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TRIAL PROTOCOL

Mild Traumatic Brain Injury Biomarker Study, a prospective cohort biomarker study of military and civilian participants with mTBI: mTBI-Predict

This protocol has regard for HRA guidance

Version Number:	**V1.0**
Version Date:	**26-Jan-2023**

PROTOCOL DEVELOPMENT

Protocol amendments

The following amendments and/or administrative changes have been made to this protocol since the implementation of the first approved version.

Amendment number	Date of amendment	Protocol version number	Type of amendment	Summary of amendment

Funding and support in kind

Funder/Supporting Organisations	**Financial and non-financial support given:**
Ministry of Defence, UK	£3,986,882
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The Funder of the study has had no role in the study design, data collection, data analysis or data interpretation. However, it is acknowledged that co-investigators employed by the Ministry of Defence are part of the study consortium.

This project is funded by the Ministry of Defence. The views expressed are those of the authors and not necessarily those of the Ministry of Defence.

PROTOCOL SIGN OFF

Chief Investigator (CI) signature page

I, the Chief Investigator, confirm that I have read and agree with the following protocol, and that I will conduct the study in compliance with the version of this protocol approved by the REC and any other responsible organisations.

I agree to ensure that the information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor.

I also confirm that I will make the findings of the study publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as stated in this and any subsequent approved protocol will be explained.

Study name:	mTBI-Predict
Protocol version number: Protocol version date:	Version: __ __ __ __ / __ __ / __ __ __ __
CI name:	Professor Alex Sinclair
Signature and date:	_____ __ __ / __ __ / __ __ __ __

Sponsor statement

Where the University of Birmingham takes on the sponsor role for protocol development oversight, the signing of the IRAS form by the sponsor will serve as confirmation of approval of this protocol.;

Compliance statement

This protocol describes the mTBI-Predict study only. The protocol should not be used as a guide for the treatment of people not taking part in the mTBI-Predict study.

The study will be conducted in compliance with the approved protocol, the UK Policy Framework for Health and Social Care Research, Data Protection Act 2018, Human Tissue Act 2004 and the Principles of Good Clinical Practice (GCP) as set out in the UK Statutory Instrument (2004/1031) and subsequent amendments thereof. Every care has been taken in the drafting of this protocol, but future amendments may be necessary, which will receive the required approvals prior to implementation.

Principal Investigator (PI) signature page

As Principal Investigator, I confirm that the following protocol has been agreed and accepted, and that I will conduct the study in compliance with the approved protocol where this does not compromise participant safety.

I agree to ensure that the information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor.

Study name:	mTBI-Predict
Protocol version number:	Version: __ __
Protocol version date:	__ __ / __ __ / __ __ __ __
PI name:	
Name of Site: Signature and date:	 _____ __ __ / __ __ / __ __ __ __

ADMINISTRATIVE INFORMATION

Reference Numbers	
Sponsor number	RG_22-004
ISRCTN reference number	< ISRCTN reference number >
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Study website	< tbc >
Study social media	< tbc >

Study organisation: Oversight

Various sub-committees will oversee this project, with the chair for each identified below. The members of these sub-committees will be outlined in each committee's terms of reference.

- **Study Management Group (SMG)**
  - Prof Alex Sinclair, Neurology Professor, (CI) will chair the SMG.
- **Study Steering Committee**
  - An independent clinician will chair a study steering committee whose remit is to provide independent oversight of the study.
- **App development committee**
  - Prof Adam Hampshire, Professor in Restorative Neurosciences, Imperial College London, will chair this committee.
- **Statistical oversight committee**
  - Dr Alice Sitch, Associate Professor of Biostatistics, University of Birmingham, will chair this committee.
- **Clinical facilitation committee**
  - Major James Mitchell, Clinical Lecturer Neurology, University of Birmingham, will chair this committee.
- **Sports oversight committee**
  - Dr Jamie Pringle, Associate Professor in Sport Science and Leadership, University of Birmingham, will chair this committee.
- **Data oversight committee**
  - Prof Hamid Dehghani, Professor of Medical Imaging, University of Birmingham, will chair this committee.

Study organisation: Workstreams

Workstream 1 – Headache	Mitchell Lyons, Sassani, Yiangou
Workstream 2 – Mental Health	Upthegrove / Rogers Palmer
Workstream 3 – Vestibular	Seemungal Reynolds, Ellmers, Golding
Workstream 4 – Cognition	Hampshire / Fernandez-Espejo Bagshaw, Brunger
Workstream 5 – Visual	Blanch Lyons, Mollan
Workstream 6 – Human brain imaging • MEG • Structural MRI • Cognitive functional MRI • 7T MRI	Jensen / Mullinger Bagshaw, Brookes, Fernandez-Espejo, Novak, Park, Witton
Workstream 7 – Fluid biomarkers & steroid hormone biomarkers	Hill Mitchell
Workstream 8 – Cerebral physiology • Near infra-red spectroscopy • Vascular functional MRI • Trans-cranial magnetic stimulation • EEG	Lucas Dehghani, Jenkinson, Mazaheri, Mullinger, Pringle, Weaver
Workstream 9 – Computer modelling & quantitative biomedicine	Terry Smith
Chief data officer	Dehghani

ABBREVIATIONS

Abbreviation	Term
ABC	Activities Balance Confidence
AE	Adverse Event
AUDIT	Alcohol Use Disorders Identification Test
BCTU	Birmingham Clinical Trials Unit
BIVSS	Brain Injury Vision Symptom Survey
BPPV	Benign Paroxysmal Positional Vertigo
BQ	Berlin Questionnaire
BSQ	Body Sensations Questionnaire
CA	Cerebral Autoregulation
CBF	Cerebral Blood Flow
CI	Chief Investigator
CRF	Case Report Form
CRP	C-Reactive Protein
CRT	Choice Reaction Time
CT	Computed Tomography
CVR	Cerebrovascular Reactivity
DCF	Data Clarification Form
DHI	Dizziness Handicap Inventory
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, 5th Edition
EEG	Electroencephalogram
EMG	Electromyography
ESS	Epworth Sleepiness Scale
FA	Fractional Anisotropy
FDI	First Dorsal Interosseous
fMRI	Functional Magnetic Resonance Imaging
fNIRS	Functional Near-Infrared Spectroscopy
FSS	Fatigue Severity Scale
GAD-7	Generalised Anxiety Disorder 7
GCP	Good Clinical Practice
GCS	Glasgow Coma Scale
GOS-E	Glasgow Outcome Scale - Extended
HbO2	Oxyhaemoglobin
HHb	Deoxyhaemoglobin
HIT-6	Headache Impact Test-6
HPA	Hypothalamic-Pituitary-Adrenal
HRA	Health Research Authority
HVF SITA	Humphrey Visual Field Swedish Interactive Thresholding Algorithm
ICA	Internal Carotid Artery
ICC	Intra-Cluster Correlation Coefficient
ICD-10	International Statistical Classification of Diseases and Related Health Problems 10th revision
ICF	Informed Consent Form

ICHD	International Classification of Headache Disorders
IL-6	Interleukin-6
ISI	Insomnia Severity Index
MCAv	Middle Cerebral Artery Blood Flow Velocity
MD	Mean Diffusivity
MEG	Magnetoencephalography
MINI	Mini-International Neuropsychiatric Interview
MPAI	Mayo-Portland Adaptability Inventory
MRI	Magnetic Resonance Imaging
mTBI	Mild Traumatic Brain Injury
NHS	National Health Service
NIHR	National Institute for Health and Care Research
NVC	Neurovascular Coupling
OCT	Optical Coherence Tomography
OCTA	Optical Coherence Tomography Angiography
OPM	Optically Pumped Magnetometer
PCAv	Posterior Cerebral Artery Blood Flow Velocity
PCL-5	PTSD Checklist For DSM-5
PCS	Post-Concussion Syndrome
PHQ-9	Patient Health Questionnaire Depression Scale
PI	Principal Investigator
PIC	Participant Identification Centre
PIS	Participant Information Sheet
PSQI	Pittsburgh Sleep Quality Index
PTH	Post-Traumatic Headache
PTSD	Post-Traumatic Stress Disorder
PVAQ	Pain Vigilance and Awareness Questionnaire
QoLiBri	Quality Of Life After Brain Injury questionnaire
QSM	Quantitative Susceptibility Mapping
REC	Research Ethics Committee
RGC	Retinal Ganglion Cell
rMEQ	Reduced Morningness-Eveningness Questionnaire
RNFL	Retinal Nerve Fibre Layer
R-PSQ	Rivermead Post-Concussion Symptoms Questionnaire
SBQ-R	Suicidal Behaviour Questionnaire-Revised
SD	Standard Deviation
SMG	Study Management Group
SOFAS	Social and Occupational Functioning Assessment Scale
SQUIDS	Superconducting Quantum Interference Devices
SRC	Sports-related Concussion
SSC	Study Steering Committee
SWI	Susceptibility weighted Imaging
TBI	Traumatic Brain Injury
TCD	Transcranial Doppler
TMS	Transcranial Magnetic Stimulation

UHF	Ultra-High Field
UoB	University of Birmingham
VA/DoD	Department of Veterans Affairs and the Department of Defense
VOR	Vestibulo–Ocular Reflex
VVAS	Visual Vertigo Analogue Scale

