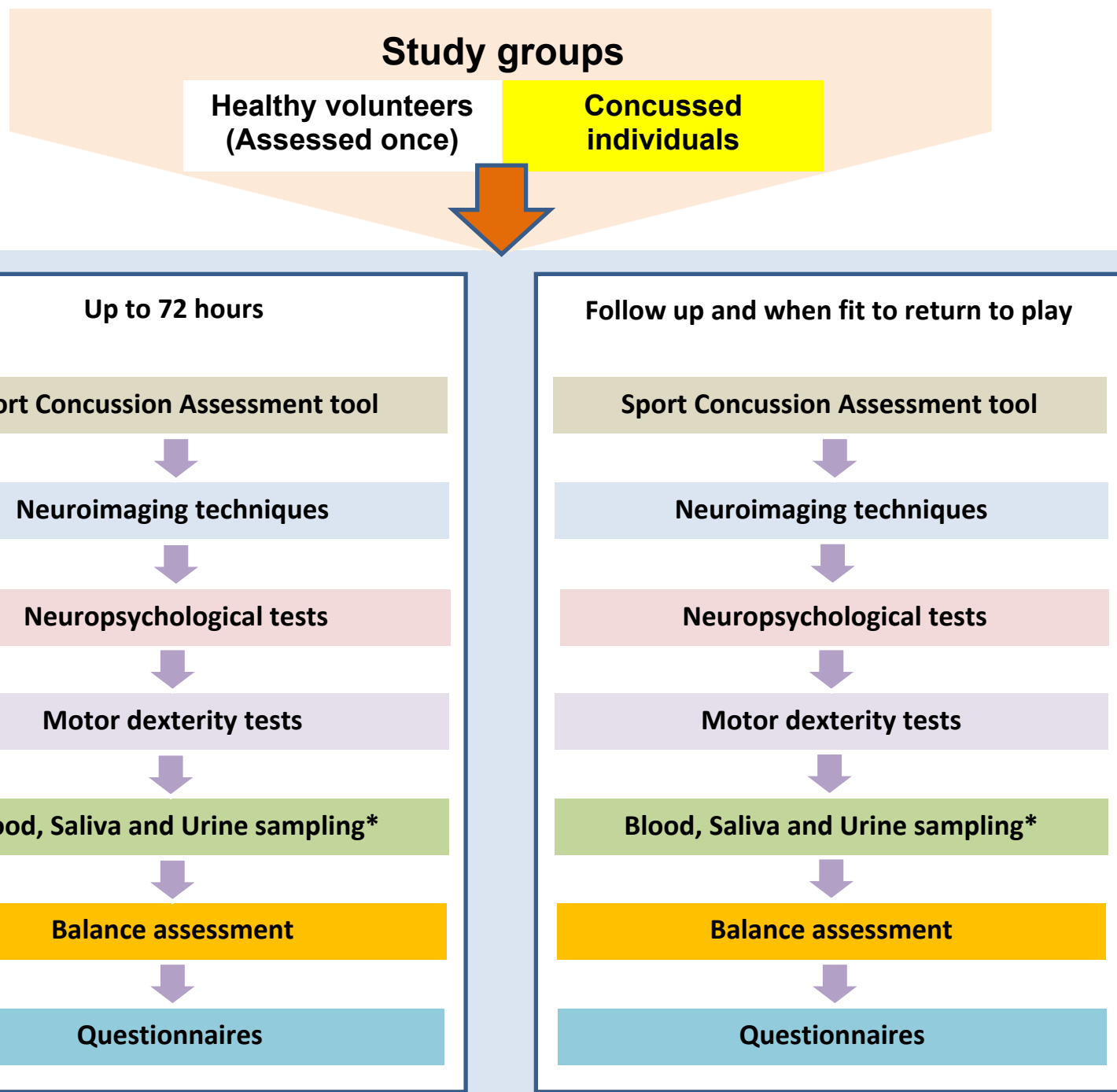
**Table 2. Analysis of biological fluids**