STUDY SUMMARY

Title	**mTBI-Predict**
Objectives	• To evaluate the accuracy and precision of candidate biomarkers (imaging, clinical, biofluid) in predicting prognosis in mTBI due to impact, blast and sport concussion. • To identify biomarkers at the time of injury that enable a rapid decision to return to play, work or duty. • To develop a multifaceted biomarker algorithm to predict prognosis in mTBI. • To assess the variability of candidate biomarkers. • To identify novel biomarkers in patients with mTBI.
Study Design	Longitudinal prospective cohort study with nested **variability** and **case-control** studies in military and civilian populations including impact, blast and sports injury. 1. **Main study: Longitudinal prospective cohort study:** 610 participants will undergo assessments of candidate biomarkers (injury day <24 hours, + 21 days and month 3 according to participant availability). Prognostic outcome assessments will follow at 6, 12 and 24 months with long term digital follow-up. 2. **Nested study 1: Candidate biomarker variability study:** biomarkers will be repeated over 12 days in 40 mTBI patients and 40 healthy controls. 3. **Nested study 2: Observational case-control prospective study:** 100 cases of mTBI will be compared to 100 healthy controls to identify novel biomarkers.
Setting	• Multi-centre through the UK mTBI Research Network: • Recruitment: Defence Medical Rehabilitation Centre Stanford Hall, University Hospitals Birmingham NHS Foundation Trust plus further sites. • Imaging: Centre for Human Brain Health, University of Birmingham; Sir Peter Mansfield Imaging Centre, University of Nottingham; Aston Institute for Health and Neurodevelopment, Aston University, plus further sites. • Clinical: NIHR/Wellcome Trust Clinical Research Facility, University Hospitals Birmingham plus further sites.
Main study inclusion criteria	• Age ≥18 & ≤60 years • mTBI: Acute (<3 months) mild traumatic brain injury (VA/DoD criteria)
Main study exclusion criteria	• Prior diagnosis of PTSD or severe mental illness (see Appendix 2) • Pregnancy • Prior brain injury (from trauma, stroke or other aetiologies) without full functional and symptomatic recovery • Inability to comply with study schedule or follow-up • Inability to provide informed consent (e.g. due to cognitive impairment) • Any progressive neurodegenerative or neuroinflammatory condition • Alcohol use disorder or drug dependence • Patients with medical conditions that are unstable or untreated

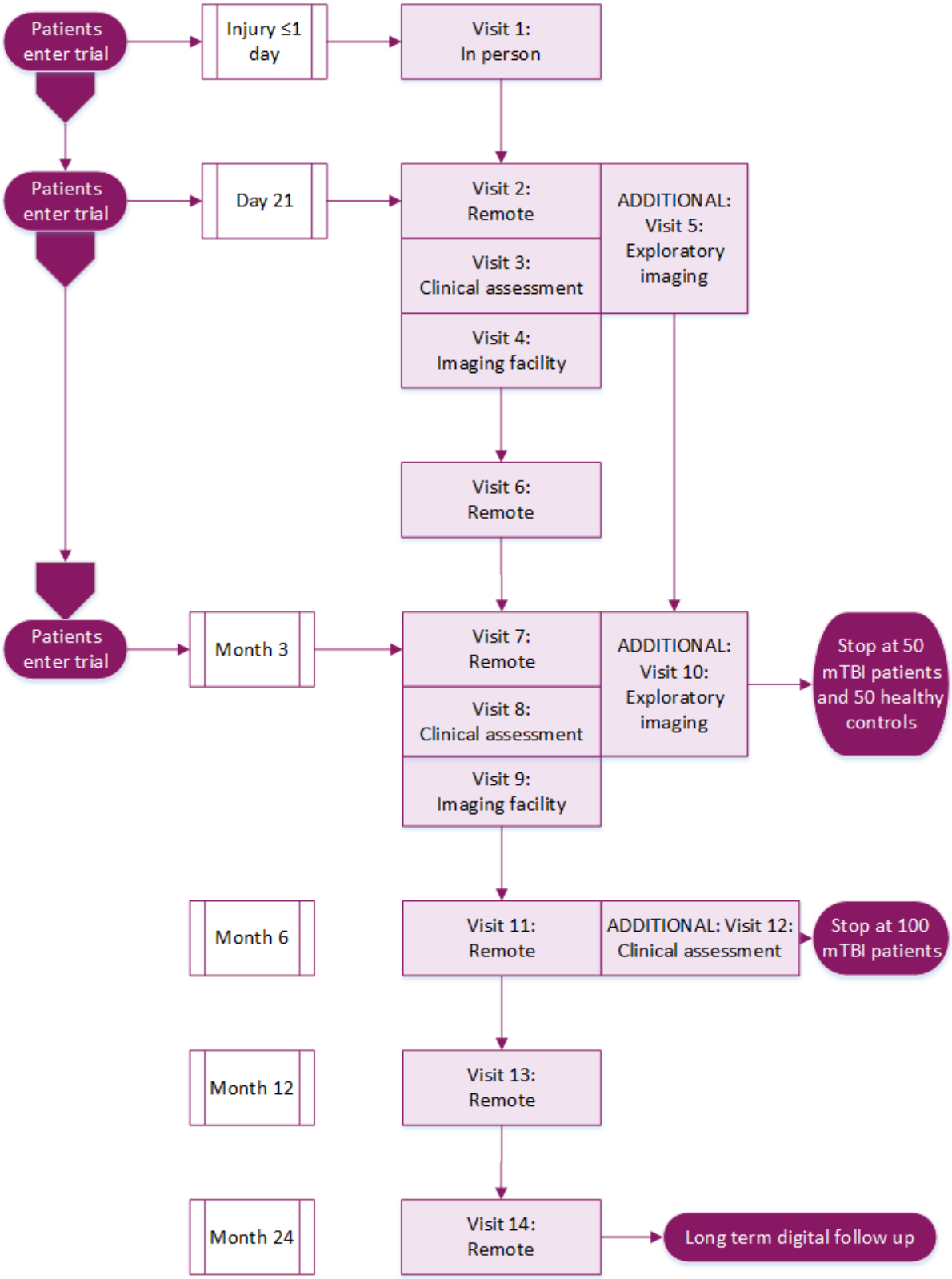
Outcome Measures	**Main study** • Primary outcome: Ability of candidate biomarkers to predict full return to play, work or duty at 6 months post-injury • Secondary outcomes: Ability of candidate biomarkers to predict global function, persistent post-traumatic headache, cognitive dysfunction, depression, PTSD, vestibular disturbances and physical function at 6 months post-injury and beyond. • Exploratory outcomes: Accuracy of a multifaceted computer modelled biomarker algorithm to predict sequelae of mTBI (full return to play, work or duty, persistent post-traumatic headache, cognitive dysfunction, depression, PTSD, vestibular disturbances, and physical function). **Candidate biomarker variability study (nested study 1)** • Primary outcome: The variability of candidate biomarkers for each workstream. **Observational case-control prospective study (nested study 2)** • Primary outcome: Identify novel candidate biomarkers. • Exploratory outcomes: Gain mechanistic insights into the candidate biomarkers.

Lay Summary

- Mild traumatic brain injury (mTBI) (also sometimes called concussion) is common, with nearly 1.2 million hospital visits due to mTBI each year in the UK. Although classed as mild, it leads to a disproportionate impact on future health, with 3 in 10 patients unable to work 12 months after their injury. The consequences of mTBI are profound, with many patients suffering long-term disability due to persistent headaches, imbalance, memory disturbance and poor mental health. We cannot yet identify those patients most at risk of these disabling consequences. This is a clear unmet need which would allow targeting of treatments to improve patient outcomes.
- Mild TBI can be caused by physical impact to the head through accident, injury or sport, or due to the effects on the brain of shockwaves propagated by explosions – blast TBI.
- We will test key biomarkers to allow identification of mTBI patients at risk of long-term health issues. Biomarkers need to be accurate, reproducible and practical to use in a clinical setting.
- Mild TBI is often interchangeable with the term 'concussion'. Sports-Related Concussion (SRC) can be defined as the immediate and transient symptoms post-TBI resulting in a large range of different symptoms. Currently, evaluating SRC on the field involves a rapid assessment during competition with a time constraint and the athlete eager to play. There is no accurate reliable test for an immediate diagnosis of SRC on the field. The current recommendation is to keep an athlete out of participation when there is suspicion of injury as signs and symptoms may be delayed. mTBI-Predict will look at biomarkers to enable a faster diagnosis and prognostic assessments which could improve treatment and long-term management of SRC, which may enable a quicker return to play for some athletes.
- We will conduct a long-term study following patients after a new mTBI. At onset, we will measure a variety of different, but complementary biomarkers including brain imaging, brain physiology, blood and saliva, headache, mental health, vision, balance and cognitive performance. We will then look at the ability of these biomarkers to predict long term complications at 6, 12 and 24 months. This will allow those with a good prognosis to rapidly return to normal activity and those likely to suffer complications to receive prompt and targeted therapy.
- The mTBI-Predict study will be driven by the Royal Centre for Defence Medicine (RCDM) and the University of Birmingham (UoB). The study will use the UK TBI Research Network encompassing multiple sites around the UK to recruit both civilian and military participants. Study analysis will involve not only leading UK biomarker biostatisticians (Biostatistics, Evidence Synthesis and Test Evaluation Research Group, UoB), but also multimodal computer modelling (Centre for Systems Modelling and Quantitative Biomedicine, UoB).
- This programme of research will deliver a step change in the care of patients with mTBI and bring much needed advances in patient management.