| Blood  | Urine  | Saliva                          |
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| 1. Metabolomics (NAA and related metabolites)<br>2. Hormones (Progesterone, Aldosterone, 11-deoxycortisol, Corticosterone, Testosterone, Androstenedione, Cortisol, 17OHP, DHEA, DHEAS, Cortisone)<br>3. Pro and anti-inflammatory cytokines (TNF $\alpha$ , IL1 $\beta$ , IL6, IL4, IL8, IL10, IL13, IL17, GM-CSF)<br>4. Proteomics<br>5. Microparticles<br>6. Biomarkers of brain injury (S100B, GFAP and NSE) | 1. Hormone profile<br>2. Brain biomarkers (NAA, S100B and GFAP)<br>3. Metabolomics<br>4. MicroRNAs | 1. MicroRNAs<br>2. Metabolomics |

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| 7. MicroRNAs |  |  |
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47 **Figure 1: Flowchart of study procedures. \*urine pregnancy test will be performed first for all female participants**



## **Outcome measures and statistical analysis**

The recruitment target is based on the size of the convenient sample that can be recruited (approximately 400); power calculation do not apply as the investigation is explorative at this stage. The sample size will be open-ended and reviewed after 3 years.

Correlations between biomarkers, 1H-MRS, fMRI, MRE, DTI, fNIRS, neuropsychological scores, motor and coordination parameters will be assessed and reported with standard parametric statistical tests after normalisation of data or non-parametric test if normalisation were not possible. Time to resolution of symptoms will be compared for all the above modalities, as well as self-reported outcomes. Biochemical and imaging data will also be analysed with standard group comparison statistics (e.g. t-test on normalised data or Mann-Whitney on non-normalisable data).

## **Patient and Public Involvement**

Links with sports academics and coaching staff have been established throughout the region through the University of Birmingham School of Sports, Exercise and Rehabilitation Sciences (SportexR) and through sports authorities such as the Rugby Football Union, the Welsh Rugby Union, GB Basketball, GB Hockey and the Football Association. Awareness of these programs will be promoted to the potential cohort of athletes through event days organised at individual recruitment sites coordinated centrally through the UoB. In terms of dissemination, the study team will regularly update the clubs on the progress of the study and will report the findings by email/newsletter and in various meetings where club representatives are in attendance.

## Ethics and dissemination

This study was approved by the East of England - Essex Research Ethics Committee on 22 September 2017 – REC 17/EE/0275; IRAS 216703.

ISRCTN, ISRCTN16974791. Registered on 16/05/2018.

All data will be collected and stored in accordance with the 1998 UK Data Protection Act, UoB and QEHB data handling and maintenance guidelines, with the minimum amount of required information recorded. The complied and analysed results will be presented at national and international conferences. Results will also be submitted for peer review and publication in the subject journals/literature.

### Data statement

All analysed data arising from the study will be kept on NHS and UoB servers. The computers on this network have restricted physical access; data are stored under coded filenames and the local network has secure password access restricted to a limited set of people. The raw MRI data are kept on a separate DICOM server on another network behind its own firewall which is only accessible by a small number of system administrators. Subject score sheet data will be kept on computers with password protected access and coded filenames, the original paper files will be secured in locked filing cabinets.

The datasets generated and/or analysed during the current study are/will be available upon request from, Professor Antonio Belli, who is the point of contact and will be able to provide anonymised data with sufficient detail to able to reproduce the analyses for up to 10 years.

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## Author Contributions

AB, MG, VDP, DH are the principal and co- investigators.

AB, MG, VDP, DJD, ZS, MF, JRB developed the protocol.

AB, MG, VDP, DJD, ZS, MF, JRB, VS, CB drafted the protocol and commented on the protocol.

KMY, ET, ZS, CB, MF, CNW, LC, AKB drafted and finalised the manuscript.

All authors have approved the final manuscript.

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## Competing interests

The University of Birmingham owns intellectual property for some of the biomarkers used in this study.

## Patient consent

Not required.

## Ethics approval

This study was approved by the East of England - Essex Research Ethics Committee on 22 September 2017 – REC 17/EE/0275; IRAS 216703.

ISRCTN, ISRCTN16974791. Registered on 16/05/2018.

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# BMJ Open

## An investigation into Repetitive Concussion in Sport (RECOS): A study protocol of a prospective, exploratory, observational cohort study

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| Keywords:                       | Concussion, Traumatic brain injury, Sport concussion, Biomarkers, Imaging, Post-concussion syndrome  |
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## **Title**

An investigation into Repetitive Concussion in Sport (RECOS): A study protocol of a prospective, exploratory, observational cohort study

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## 39 **Keywords**

40 Concussion; Traumatic brain injury; Sport concussion; Biomarkers; Imaging; Post-  
41 concussion syndrome

## 50 **Abstract**

### 51 **Introduction**

52 Sport-related concussion (SRC) management remains a diagnostic dilemma to  
53 clinicians in all strata of care, coaching staff and players alike. The lack of  
54 objective diagnostic and prognostic biomarkers and over-reliance on subjective  
55 clinical assessments carries a significant health risk of undiagnosed concussive  
56 episodes and early return to play before full recovery increasing the risk of  
57 sustaining additional concussion, and leading to long-term sequelae and/or  
58 unfavorable outcome.

### 59 **Objective**

60 To identify a set of parameters (neuroimaging with neurophysiological, biological  
61 and neuropsychological tests) that may support pitch-side and outpatient clinical  
62 decision making in order to objectively diagnose concussion, determine the  
63 severity of injury, guide a safe return to play and identify the potential predictors of  
64 the long-term sequelae of concussion.

### 65 **Methods and analysis**

66 An exploratory, observational, prospective, cohort study recruiting between 2017  
67 and 2020.

68 The participants will have a baseline pre-season screening (brain imaging,  
69 neuropsychological assessments, serum, urine and saliva sampling). If a screened  
70 player later suffers a concussion and/or multiple concussions then he/she will be  
71 assessed again with the same protocol within 72h, and their baseline data will be  
72 used as internal control as well as normative data. Inferential statistical analysis

73 will be performed to determine correlations between biological, imaging  
74 techniques and neuropsychological assessments.

### 75 **Ethics and dissemination**

76 This study was approved by the East of England - Essex Research Ethics  
77 Committee on 22 September 2017 – REC 17/EE/0275; IRAS 216703.

78 The results of this study will be presented at national and international conferences  
79 and submitted for publication in peer reviewed journals.

## 80 **Article summary**

### 81 **Strengths and limitations of this study**

- 82 - Prospectively recruiting from across the contact sporting continuum, this  
83 study will collect baseline and post-concussion biological samples with  
84 corresponding neuropsychological, neurophysiological and neuroimaging  
85 tests.
- 86 - The study will help to establish a multidisciplinary approach to objectively  
87 diagnose sport-related concussion and guide a safe return to play following  
88 single and repetitive concussions.
- 89 - To inter-correlate neuroimaging with neurophysiological, biological and  
90 neuropsychological tests for the prospective development of potential pitch-  
91 side and ambulatory technologies.
- 92 - This study will recruit from a population of adult athletes and therefore  
93 applications of study findings to other populations, such as non-athletes, will  
94 require further validation.
- 95 - Neuroimaging may not be suitable for all the participants, such as pregnant,  
96 claustrophobic athletes, and those with metallic implants.