STUDY SCHEMA

NB: mTBI patients may enter study at one of three timepoints after injury



List of primary biomarkers by workstream

Workstream	Assessment	Primary biomarker
Global		
	Return to work/duty/play	Yes/No
	Mayo-Portland adaptability inventory	Score
Headache		
	Headache diary	Monthly headache days
Mental health		
	PCL-5 (Post-Traumatic Stress Disorder checklist for DSM-5)	PTSD (Post-traumatic stress disorder) Yes/No
Vestibular		
	Vestibular perceptual thresholds	Score
Cognition		
	Cognitive battery	Corrected Global Composite Score
Visual		
	OCT (Optical coherence tomography)	RNFL thickness (Retinal nerve fibre layer thickness)
Imaging		
	MEG (Magnetoencephalography)	Delta/theta waves
	Structural MRI (Magnetic resonance imaging)	FA (fractional anisotropy) / MD (mean diffusivity)
	Functional MRI	Dynamic connectivity (mean dwell time)
	Physiology fMRI	Cerebrovascular reactivity (CVR)
Fluid / hormone		
	Blood	Glial Fibrillary Acidic Protein
	Blood	Cortisol
Cerebral physiology		
	Doppler	Cerebrovascular reactivity (CVR)
	fNIRS (Functional near-infrared spectroscopy)	Neurovascular coupling (relative change in oxy- and deoxyhaemoglobin)
	TMS (Transcranial magnetic stimulation)	Cortical silent period
	EEG (Electroencephalogram)	Delta/theta waves
	Physical function	6-Minute Walk Test
	Exercise capacity	Maximal voluntary contraction / muscular endurance

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1. BACKGROUND AND RATIONALE

There are 1.4 million hospital visits annually in England and Wales due to head injury, and of these around 200,000 people are admitted [1]. Of these hospital visits, 85% (1.2 million) will be classified as mild traumatic brain injury (mTBI) (encompassing concussion), with 10% moderate and 5% severe [1]. Whilst many individuals with mTBI recover, a significant proportion will have disabling long term sequelae. The most common and highly disabling consequence of mTBI is post-traumatic headache (PTH) (up to 54% affected at 1 year) [2, 3]. Cognitive dysfunction, psychiatric morbidity including post-traumatic stress disorder (PTSD) and intrusive dizziness are also common sequelae which contribute to poor quality of life and impair ability to return to play, work or duty. Consequently, compared to moderate and severe traumatic brain injury (TBI) and as a result of its high prevalence, mTBI leads to disproportionate morbidity and healthcare utilisation [4].

There are no objective measures to quantify injury following mTBI. Additionally, measures to accurately predict those who will develop long-term complications are not established. There is therefore a pressing need to develop accurate, reproducible biomarkers of mTBI that predict long-term complications. These biomarkers need to be relevant to assessments immediately at the time of injury as well as over the months following injury. These biomarkers will enable a personalised medicine approach to target early intervention to those most in need, but also identify those with a good prognosis who can return rapidly to play, work or duty.

The aim of this program of work is to identify accurate, reproducible biomarkers in mTBI that will predict the most common and disabling consequences: primarily, delayed return to play, work or duty.

1.1. Background

TBI is an alteration in brain function, or other evidence of brain pathology, caused by an external force [5]. It is commonly divided into mild, moderate and severe. Mild TBI is often referred to as concussion or minor head injury. It can temporarily disrupt brain function with increasing evidence associating it with significant morbidity [6]. The National Institutes of Health declared in 1999 that mTBI was a major public health problem, being underdiagnosed and with a great societal burden [7]. Mild TBI is a clinical diagnosis, most commonly diagnosed using the Department of Veterans Affairs and the Department of Defense (VA/DoD) diagnostic criteria [8].

The leading causes of TBI in Europe are road traffic accidents and falls [9]. Seventy to 90% of treated brain injuries are classified as mild, despite many cases of mTBI not being treated at hospitals. Estimates of the true mTBI population-based rate suggest it is likely to be above 600/100,000 [10]. Around 63% of mTBI occur in adults of working age, between 16 to 64 years old [11].

Sports-Related Concussion (SRC) is often defined on the basis of the immediate and transient symptoms post-TBI. SRC, like mTBI, can result in a wide variety of different symptoms and may be associated with concurrent neck injuries or disruption to the body's balance mechanisms [12]. As a result, a range of treatments may be necessary, including physical and psychological therapies.

Recognising and evaluating SRC on the field is a challenging responsibility for the healthcare provider. Performing this task often involves a rapid assessment in the midst of competition with a time constraint, and on an athlete that is eager to play. Side-line evaluations are currently based upon the recognition of injury, assessment of symptoms, memory and higher nerve function, and balance. Repeated assessments may be necessary as SRC is often an evolving injury. Signs and

symptoms may be delayed, so, in the absence of reliable biomarkers, erring on the side of caution (i.e. keeping an athlete out of participation when there is any suspicion of injury) is important and the mainstay of current management strategies. There is no accurate diagnostic test that can be relied upon for an immediate diagnosis of SRC on the field.

The 'Consensus Statement on Concussion in Sport' from the 5th International Conference on Concussion in Sport (Berlin, October 2016) concluded that further research evaluating rest and active treatments within rehabilitation should be performed. The consensus recommended the use of high-quality study designs that account for potential confounding factors and have matched controls and effect modifiers to best inform clinical practice and facilitate recovery after SRC [13]. mTBI-Predict addresses the consensus recommendations. It is designed to identify ground-breaking evidence to enable a faster diagnosis and prognostic assessments which could improve treatment and long-term management of SRC. This may enable a quicker return to play for some athletes, but also support a healthcare provider and team manager's duty of care by confirming when an athlete with SRC is unfit to return to competition.

Alongside mTBI in the sporting community, there is also significant incidence of mTBI amongst military personnel. Amongst deployed UK military personnel, the estimated prevalence of mTBI is 4.4%, and 9.5% in those with a combat role [14]. This is lower than that observed in the US military, where estimates range from 12% to 23% [15, 16]. In the UK military epidemiology study [14], mTBI was found to be more prevalent in the youngest, those with a lower level of education, lower ranks, combat roles and those who spent more time outside of base. Associations have been found between experiencing mTBI and symptoms of PTSD, as well as co-morbid depression and anxiety, alcohol misuse and multiple physical symptoms (headache, double vision, dizziness). Blasts, fragments and vehicle incidents were the mechanisms of injury, with blast injuries being the most frequent [14].

Blast represents a form of injury that has a distinct mechanism from non-blast injury. A high-explosive detonation causes nearby solids or liquids to convert to gas, which subsequently expand rapidly forming a high-pressure wave. After this the pressure drops causing a relative vacuum which leads to a momentary reversal of air flow. There is then a second lower intensity positive pressure wave before atmospheric pressure returns to normal [17]. The Department of Defense defines five types of mechanisms of injury: primary (direct effect), secondary (result of debris), tertiary (body displacement), quaternary (effects of explosion i.e. burns, toxins) and quinary (environmental contaminants i.e. radiation) [18]. Close proximity to high-pressure blast can cause moderate-to-severe TBI (typically secondary and tertiary injuries)[17]. Whilst experimental animal studies have shown that primary injury can induce a brain injury, it is less clear the degree in which this can produce long-term effects, especially in mTBI [19, 20].

Whilst many recover following mTBI, a significant proportion develop long-term sequelae (Table 1). This constellation of symptoms, most often seen in prolonged mTBI, can collectively be known as post-concussion syndrome (PCS) [21]. ICD-10 defines PCS as consisting of three out of eight symptoms and functional changes (headache, dizziness, fatigue, irritability, insomnia, concentration difficulty, memory difficulties, and intolerance of stress, emotion and/or alcohol) [22]. Persistent PCS is when these symptoms persist after 3 months and is estimated to affect 15% of mTBI patients, although this could be an underestimate, especially of cognitive impairment. Persistent PCS has lasting effects on executive function, cognition, memory and learning [23]. Predictors of poorer

outcome at 12 months include history of brain injury, having a least one comorbidity, living alone, non-white ethic group, being female, and alcohol and medication use [24].

Table 1: Symptoms of post-mild traumatic brain injury in order of frequency at 3 months, according to the Rivermead Post-Concussion Symptoms Questionnaire [25]

Symptom *(Highest frequency at the top)*	
Fatigue	23%
Headache	22%
Dizziness/Balance issues	16%
Poor memory	16%
Irritability	15%
Sleep disturbance	14%
Poor concentration	14%
Frustration	12%
Longer to think	11%
Restlessness	11%
Depression	10%
Noise sensitivity	9%
Light sensitivity	7%
Nausea	6%
Blurred vision	6%
Double vision	2%

Mild TBI, compared to moderate and severe TBI, leads to disproportionate morbidity and healthcare utilisation. Studies have shown that, in the longer term post-mTBI, there are increased rates of unemployment, productivity loss and work limitations [24]. Although classified as 'mild', it leads to a disproportionate impact on future health with half complaining of functional limitations 12 months after the event [26]. Delayed return to work, results in lost earnings and long-term treatment costs. This is particularly important as 32% of the population experience at least one TBI prior to 25 years old.