## 97 **Introduction**

98 Sport-related concussion (SRC) is defined as a brief period of loss of  
99 consciousness (LOC), memory loss, feeling dazed or confused following trauma to  
100 the head, face or neck, and is a common cause of mild traumatic brain injury  
101 (mTBI).<sup>1,2</sup> In the US, the Centers for Disease Control and Prevention estimate that  
102 1.6 to 3.8 million concussions occur in sports and recreational activities annually,  
103 but there is fear that the number may be much larger, as the majority of incidents  
104 are unrecognized or unreported.<sup>3</sup>

105 The majority of patients improve rapidly following a single concussion, but 10-  
106 20% of individuals have persistent symptoms (e.g. headache, dizziness, fogginess,  
107 imbalance, and anxiety) at three months. In a minority (2-4%) the symptoms may  
108 become permanent.<sup>4,5</sup> There is a higher risk of sustaining further concussion if they  
109 returned to play before full recovery which prolongs recovery time after each  
110 incident.<sup>4,5</sup>

111 Certain groups of patients, such as athletes, soldiers and children are at greater risk  
112 of repetitive mild traumatic brain injury (mTBI); potentially leading to a  
113 catastrophic form of brain injury known as second impact syndrome (SIS), thought  
114 to be due to the second insult occurring during a window of metabolic vulnerability  
115 of the brain.<sup>6-8</sup>

116 In susceptible individuals, repetitive head trauma has been linked to early  
117 neurodegenerative conditions such as Parkinson's, amyotrophic lateral sclerosis  
118 and Alzheimer's disease. The risk of neurodegenerative conditions has been  
119 demonstrated to treble in retired professional American football players<sup>9</sup> and  
120 following the media attention dedicated to this issue, the link between mTBI and  
121 chronic traumatic encephalopathy (CTE) has become a major public concern.<sup>10,11</sup>

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3 122 Compounding this problem is the fact that athletes, like soldiers, are highly trained,  
4  
5 123 young, fit, motivated individuals proven to under-report concussive symptoms.<sup>12, 13</sup>  
6  
7

8 124 Athletes are, therefore, at risk of SIS and CTE. Currently, there is no validated  
9  
10 125 clinical biomarker available to assess mTBI, SIS and cumulative brain damage,  
11  
12 126 leading to an over-reliance on self-reporting of symptoms in the management of  
13  
14 127 mTBI.<sup>14</sup>  
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16  
17 128 At present, the mainstream assessment of mTBI both in sports and in the  
18  
19 129 primary/secondary healthcare setting involves the functional and symptomatic  
20  
21 130 assessment of an individual using neurocognitive tests.<sup>15</sup> These tests have  
22  
23 131 significant limitations, particularly the lack of baseline/premorbid measurement in  
24  
25 132 terms of sensitivity and specificity<sup>16, 17</sup> and the susceptibility to multiple  
26  
27 133 confounding factors<sup>18</sup> such as musculoskeletal injury and/or pre-morbid disability.  
28  
29 134 This together with their requirement for a high level of engagement and  
30  
31 135 concentration limits their application in outpatient clinic or pitch-side.  
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## 37 137 **Methods and analysis**

### 39 138 **Study design**

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42 139 A cohort of contact sport athletes (e.g. rugby, football, American football, etc.) will  
43  
44 140 be recruited throughout the West Midlands region and through referrals from  
45  
46 141 sports clubs anywhere in the Great Britain.  
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49 142 The assessments are carried out in two settings, (1) the sports clubs with clinical  
50  
51 143 areas dedicated to sample taking and data collection and (2) Birmingham  
52  
53 144 concussion clinic at the Queen Elizabeth Hospital Birmingham (QEHB) or at the  
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55 145 University of Birmingham (UoB).  
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3 146 Eligible individuals will be identified by key members of their coaching staff who  
4  
5 147 have been trained regarding the inclusion specifications and the procedures  
6  
7 148 involved in recruitment (Table 1). Athletes who sustain a head injury during  
8  
9 149 competition or training will be advised to attend an assessment session between  
10  
11 150 48h and 72h after their concussion at the concussion clinic at QEHB, although  
12  
13 151 attending directly or as soon as is practically possible will be advised. Should the  
14  
15 152 injury be sufficiently serious that the attending staff believes immediate medical  
16  
17 153 assistance or transfer to the nearest emergency department is required, then  
18  
19 154 standard clinical procedures should be followed in these cases.

21  
22 155 Consent to participate in the study will be obtained from the participants in a  
23  
24 156 standardised written consent form. Details of the consent will be maintained as per  
25  
26 157 the UoB and University Hospitals Birmingham NHS Foundation Trust (UHBNFT)  
27  
28 158 guidelines, and maintained for the study period and archived thereafter.

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31 159 Consented non-concussed athletes will participate in a baseline screening  
32  
33 160 consisting of same assessments as for the concussed players, although these may  
34  
35 161 not include Magnetic Resonance Imaging (MRI) screening in all subjects due to  
36  
37 162 resources constrains. The uninjured cohort provides normative data, as well as  
38  
39 163 internal control data if a screened player later suffers a concussion during the  
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41 164 season. Pre-season screening assessments will be conducted at either the  
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43 165 University of Birmingham site or the QEHB site or at the club facilities.

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3 171 Up to 400 singly or multiply concussed athletes (where the second concussion has  
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5 172 occurred within 21 days of the first event) will be recruited, consented and  
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7 173 assessed by:

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10 174 - Sampling of 25ml venous blood, 2ml saliva and 5ml urine for metabolomics  
11  
12 175 and genomic analysis (Table 2).  
13  
14 176 - The Immediate post-concussion assessment and cognitive testing (ImPACT)  
15  
16 177 is a computerised neurocognitive test for evaluating sport-related  
17  
18 178 concussion. It measures multiple aspects of cognitive functioning, and  
19  
20 179 consists of five testing composites, i.e. verbal memory, visual memory,  
21  
22 180 processing speed, reaction time, and impulse control.<sup>19</sup>  
23  
24 181 - The Screening Module of Neuropsychological Assessment Battery (NAB)  
25  
26 182 (Digit Span).<sup>18</sup>  
27  
28 183 - Wechsler Adult Intelligence Scale Version 4 (WAIS-IV) (Digit Symbol  
29  
30 184 Coding and Symbol Search).<sup>20</sup>  
31  
32 185 - Nine Hole Peg Test (NHPT) to assess fine motor skills; the individual will  
33  
34 186 be asked to place and remove nine pegs at one time, as quickly as possible,  
35  
36 187 from nine holes in a peg board. This test evaluates coordination of upper  
37  
38 188 limbs and hand dexterity.<sup>21</sup>  
39  
40 189 - The Medical Symptom Validity Test (MSVT) is a validated and  
41  
42 190 computerized performance validity test. It is designed to assess the degree to  
43  
44 191 which the participant has engaged appropriately in the testing process.<sup>22</sup>  
45  
46 192 - Balance assessments including virtual reality system, gait analysis, Modified  
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48 193 Balance Error Scoring System (mBESS).<sup>20-27</sup>  
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50 194 The mBESS test will be performed whereby a trained member of the  
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52 195 RECOS team will assess the number of errors participants make whilst  
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3 196 undertaking eyes closed dual, single leg and a tandem stances for 30 seconds  
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5 197 respectively.<sup>27</sup>  
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7 198 - An Immersion questionnaire to complete after the virtual reality system to  
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9 199 investigate their level of immersion and sense of presence in the virtual  
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11 200 reality environment and questionnaire using the Metabolic Equivalent of  
12  
13 201 Tasks (MET) will be used to assess participants' physical activity levels prior  
14  
15 202 to the sustaining of a concussion.<sup>28</sup>  
16  
17 203 - MRI to assess neuronal energy metabolism, functional brain network and  
18  
19 204 tissue mechanical parameters.  
20  
21 205 - Functional Near-Infrared Spectroscopy (fNIRS) to assess brain activation  
22  
23 206 patterns during neurocognitive tasks.