There can be significant costs involved in mTBI, both directly and indirectly. In 2006, in the United States, it was estimated that the annual direct cost of TBI (all severities) was $9.2 billion and the indirect cost was $51.2 billion (through missed work and lost productivity) [27].

The overarching aim of this program of work is to identify accurate, reproducible biomarkers in mTBI that will predict the most common and disabling consequences of mTBI: primarily return to play, work or duty, as well as persistent PTH, cognitive dysfunction, depression, PTSD and vestibular disturbances. This will be achieved through a harmonised program of detailed clinical phenotyping of acute mTBI patients coupled with state-of-the-art multimodal biomarker evaluation (brain imaging, fluid biomarkers, steroid hormones, visual, vestibular, and cerebral physiology). The

program will recruit 610 participants aged between 18 and 60 years, with a diagnosis of mTBI within 3 months of injury. The participants will include civilians, military personnel, and athletes. As identified above, the sequelae post-mTBI are varied, and this is reflected through the nine workstreams through which each participant will be assessed. This will enable us to focus on a variety of biomarkers, reflecting the clinical symptoms and imaging results that we see in clinical practice. Through doing this we will be able to identify which biomarkers, whether in isolation or combination, are most reliable and indicative of prognosis for those with mTBI.

There are four main existing consortia worldwide looking at TBI, three based in the United States (TRACK-TBI, CONNECT-TBI, LIMBIC-CENC), and one based in Europe (CENTER-TBI). TRACK-TBI, CONNECT-TBI and CENTER-TBI cover the spectrum of TBI, with LIMBIC-CENC focusing only on mTBI. However, they do not specify time of entry into programme as an inclusion criterion. Our program highlights a gap in the research looking only at the acute mTBI population within 3 months of injury and then follows these individuals up prospectively. Where there are areas of overlap, we will exploit the data through the formation of an international consortia network that will facilitate cross validation and meta-analysis.

1.2. Study rationale

- There is a lack of biomarkers that are accurate, reproducible and practical for use in a clinical setting.
- There is no accurate and reliable test for an immediate diagnosis of SRC on the field.
- There is no predictive model for mTBI in the acute and sub-acute setting.
- This study is important because targeted biomarkers can help orientate prioritisation for clinical care.

1.2.1. Justification for participant population

- Why mTBI? Mild TBI is common and leads to a disproportionate impact on future health with up to 37% of mTBI patients being unable to work at 6 months and 24% at 12 months. There are profound consequences of mTBI with many suffering long-term disability. There is currently no way to identify those patients most at risk; there is an unmet need to be able to target treatment.
- Why acute? An acute cohort enables us to measure biomarkers early and track patient outcomes in the long term via NHS Digital follow-up.
- Why military? Mild TBI is the most common traumatic injury affecting military personnel. Some mTBI have long-term debilitating effects, thereby affecting return to field for military personnel.
- Why civilian? Civilian participation will allow us to translate the research into the general population via the NHS.
- Why sport? Sports injuries account for a large proportion of both civilian and military TBI and have unique challenges.
- Why blast TBI? Blast exposure is common in some groups of military personnel and the sequelae are not well understood.

1.2.2. Justification for design

- Why prospective cohort study? A prospective cohort design allows us to observe the development and resolution of symptoms in real-time. By including all new injuries where possible, we will minimise selection bias and symptom recall error.
    - We exclude previous head injuries with ongoing sequelae as this would make the cohort too heterogeneous and risk failure to identify useful biomarkers.
    - We exclude patients with a prior diagnosis of PTSD to allow us to identify biomarkers which increase the likelihood of developing PTSD following mTBI.
- Why variability study? Understanding the variability of biomarkers is critical to later using them in a predictive model; this study will allow us to collect this vital data.
- Why case-control cohort study? Identifying new biomarkers in someone with mTBI and a healthy control will allow us to track changes in these biomarkers over the course of recovery. We should get a reliable measure of 'normal' variability in outcomes across the course of the study.

1.2.3. Justification of choice of primary outcome

Return to and participation in normal play, work or duty is an outcome fundamental to an individual's wellbeing, so we will primarily concentrate on this, but other outcomes remain important (e.g. headache, cognition, sleep, vestibular and mental health, and physical function).

An important outcome for mTBI patients is return to work, which is a surrogate marker of functional recovery. A 2018 systematic review examining return to work post mTBI [28] identified 14 studies to date, with 12 reporting the proportion of patients that return to work at specific time points. Two studies reported 66-79% of participants returning to work at 3 months [29, 30] and at 6 months this was 63-93% (4 additional studies) [31-34]. The lower rates of return to work were 63% by Dikmen et al [34] and 76% in Stulemeiher et al [33], but they had one of the highest proportions of patients with complicated mTBI (CT brain changes). Dikmen and colleagues did not report further detail regarding injury severity within the mTBI subgroup [34]. Seven studies reported an average proportion of 76-97% of patients returning to work at 12 months [29-31, 34-37]. The authors commented that there was heterogeneity in this review as outcome reporting in mTBI is variable. The TRACK-TBI Pilot study found that their return to work rates were 77.6% at 3 months and 78.9% at 6 months, although 39% of their 'mild TBI' cohort had a computed tomography (CT) intracranial lesion [38]. A study in 2022 found that 11 of their 113 patients were still on 100% sick leave at 12 months (return to work 90%). This study included moderate TBI, but the majority (94%) were mTBI. They found that a higher PCS burden, using the Rivermead Post-Concussion Symptoms Questionnaire, was negatively associated with work participation at 12 months (p=0.04). Kraemer et al collected retrospective return to work data in mTBI patients with PTH (n=91) [39]. At 3 months, return to full-time work differed significantly in those with persistent PTH vs recovered acute PTH (69 vs 94%, p=0.025), and also between persistent PTH and non-PTH (69 vs 93%, p=0.035). At 12 months follow-up, all participants, aside from one with persistent PTH, had returned to work [39].

1.3. Workstream 1, headache

1.3.1. Background

PTH is very common following mTBI with prevalence reported as between 30%-90% [40]. Although the International Classification of Headache Disorders (ICHD) classifies PTH as occurring within 7 days of injury, it is not uncommon for PTH to occur later in the time course [2]. The incidence of PTH varies between literature, with an incidence of 15 to 78% [2, 3, 39, 41-52] at 3 months and 15 to 65% at 1 year [2, 3, 39, 41, 43, 53-55] . This range may be largely due to the differing definitions of PTH. Kraemer et al used the ICHD PTH diagnostic criteria of the headache having to be less than 7 days post injury and reported lower incidence rates at 12 months [39]. Some have stated that the ICHD-2 diagnostic criteria of PTH is arbitrary and is likely to result in under-diagnosis [45, 46, 50]. One study showed an increase in the incidence of reported headaches 2 to 5 years after trauma [2]. Beswick-Escanlar et al reported an incidence of PTH of 15.2% at 12 months, but this was a retrospective study, hence we need to consider recall bias [54]. A large prospective cohort study in China with 97% mTBI (n=526) found 49.4% of their cohort reporting PTH, defined as a new or worsening headache after injury (no time frame) [50]. A prospective military study used the VA/DoD criteria and found an incidence of 15-28% at 3 months and 17-23% at 12 months. They included different types of headaches from ICD-9-CM codes, not only PTH [41]. A Canadian study noted that whilst their incidence of PTH at 12 months was only 18.6%, 74% of their cohort sought care from a health care professional [43]. Conversely, a Lithuanian prospective study found that at 3 months (p=0.25) and 1 year (p=0.98) they did not find a significant difference in 'headache during last month' between head-injured participants and the non-head-injured controls. This study only included very mild head injuries with loss of consciousness <15 minutes, so cannot be applied to all mild head injuries (loss of consciousness 15-30 minutes) [3].

PTH most commonly has a phenotype of migraine, but tension-type-like headache is also found in a proportion of patients [56, 57]. Albeit much rarer, there are reports of cluster-like, chronic paroxysmal hemicrania-like, and hemicrania continua-like PTH in the literature [58]. Heterogeneity of PTH illustrates the complexity of this symptom and renders deep phenotyping of headache crucial to this study. PTH can lead to significant morbidity from cognitive dysfunction [59], behavioural changes, and social functional ability. Patients with pre-injury headache and PTH had substantially worse physical and cognitive symptoms than participants with no headache, as demonstrated by a large cross-sectional study [59]. Moreover, PTH intensity has an adverse impact on the ability to return to work or play following injury [60]. New headache therapies for PTH have emerged [61], but accurate stratification and identification of those who will go on to develop long term sequelae is still lacking. [2]

1.3.2. Rationale for main study

PTH is an important outcome of TBI but can also be considered a biomarker as there is some evidence that presence of PTH predicts worse functional outcome following injury [39]. Prognostic models using biomarkers to predict outcome and to identify those at risk of persistent PTH would enable earlier, more appropriate treatment and potentially improve long-term outcome. To date, there are no models to accurately predict mTBI outcome for PTH [62].

1.4. Workstream 2, mental health

1.4.1. Background

People who have suffered TBI experience higher rates of co-morbid mental health outcomes. In mTBI, 1 in 5 experience mental health disorders at 6 months post injury [63], with a 3-fold increase in the risk of developing a mental illness in the first 6 months post-mTBI [64]. Further, military personnel who experience mTBI are at a significantly increased risk of PTSD, depression and anxiety 3 to 6 months after deployment [65]. PTSD and depression are the most frequent, occurring in up to 50% and 60% of patients respectively [66]. PTSD, as per the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) criteria is characterised as exposure to a traumatic event with the presence of intrusion symptoms, avoiding stimuli associated with the event, negative alterations in cognition and mood, and hyper-arousal [67]. We have previously demonstrated several clinical variables that are associated with increased risk of PTSD post-mTBI, including mode of injury (e.g. assault), severity of injury, and pre-existing mental health disorder [68]. Rates of completed suicide are also significant, with the relative risk of suicide post-TBI almost 3 times higher than the general population [69]. Poor mental health contributes uniquely to the burden of living with TBI, with some indication that mental health problems precede functional decline. Hence, better understanding of the aetiology of PTSD and depression post-mTBI could lead to targeted preventative treatments with significant and wide impact.

Whilst we have previously shown that self-reported outcomes of TBI are more sensitive to patient needs than objective outcome scores [70], the subjective nature of conventional diagnostic methods for PTSD and the high comorbidity of depression mean that an objectively measurable biomarker for diagnosing PTSD would be particularly impactful to clinicians and researchers.

To date, there is limited exploration of biomarkers that may be utilised in the identification and prediction of PTSD and depression in the context of mTBI, although several biomarkers may have useful translational use. For instance, there is an increasingly recognised role of low grade innate immune activation in mental illnesses. Interleukin-6 (IL-6) and C-reactive protein (CRP)are reliably elevated in patients with PTSD and depression, which is associated with poorer outcomes [71]. Within neuroendocrine biomarkers, depression is associated with hypercortisolaemia [72], although evidence also suggests that in PTSD cortisol may be lowered. It is possible this reflects a state of hypothalamic-pituitary-adrenal (HPA) exhaustion after prolonged hyperarousal, with a decrease in hormonal reactivity seen in acute stress and increased response to suppression tests. However, most studies have been cross-sectional, with longitudinal research needed to investigate fluctuations in the HPA axis in response to certain tasks i.e. face recognition tasks [73]. HPA axis (dys)function is also known to be influenced by exposure to adverse events early in life, which could also be linked to increased risk of PTSD [74].

PTSD may be increasingly understood in the context of aberrant neural networks [75, 76]. Studies have revealed altered neurophysiological processing of threat-related emotional stimuli in individuals with PTSD compared to matched trauma-exposed and trauma-unexposed groups [77, 78]. PTSD groups also show heightened threat responses for angry but not happy faces, as measured by increased functional connectivity and clustering in regions critical to emotional processing, including the amygdala and medial prefrontal cortex [79, 80]. However, less is known about how mTBI impacts PTSD network dynamics of emotional processing, with the neurophysiological ‘threat’ response remaining unclear. In PTSD, individuals without co-existing TBI have shown increased

synchronisation during electrophysiological resting-state recordings, which is distinguishable from TBI-related PTSD [79, 81].

1.4.2. Rationale for main study

Prediction of the development of mental health disorders, including PTSD, and the ability to intervene early would be facilitated by the identification of prognostic biomarkers. Previous work illustrates the potential to use biomarkers integrated with computational modelling of mental illness symptoms driven by TBI [82, 83]. We will extend this work to develop biomarkers in conjunction with data-driven analytical approaches to enable specific prognostication in different mental health sequelae including PTSD, depression, and other commonly comorbid mental health symptoms after mTBI. Previous work has identified the potential importance of serum cortisol, inflammatory biomarkers (e.g. IL-6), neurophysiological measures of hyperarousal and neuroimaging.

A particularly important focus as an outcome is PTSD. Accurate biomarkers to predict PTSD could enable earlier treatment, and so drive better patient outcomes and return to play, work or duty.

1.5. Workstream 3, vestibular

1.5.1. Background

TBI is the commonest cause of chronic disability in young adults [84]. Imbalance post-TBI is a key predictor of failure to return to work [85, 86]. Even in mTBI, patient return to work rates at 6 months are reduced from 75% in those without vestibular features to 33% in those with vestibular dysfunction [85]. TBI is an independent predictor of falls even in young adults [86, 87], perhaps explaining why imbalance in TBI also predicts return to work rates. Despite its importance in recovery from TBI, the mechanisms underlying chronic post-TBI imbalance and vestibular functioning in general are poorly understood, with one large study unable to identify any specific cause in 25% of chronic TBI patients [88]. There is a lack of biomarkers to predict ongoing imbalance.

Accurate biomarkers for imbalance are hindered by a lack of understanding of imbalance in TBI. There are no prospective studies assessing vestibular dysfunction in acute TBI. There has been one cross-sectional study assessing instrumented measures of balance, confirming manifest imbalance in subacute TBI [89]. We have reported two separate cross-sectional clinical studies in ambulant acute TBI patients: one showing that 62% were unbalanced [90], of whom half did not report feeling unbalanced; and the other confirming the lack of correlation between objective signs of imbalance and vestibular symptom scores [91].

Another common diagnosis in acute TBI patients is benign paroxysmal positional vertigo (BPPV), affecting 40% of cases in our acute cross-sectional series [90]. Consistent with our documented observation of a dissociation between objective and subjective features of vestibular dysfunction in acute TBI [90, 91], we noted several acute TBI patients who denied vertigo sensation despite an obvious vestibular ocular reflex (VOR) response (e.g. during the positional manoeuvre used to diagnose BPPV), i.e. a loss of the vestibular perception of self-motion or ‘vestibular agnosia’. Our observation in acute TBI patients on a major trauma ward suggested that those patients with a clinically apparent vestibular agnosia (i.e. a vestibular agnosia that is sufficiently severe to be visible on bedside testing) were also those with worse balance function. This observation was confounded, however, by the clinical situation where the stimulus to the peripheral vestibular apparatus required to reveal both a prominent nystagmus and simultaneous lack of vertigo sensation could only occur in the setting of a co-existing inner ear disorder, such as BPPV (or an acute vestibular nerve injury that affected 19% of acute TBI cases) [90]. In inner ear conditions, in addition to a reflex vestibular nystagmus, patients with a healthy brain (i.e. not acute TBI cases) complain of severe vertigo. To

assess whether vestibular agnosia was directly linked to imbalance required the formal testing of vestibular reflex and perceptual function in acute TBI patients in whom there was no inner ear dysfunction, along with assessment of balance function.

Our general hypothesis linking vestibular agnosia and imbalance was that reduced vestibular signalling at the cerebral cortical level would manifest both in a vestibular agnosia and imbalance (given the cortical dominance of postural control). An additional question was whether there are cortical regions that co-localise the functions of vestibular perception and vestibular-mediated balance control, which is of potential interest to the understanding of the brain's control of balance since balance control mechanisms in humans are poorly understood.

The bedside observation of a loss of vertigo sensation in patients with preserved inner ear functioning (vestibular agnosia) has received scant attention but has hitherto only been empirically reported in elderly patients, typically with cerebral small vessel disease [92-94]. Conversely, prospective laboratory assessment in acute (within 2 weeks) focal stroke patients [95] found no evidence of a vestibular agnosia. The mechanism linking vestibular agnosia with elderly small vessel disease and younger patients with acute TBI may relate to the hypothesis that the vestibular sensation of self-motion is mediated by a distributed cortical network [96, 97] that becomes disconnected, potentially explaining why acute TBI cases may be susceptible to vestibular agnosia since this patient group exhibit cognitive deficits that often relate to the disruption of cortical networks [98].

1.5.2. Rationale for main study

TBI patients have numerous features of vestibular dysfunction such as headache, dizziness and imbalance [90]. One of the problems that TBI patients face is attenuated vestibular perception. We have identified vestibular agnosia in acute TBI patients who denied feeling vertigo sensation and also had poor balance despite having intact peripheral vestibular function [99]. Assessing mTBI patients for their vestibular perception of self-motion and imbalance is necessary as both may contribute to falls. Vestibular assessments have the potential to provide useful biomarkers to predict other common sequelae of mTBI (e.g. PTH, cognitive dysfunction and mood disturbance). Early assessment of vestibular biomarkers also has the potential to predict those likely to suffer with longer term disabling vestibular sequelae and hence those that might benefit from early treatment.

1.6. Workstream 4, cognition

1.6.1. Background

Cognitive deficits after mTBI can manifest in different ways, but typically include impairments in attention, processing speed, memory, and executive function [100]. In many cases these symptoms will return to baseline after 3 months [101]. However, up to 30% of cases will continue to experience symptoms beyond this point and develop PCS [102]. Without proper management, PCS symptoms can last for many months or years [103] and lead to a decreased quality of life and, in many cases, a secondary decline in mental health. The complex relationship between mental health and cognitive symptoms has contributed to a great deal of controversy, with some arguing it results from neurological damage vs premorbid psychiatric conditions or personality traits [104]. Meta-analyses have revealed high heterogeneity in the assessment tools and cognitive taxonomies employed across studies to evaluate cognition in mTBI [101]. This lack of consistency also maps into clinical practice, where the lack of guidelines results in many neurotrauma centres relying on clinical judgment instead of comprehensive cognitive evaluations to diagnose mTBI [105].