26 207 The Sport Concussion Assessment Tool – 5<sup>th</sup> Edition (SCAT5)<sup>27</sup> may be taken  
27  
28 208 within the sport club facilities immediately after concussion. Any other data will be  
29  
30 209 collected during the pitch-side clinical assessment on concussed athletes within the  
31  
32 210 Rugby Football Union guidelines, i.e. the Injury Surveillance Programme, may be  
33  
34 211 used in the study. After the match the concussed participants will visit QEHB or  
35  
36 212 the UoB or dedicated sport club facilities where all the assessments will be  
37  
38 213 completed with short breaks in-between when necessary (figure 1).

41 214 Magnetic Resonance (MR) scanning (approximately 1h) will be performed using a  
42  
43 215 3T MR scanner at the QEHB. First of all, a standard structural MR scan will be  
44  
45 216 acquired (T1-, T2-, and T2\*-weighted and FLAIR images), followed by MR  
46  
47 217 Spectroscopy (1H-MRS) (NAA/Choline, NAA/Creatine and Choline/Creatine  
48  
49 218 ratios.)<sup>29</sup>, resting-state functional MRI (fMRI) (subjects are awake, eyes closed,  
50  
51 219 motionless), Diffusion tensor imaging (DTI), and Magnetic Resonance  
52  
53 220 Elastography (MRE) on brain will be performed to obtain quantitative values for  
54  
55 221 tissue mechanical parameters.<sup>30, 31</sup>

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3 222 Prior to any female participant undergoing MR imaging, a pregnancy test will be  
4  
5 223 performed after the expressed consent of the individual.  
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8 224 These assessments will be repeated in the QEHB or sports facilities during the  
9  
10 225 follow up and when the individual is deemed fit to return to play, until  
11  
12 226 normalisation of the MR Spectroscopy (1H-MRS) or self-reported symptoms are  
13  
14 227 observed.  
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17 228 The Near-Infrared Spectroscopy (NIRS) is a safe non-invasive brain imaging  
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19 229 technique that utilises Near-Infrared (NIR) light absorption to assess brain oxygen  
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21 230 saturation. The NIR light is currently widely used in clinical practice (e.g. pulse  
22  
23 231 oximeter and brain monitoring during cardiac surgery). fNIRS measurements are  
24  
25 232 performed once within 48-72 hours from the injury using a frequency domain  
26  
27 233 NIRS device (ISS™ IMAGENT; Champaign, Illinois, USA) and a digitizer  
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29 234 (POLHEMUS FASTRAK®; Colchester, Vermont, USA).<sup>32,33</sup>  
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## 245 **Outcome measures and statistical analysis**

246 The recruitment target is based on the size of the convenient sample that can be  
247 recruited (approximately 400); power calculation do not apply as the investigation  
248 is explorative at this stage. The sample size will be open-ended and reviewed after  
249 3 years.

250 Correlations between biomarkers, 1H-MRS, fMRI, MRE, DTI, fNIRS,  
251 neuropsychological scores, motor and coordination parameters will be assessed  
252 and reported with standard parametric statistical tests after normalisation of data or  
253 non-parametric test if normalisation were not possible. Time to resolution of  
254 symptoms will be compared for all the above modalities, as well as self-reported  
255 outcomes. Biochemical and imaging data will also be analysed with standard group  
256 comparison statistics (e.g. t-test on normalised data or Mann-Whitney on non-  
257 normalisable data).

## 258 **Patient and Public Involvement**

259 Links with sports academics and coaching staff have been established throughout  
260 the region through the University of Birmingham School of Sports, Exercise and  
261 Rehabilitation Sciences (SportexR) and through sports authorities such as the  
262 Rugby Football Union, the Welsh Rugby Union, GB Basketball, GB Hockey and  
263 the Football Association. Awareness of these programs will be promoted to the  
264 potential cohort of athletes through event days organised at individual recruitment  
265 sites coordinated centrally through the UoB. In terms of dissemination, the study  
266 team will regularly update the clubs on the progress of the study and will report the  
267 findings by email/newsletter and in various meetings where club representatives  
268 are in attendance.