A major challenge to addressing these issues is the historic lack of a TBI-optimised assessment battery. Several solutions are available, but many have been commercialised, presenting a barrier to broader deployment. Furthermore, they lack precision to differentiate at a fine grain between deficits that arise from damage to the brain systems that underlie different dimensions of cognition. Building on previous work, and with National Institute for Health and Care Research (NIHR) support, we applied an optimisation process to identify a battery of cognitive tests for repeated precision assessment of mild to moderate TBI patients. The tests can be delivered online via computer, tablet and smartphone devices. They can be deployed over many repeat sessions to track cognitive change. Patients with moderate motor and cognitive impairments can engage with them. They show maximal sensitivity to TBI, greatly outperforming gold standard pen and paper neuropsychological tests. Critically, they are decorrelated from each other, being selected from a large superset to measure different dimensions of cognition that are: a) affected in mild to moderate TBI during the chronic phase; b) insensitive to the type of device being used for assessment; and c) may be delivered at many repeat timepoints.

The logical next step is to apply these precision diagnostic tools longitudinally in an mTBI cohort at sufficient scale to examine how dimensions of cognitive deficit change across time, covary with brain metrics, and predict symptoms and real-world outcomes.

Most of our current understanding of the clinical consequences of mTBI comes from studies including exclusively male participants. There is preliminary evidence highlighting sex and gender differences in the brain response to mTBI. By recruiting a large sample with a good balance across male and female participants, we will be able to characterise sex differences in acute cognitive profiles, recovery trajectories, and long-term outcomes for the first time.

There is some preliminary evidence from animal research suggesting that sex hormones play a role in the brain's response to mTBI, and the cognitive and sensorimotor symptoms that follow the injury [106]. In humans, the relationship between TBI and sex hormones is complex and very poorly understood. However, hormonal changes after TBI appear to correlate with clinical outcome [107], hormonal contraceptives seem to have a protective effect and are associated with less severe symptoms [108], and there is evidence that outcomes are worse when the injury occurs during the luteal phase of the menstrual cycle [109, 110]. Together, this evidence indicates that the specific hormonal profile at the time of injury will impact clinical and cognitive outcome. In female participants, we will look at whether the severity of the cognitive symptoms correlates with baseline measures (including use of hormonal therapies, contraceptives, etc., and menstrual phase when the injury took place), as well as hormonal imbalances after injury (pituitary dysfunction).

1.6.2. Rationale for main study

As outlined above, our battery is highly sensitive to cognitive deficits in mTBI. The next logical step is to apply the precision cognitive diagnostic tools longitudinally in an mTBI cohort at sufficient scale to apply a multivariate treatment of the data. This would enable examination of how dimensions of cognitive deficit change across time, covary with brain metrics, and predict symptoms and real-world outcomes. This in turn can inform better prognostication, and the development of individually tailored therapies.

1.7. Workstream 5, visual biomarkers

1.7.1. Background

Up to 80% of patients with mild to moderate TBI complain of visual dysfunction, with both civilians and service personnel reporting difficulty with reading and near work, spatial perception and photophobia [111, 112].In this context, it is vital to define visual function in TBI patients, as limitation of vision has the potential to be a confounder for the other tests evaluating TBI. For example, a patient performing poorly on neuropsychological assessments with visual impairment may be incorrectly diagnosed with cognitive problems when in fact the limitation is visual.

The frequency of visual symptoms in TBI reflects the high proportion (~30%) of the cerebral cortex devoted to visual function [113], which is also readily assessable using objective tests. Ophthalmic manifestations assessed by objective assessment of visual structure and function also provide objective and reproducible disease biomarkers, termed “oculomics”. Alterations in afferent and efferent visual pathways may therefore both underlie and predict post-traumatic visual dysfunction as well as providing biomarkers for the severity of brain injury.

The optic nerve is a simple unidirectional central nervous system tract comprised of retinal ganglion cells (RGC) whose axons pass throughout the midbrain and brainstem, but whose cell bodies are all located in the retina, where they may be readily imaged and functionally characterised. Ocular imaging using Optical Coherence Tomography (OCT) is a non-invasive and rapid modality that has the potential to provide a diagnostic and prognostic biomarker for mTBI as it readily assesses RGC structure. OCT angiography (OCTA) allows non-contact assessment of retinal blood flow, which has the potential to be a surrogate for cerebral blood flow (CBF) [114]. Visual acuity, contrast sensitivity, colour vision, pupillometry and visual field analysis also assess RGC function.

Optic nerve damage caused by trauma is termed traumatic optic neuropathy. Historic data suggests that the prevalence of traumatic optic neuropathy causing severe visual loss is between 0.5 and 5% in TBI patients; more recent case series suggest that more subtle retinal and visual changes after TBI may be much more frequent, being present in many elite athletes after sports head injuries [113, 115, 116]. The OCT measures of peripapillary retinal nerve fibre layer (RNFL) thickness, macular RGC layer and choroidal thickness were both thinner in patients with mTBI than controls, demonstrating loss of RGC axons and a global reduction in blood flow respectively [117, 118]. Both global RNFL and the macular RGC nuclear layer were thicker in athletes with previous concussion than those without, suggesting long-term gliosis, and these changes were better able to distinguish these two groups than balance and reaction time performance tests.

Evidence for functional biomarkers in mTBI is seen in the pupil-light reflex. This assesses both afferent (RGC) and efferent (cranial nerve III) function and is frequently abnormal after mTBI with multiple metrics including 75% recovery time and constriction latency separating strongly between mTBI and control patients [119].

Imaging of the optic nerve head shortly after injury using cutting edge high magnification OCT has the potential to detect early structural changes such as axonal swelling after injury that is not detectable with standard imaging and analysis of optic nerve head blood flow (which is in part activity dependent), and may predict subsequent neurodegeneration [120, 121].

1.7.2. Rationale for main study

A complete assessment of visual structure and afferent and efferent function including OCT, OCTA, visual acuity, colour vision, contrast sensitivity, pupillometry, accommodation and visual field

assessment will be key to interpretation of the other biomarkers as well as providing potential prognostic information.

1.8. Workstream 6, brain imaging

1.8.1. Background

1.8.1.1. Magnetoencephalography (MEG)

MEG measures electrical brain activity via assessment of the magnetic fields generated outside the head by neural current flow. Mathematical modelling of these fields (a process called source reconstruction) enables formation of 3D images showing moment-to-moment changes in electrophysiological brain activity. This provides a powerful technique for functional imaging, with millimetre spatial and millisecond temporal resolution, where brain networks can be seen to form and dissolve in real time, as the brain responds to cognitive demand.

In mTBI, the predominant hypothesis is that impact causes damage to axonal pathways in the white matter. Such damage is likely to disrupt functional connectivity. We predict that the oscillations observed in MEG, and indeed the functional connectivity that they underpin, may be disrupted post-injury. Furthermore, findings support that these changes in functional connectivity are clearly discernible with MEG [122-125]. Importantly, MEG also allows for localising the neuronal sources generating the electrophysiological activity. This makes it possible to identify which brain regions are generating the slow wave activity associated with an injury [126]. Consequently, MEG is a promising technology for assessment of mTBI. However, moving from initial promise to reliable robust biomarkers with a prognostic aim requires significant further work.

1.8.1.2. Structural Magnetic resonance imaging (MRI)

MRI using 3T magnetic field strength is a standard technique to assess structural brain damage following TBI. MRI is a flexible technique which can be used to investigate a variety of aspects of brain structure, with a typical clinical pipeline incorporating several different types of scans which can provide information about different physiological features and hence increase the likelihood of identifying abnormalities (e.g. T1, T2, T2 FLAIR etc.). In clinical practice, MRI scans are generally analysed visually: a neuroradiologist examines the scans and determines the extent of the damage. However, more recent developments allow several different quantitative analyses of these scans, while also providing several newer scan sequences (such as diffusion weighted imaging [127] and quantitative susceptibility mapping (QSM) [128]) which have shown promise in prognosing TBI. Ultra-high field (UHF) MRI (7T) has increased sensitivity for identifying abnormalities which can help to inform assessment and analyses and develop new potential biomarkers. In the observational case-control prospective study, we will use a combination of standard (3T) and ultra-high (7T) field MRI to identify the most informative prognostic markers.

1.8.1.3. Functional MRI (fMRI)

In addition to structural changes, MRI can provide quantitative markers demonstrating the impact of mTBI on brain function and its neuropsychiatric sequelae. fMRI is a widely used method that can be used to identify brain regions that are active during a task (task-based fMRI), or to investigate interactions between brain regions at rest (resting fMRI). Both approaches have demonstrated sensitivity to the impact of mTBI and have also been recognised as one of the most promising techniques to prognosticate outcome in mTBI [129]. While fMRI does not have the temporal resolution afforded by MEG, it provides a window to subcortical structures like the thalamus that are key to understanding the dysfunctions that follow mTBI.