269

## 270 **Ethics and dissemination**

271 This study was approved by the East of England - Essex Research Ethics  
272 Committee on 22 September 2017 – REC 17/EE/0275; IRAS 216703.

273 ISRCTN, ISRCTN16974791. Registered on 16/05/2018.

274 All data will be collected and stored in accordance with the 1998 UK Data  
275 Protection Act, UoB and QEHB data handling and maintenance guidelines, with  
276 the minimum amount of required information recorded. The complied and  
277 analysed results will be presented at national and international conferences.  
278 Results will also be submitted for peer review and publication in the subject  
279 journals/literature.

## 280 **Data statement**

281 All analysed data arising from the study will be kept on NHS and UoB servers.  
282 The computers on this network have restricted physical access; data are stored  
283 under coded filenames and the local network has secure password access restricted  
284 to a limited set of people. The raw MRI data are kept on a separate DICOM server  
285 on another network behind its own firewall which is only accessible by a small  
286 number of system administrators. Data spreadsheets will be kept on computers  
287 with password protected access and coded filenames, the original paper files will  
288 be secured in locked filing cabinets.

289 The datasets generated and/or analysed during the current study are/will be  
290 available upon request from, Professor Antonio Belli, who is the point of contact  
291 and will be able to provide anonymised data with sufficient details to able to  
292 reproduce the analyses for up to 10 years.

293

## 294 **Acknowledgements and Disclaimer**

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296 like to thank all the sports authorities, clubs and academics that agreed to participate in the study.

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299 the NIHR or the Department of Health and social care.

## 300 **Author Contributions**

301 AB, MJG, VDP, DH are the principal and co- investigators.

302 AB, MJG, VDP, DJD, ZS, MF, JB developed the protocol.

303 AB, MJG, VDP, DJD, ZS, MF, JB, VS, CB drafted the protocol and commented on the protocol.

304 KMY, ET, ZS, CB, MF, CNW, LC, AKB drafted and finalised the manuscript.

305 All authors have approved the final manuscript.

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308 Reconstruction and Microbiology Research Centre (SRMRC).

## 309 **Competing interests**

310 The University of Birmingham owns intellectual property for some of the biomarkers used in this study.

## 311 **Patient consent**

312 Not required.

## 313 **Ethics approval**

314 This study was approved by the East of England - Essex Research Ethics Committee on 22 September 2017 – REC  
315 17/EE/0275; IRAS 216703.

316 ISRCTN, ISRCTN16974791. Registered on 16/05/2018.

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4 420 **Figure legends/caption**

5  
6 421 Table 1

7  
8 422 Summary of eligibility criteria  
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| Inclusion criteria   | Exclusion criteria   |
|--|--|
| Male or female athletes participating in contact sports, aged 16-65 years, with fluent English speaking. | Individuals who require hospital admission after initial assessment for their TBI.   |
| Single or repetitive mTBI sustained in contact sport less than 72 hours prior to assessment.             | Intracranial haemorrhage, brain tissue injury, or non-TBI related pathologies on initial CT/MRI scan.  |
| Normal neurological objective examination at the time of enrolment.                                      | Pregnancy (urine pregnancy test will be performed for confirmation).   |
|  | Any history of neurodegenerative pathology or any recent or ongoing illness affecting the central nervous system (e.g. Parkinson's, multiple sclerosis, meningitis, epilepsy, neoplasm). |
|  | History of chronic alcoholism or drug abuse.   |
|  | Any other sustained injury that requires hospital admission.   |