By combining MEG and fMRI measures, we will be able to capture the full range of features that characterise neural dysfunctions after mTBI. Resting-state fMRI works on the principle that most of the brain's activity is intrinsically generated, rather than tied to a particular cognitive or sensory task. As such, mTBI's impact can be quantified by investigating how the whole brain network or specific individual sub-networks interact [130, 131]. This approach has proven successful at predicting outcome after TBI and complements independent molecular biomarkers for TBI severity [132].

Alongside resting fMRI, it is possible to record brain activity generated by a particular cognitive task to assess the neural bases that underlie specific cognitive functions. Sustained attention and processing speed deficits are some of the main cognitive sequelae after mTBI and have been vastly studied with fMRI paradigms. One of the paradigms most commonly employed in this clinical group is the Choice Reaction Time (CRT; see e.g. [133, 134]). The CRT is a simple cognitive task with which TBI patients are able to engage with very high (>90%) levels of accuracy [135], making it ideal for an intensive schedule of assessments like the one proposed here. The CRT requires a sustained and consistent configuration of key neural networks [136] and is proven to be very sensitive at characterising functional impairments after TBI [134]. Crucially, alterations to functional activity and connectivity during CRT in TBI map into structural changes identified with diffusion imaging [135, 137], providing a framework for the combination of both techniques.

1.8.1.4. Electroencephalogram (EEG)

EEG is a portable, relatively cost-effective non-invasive neuroimaging technique that detects the electric potentials generated by active neurons. EEG and MEG essentially measure the same physiological activation but have different sensitivities to brain areas they can pick up activity from, as well as unique advantages. EEG is much more cost-effective and widely available as a clinical tool than MEG. However, it is much easier to localise the source of MEG signals in the brain than EEG, since unlike electrical fields, magnetic fields are not affected by the tissue between the cortex and the sensors (e.g. bone, skin, cerebrospinal fluid). The measurement of both MEG and EEG in a patient allows for broad coverage of brain activity in the gyri and sulci of the neocortex, and potentially in subcortical regions.

Here our use of EEG will be two-fold:

- Resting EEG will allow us to capture the brain's underlying functional architecture in an individual and look for signs for anomalies that are predictive of neural degeneration.
- Task-based EEG will allow us to capture how the brain of the individual is functioning during a specific cognitive task, allowing us to gauge the fidelity of brain networks involved in carrying out the task (see Workstream 8, cerebral physiology).

1.8.2. Rationale for main study

As noted above, evidence suggests that MEG and MRI can provide accurate diagnostic classification of mTBI in case-vs-control demonstrations. Such demonstration is useful for diagnosis and for understanding the neurophysiology and functional anatomy of sequelae. However, mTBI will remain a clinical diagnosis, and to have genuine utility MEG and MRI measurements must offer prognostic information which impacts the treatment pathway. Therefore, the crux of this investigation needs to probe the ability to stratify patients into (yet unknown) phenotypes and consequently differentiate based on likely treatment pathway. At the most basic level, an assessment based on imaging conducted soon after injury could differentiate those who will recover fully from those who will continue to have ongoing problems.

1.9. Workstream 7, fluid and steroid biomarkers

1.9.1. Background

There has been much research into biomarkers for neurotrauma, which has yet to lead to a change in clinical practice. Previous research has assessed potential biomarkers, for instance creatine kinase, glial fibrillary acidic protein, myelin basic protein and S100β. S100β is the most studied and has been shown not to be specific to central nervous system damage. A panel of 6 fluid biomarkers was assessed in the CENTER-TBI study; glial fibrillary acidic protein had the highest sensitivity for detecting CT abnormality; combinations of markers did not further improve discrimination [138].

Several research studies have confirmed the risk of pituitary gland dysfunction following TBI which can potentially have a negative impact on the morbidity and rehabilitation of these patients. In a meta-analysis of 19 studies including TBI cases from mild to severe, the prevalence of post-traumatic hypopituitarism was 27% [139]. Proposed mechanisms include vascular injury to the hypothalamo-pituitary unit resulting in infarction, direct trauma to the pituitary following skull base fracture, or secondary insults due to hypoxia, hypotension or raised intracranial pressure [140]. The most clinically significant hormonal abnormalities in the acute phase of TBI are corticotropin/cortisol deficiency, as well as antidiuretic hormone deficiency leading to water and salt imbalance[141]. Both conditions can contribute to acute morbidity, and if cortisol deficiency is left untreated it can be life-threatening. In the chronic phase, further pituitary hormone deficits can be detected (growth hormone, gonadotropins leading to hypogonadism and thyrotropin hormone leading to hypothyroidism) which if not appropriately replaced can have a plethora of adverse sequelae, e.g. unfavourable metabolic profile and body composition, increased cardiovascular risk, reduced muscle mass and exercise capacity, compromised bone mineral density and osteoporosis, fatigue, social isolation, diminished sense of well-being and quality of life, and infertility [142]. Symptoms associated with pituitary dysfunction overlap significantly with those of PTSD, so early detection and management of hypopituitarism is of major importance for the optimal prognosis of these patients.

Alterations in the neuroendocrine function following TBI (as reflected by e.g. cortisol measured in blood or urine and plasma copeptin) may be predictive of PCS, neurological, functional, psychiatric or other long-term clinical outcomes [143, 144]. Furthermore, it has been suggested that metabolites in the peripheral blood may be potential markers of pathological processes in TBI [145], and plasma metabolomics profiling upon admission and in the first 7 days post-mTBI has led to the identification of metabolite panels classifying acute mTBI from controls [146]. It has also been proposed that specific plasma metabolites can associate with functional outcomes in these patients [147]. Therefore, the identification and validation of hormonal/metabolic biomarkers predicting the long-term prognosis of patients with mTBI and informing rehabilitation strategies will increase optimal management opportunities.

1.9.2. Rationale for main study

Although there has been great interest in an array of fluid biomarkers, none has yet been adopted into clinical practice. There have been attempts to utilise multiple fluid protein biomarkers [138] without an increase in diagnostic accuracy above a single marker. However, the utility of multimodal models encompassing biomarkers has not been studied.

1.10. Workstream 8, cerebral physiology

1.10.1. Background

Mild TBI is characterised by a period of increased cerebral vulnerability post-injury, where the brain is more sensitive to additional trauma [148]. The increased observed post-mTBI may reflect a fundamental difference between physiological recovery and clinical recovery – heavily based on medical symptom resolution, which guides return to duty and play decisions. Studies have reported alterations in physiological parameters that persist far beyond the typical 7-10 days of clinical recovery. Among other physiological markers, CBF [149], myelin content [150], and cerebral metabolites [151] have demonstrated recovery profiles in the order of 30+ days. Alterations in CBF and its regulation are thought to play an important role in the pathophysiology underlying mTBI [152]. More broadly, vascular responsiveness is an established biomarker of vascular dysfunction, with the sensitivity to detect the first signs of dysfunction [153]. Given this, targeting measures of cerebrovascular responsiveness to quantify and track mTBI severity and recovery are key.

The neurometabolic cascade identified above is known to have an impact on neurotransmission that results in an imbalance of the major excitatory (glutamate) and inhibitory (gamma-aminobutyric acid) transmitters in the brain [153, 154]. This imbalance can be measured on a physiological level using transcranial magnetic stimulation to measure the excitatory state of the motor cortex. These measures have been shown to be sensitive to changes in acute mTBI and in PCS.

1.10.1.1. Transcranial Doppler (TCD)

TCD allows for a dynamic assessment of the cerebrovascular response, which is a key advantage when assessing vascular dysfunction beyond the resting state. Indeed, the dysfunction may only be revealed during tests that require movement and/or perturbation of the resting state. For this reason, the inclusion of Doppler and tasks related to common and real-life behaviours is important to determine the impact of mTBI on cerebrovascular health.

1.10.1.2. Magnetic resonance imaging (MRI)

MRI is a powerful tool to assess the vascular status of the brain with excellent spatial resolution, including the decoupling between metabolic demand and blood supply that may occur as a consequence of mTBI [155]. While Doppler techniques provide excellent temporal resolution to assess the dynamic response functions in arteries, these may be underpinned by damage of the microvasculature. MRI provides the ability to probe macro and microvascular structure and function and identify changes present at rest and during a stimulus-response challenge (e.g. CO2 reactivity). CBF has been shown to change in mTBI but, due to methodological differences between studies, a consensus of the importance and its potential as a biomarker is lacking [155, 156]. There is promising evidence that CO2 cerebrovascular reactivity (CVR) is changed in TBI and may be a more useful biomarker than CBF [155, 157]. However, the use of MRI-based CVR measures for TBI are currently limited and the utility as a prognostic biomarker in mTBI unknown. We have shown that combining Doppler and MRI to assess CVR produces different outcomes [158], related to where in the vascular tree the different imaging approaches obtain their responsiveness measure. We will use this approach to gain a greater understanding of how this regulatory process for the cerebrovasculature is impacted by mTBI, and which of these measures is most sensitive as a biomarker.