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3 430 Table 2

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5 431 Analysis of biological fluids

| Blood   | Urine  | Saliva                          |
|---|--|---------------------------------|
| 6<br>7<br>8<br>9 1. Metabolomics (NAA and related metabolites)<br>10<br>11 2. Hormones (Progesterone, Aldosterone, 11-deoxycortisol, Corticosterone, Testosterone, Androstenedione, Cortisol, 17OHP, DHEA, DHEAS, Cortisone)<br>12<br>13 3. Pro and anti-inflammatory cytokines (TNF $\alpha$ , IL1 $\beta$ , IL6, IL4, IL8, IL10, IL13, IL17, GM-CSF)<br>14<br>15 4. Proteomics<br>16<br>17 5. Microparticles<br>18<br>19 6. Biomarkers of brain injury (S100B, GFAP and NSE)<br>20<br>21 7. MicroRNAs<br>22<br>23<br>24<br>25<br>26<br>27<br>28<br>29<br>30<br>31<br>32<br>33<br>34<br>35<br>36<br>37 | 1. Hormone profile<br>2. Brain biomarkers (NAA, S100B and GFAP)<br>3. Metabolomics<br>4. MicroRNAs | 1. MicroRNAs<br>2. Metabolomics |

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40 434 Figure 1

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43 435 Flowchart of study procedures

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46 436 **\*urine pregnancy test will be performed first for all female participants**

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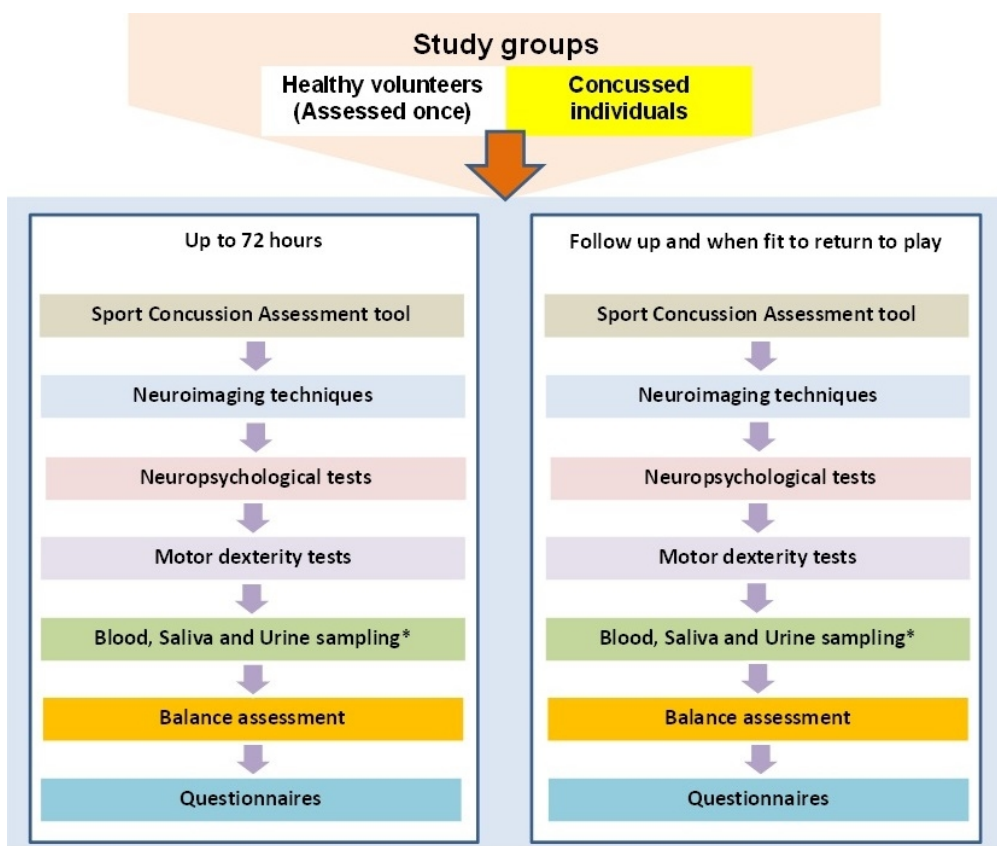


Figure 1/ Flowchart of study procedures  
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