1.10.1.3. Functional near-infrared spectroscopy (fNIRS)

fNIRS provides a means for assessing regional cerebral oxygenation, with measures of oxygenated and deoxygenated haemoglobin (HbO2 and HHb, respectively) acting as surrogate markers for CBF [159, 160]. These measures can be assessed at a relatively high frame rate (~40 Hz) during cognitive tasks to assess regional changes in cerebrovascular and brain function in response to neuronal activity, a method that has been used in clinical settings previously [161]. Studies in TBI populations have demonstrated the utility of fNIRS in the assessment of neurovascular function in specific regions of the frontal cortex during cognitive task completion, including assessment of working memory and attention [162-165]. Although these studies have identified clear differences in a detectable contrast of HbO_2, HHb and CBF between mTBI and control participants (highlighting the relevance and utility of fNIRS in relation to mTBI), quantified "cut-off" values between those with and without mTBI are yet to be established.

In this study, we aim to establish population thresholds in fNIRS responses associated with diagnosis of mTBI and so assess the viability of NIRS-based assessment strategies as biomarkers for mTBI. Further, we will combine these functional haemodynamic measures with EEG measures of neural activity during cognitive tasks. This combined approach yields simultaneous neural and vascular insight into brain dysfunction by assessing how mTBI may alter neurovascular coupling processes and provides scope to develop the technology for this assessment to be utilised in the field – if shown here to be a useful biomarker.

1.10.1.4. Transcranial magnetic stimulation (TMS)

Following mTBI, one of the most consistently observed changes is an elongation of the TMS measure – the cortical silent period [166-169]. These changes are seen immediately following sub-concussive impacts (e.g. heading a football [170]) and continue for months to years following concussive injury [167, 171-174], and represent changes to the excitatory/inhibitory balance in the cortex.

1.10.2. Rationale for main study

To date, studies have used different imaging modalities (Doppler, MRI, fNIRS) and stimulus-response approaches (vasoactive stimuli [e.g. inspired CO_2], postural challenges [e.g. repeated stand-squats], cognitive based tasks, exercise) to assess the impact of mTBI on measures of cerebrovascular responsiveness (e.g. CVR, cerebral autoregulation (CA), neurovascular coupling (NVC)). A number show promise as potential biomarkers, but which is the most sensitive is unclear as studies to date typically only use one modality and/or target only one regulatory process. Furthermore, a longitudinal, prognostic study has not been done to predict how any of these potential biomarkers relate to clinical outcomes. We will assess the utility of these functional responsiveness measures on the cerebral physiology across the range of imaging modalities and functional tests.

1.11. Workstream 9, computer modelling and quantitative biomedicine

1.11.1. Background

The purpose of this workstream is to develop a mathematical modelling and computational analysis pipeline to enable diagnostic and prognostic outcome markers to be revealed from data collected in other workstreams.

1.11.2. Imaging data

There has been a fundamental re-appraisal of the mechanisms underpinning neurological disorders. Simplistic concepts of single brain regions being responsible for disease are being updated with

connectomics – the large-scale networks within which brain function arises – increasingly implicated in neurological disorders [175]. We will integrate dynamic network models (computer models that describe both the neural activity within brain regions, as well as the connections between them) and the fMRI, MEG and EEG data collected in workstreams 6 and 8. Up to 400 participants will be used for biomarker identification and up to 200 participants for biomarker validation. Using algorithms for inferring network structures from these imaging modalities that we have developed in the context of studying epilepsy [176, 177], we will construct large scale brain networks of order 30-150 nodes. We will pursue two approaches to interrogating these networks: hypothesis free and hypothesis driven. In the first approach, we will use a range of quantitative network measures (see e.g. [178] for a review) to reveal candidate markers of severity of TBI and the likelihood of developing specific outcomes, including mental health, cognitive (dys)function and headache. Using longitudinal recordings, we will study the dynamic variation in these markers over time and so assess and refine their prognostic capacity.

Our second approach is to define network markers based on specific hypotheses from the literature. For example, Nuwer et al and Haneef et al [179, 180] have defined quantitative features of EEG in mTBI, such as frequency, amplitude, power, and phase. We can define a mathematical model on each node, whose parameters can be calibrated from these quantitative measures from each channel. Alternatively, Kuceyeski et al [181] developed a mathematical approach that revealed a network level mechanism for measuring the recovery of consciousness following severe brain injury. This study revealed speed of diffusion across the network as a strong marker of recovery. Speed of diffusion can be captured, both in terms of network markers (such as the propagation index introduced in Woldman et al [182]) as well as in the brain dynamics of each node. We will use similar knowledge of network alterations in outcomes such as PTSD and headache [175] and cognition [183] to predict outcomes. Here we will study the presence of these markers in initial recordings, as well as their dynamic evolution as inferred from longitudinal recordings.

1.12. Rationale for biomarker variability study (nested study 1)

The vestibular, visual, brain imaging, fluid biomarkers and cerebral physiology workstreams will all be involved in the variability study. This is due to the need to estimate the reliability of the techniques used and allow for this in subsequent use of measurements and interpretation of results. Many measures have not been validated. We will validate the techniques used in order to establish the variation between participants and the variation within participants. It is important for biomarkers to be reproducible. This will inform the main study, as biomarkers that are not fit for purpose due to excess variability may be dropped from further analyses, as we would suggest the need for refinement of these measurements.

Headache, mental health and the cognition workstreams are not included in the variability study. Headache is assessed utilising clinical history to assign phenotype, diaries to describe a patient's experience of their symptoms and patient reported outcome measures (such as the Headache Impact Test-6, or HIT-6) to describe disability. These are already validated tools (HIT-6) and hence variability does not need assessing [184]. The questionnaires involved in the mental health workstream have also been validated [185-191]. Cognitive outcomes will not be looked at due to test-retest reliability having already been determined by the Department of Brain Sciences at Imperial College London (unpublished data) for the cognitive tests proposed.

1.13. Rationale for case-control study (nested study 2)

The case-control study takes patients with mTBI and compares them to healthy controls. This will give us the opportunity to look for biomarkers specific to head injury and the option to perform exploratory work looking at novel biomarkers that can be evaluated in the longitudinal main study. All workstreams will be looking for TBI-specific biomarkers in addition to the exploratory work.

1.13.1. Rationale for exploratory work within case-control study

The brain imaging workstream will have optional exploratory assessments using optically pumped magnetometer (OPM) and 7T MRI measures. Conventional MEG measures the fields from the brain using superconducting quantum interference devices (SQUIDs). These cryogenically cooled detectors are sufficiently sensitive to measure the extremely small (in the femto-Tesla range) magnetic fields generated by neural current. However, the requirement for cryogenic cooling of superconductors means that detectors must be kept ~2 cm from the head which dramatically lowers sensitivity as the magnetic field decreases with the square of the distance. Furthermore, sensors are fixed in place meaning a one-size-fits-all device in which patients must remain still for long periods of time. This produces an environment often poorly tolerated by patients. However, developments have led to the emergence of new MEG systems built from quantum sensors called OPMs.

These small (Lego-brick sized) devices do not require cryogenic cooling and can be mounted flexibly on the head, adapting to head shape/size, and moving with the subject (thereby negating subject movement). These 'wearable' MEG systems also offer significant improvements in sensitivity and spatial resolution, as well as significant reduction in purchase and running costs. Flexibility means that unlike conventional systems which are large, immovable, and require significant infrastructure, an OPM-MEG could realistically be placed in the back of a vehicle and driven either to specialist centres across the UK, or e.g. to sports grounds where mTBI is commonplace. It is likely that the coming years will see the replacement of conventional cryogenic devices with these new systems. Therefore, another aim of the case-control study will be to record data from patients using a 50 sensor OPM system and compare it to the conventional MEG data.

Brain imaging techniques are constantly evolving and improving. While 3T scanners are now the standard in research centres (and specialised clinical facilities) across the world, 7T UHF scanners are becoming increasingly available. To provide a degree of futureproofing, participants in the case-control study will undergo MRI at 7T UHF. UHF MRI can provide additional sensitivity to subtle damage and, in doing so, can help identify markers that could then be assessed at 3T, thus informing the analysis of the data acquired as part of the main protocol. For example, one of the most observed structural abnormalities reported by MRI studies is cerebral microhaemorrhage. This abnormality can be detected using susceptibility weighted imaging (SWI) sequences in MRI but is only successful in a minority of cases at 1/1.5T/3T. However, SWI improves dramatically at 7T UHF MRI, meaning the potential for imaging subtle abnormalities is increased. The potential has been demonstrated for mTBI in a small case study [192] but further investigation is needed. We will therefore acquire multi-echo gradient echo data using the 7T system at the University of Nottingham from which we will be able to derive SWI, QSM and T2* maps. QSM data will be able to inform us of the venous oxygenation levels which have been shown to be perturbed in TBI [155], providing complementary data to the cerebral physiology assessments (Workstream 8). In the same session, we will acquire other structural scans (MP2RAGE) and investigate whether the enhanced spatial resolution and signal-to-noise ratio provided by 7T can identify additional abnormalities and/or inform the analysis at 3T. In addition, we will explore the benefits of 7T for new biomarkers, namely: image cerebrovasculature to interrogate blood flow across the vascular tree (4D PCA) –

complementing Workstream 8 – and provide indices of neuroinflammation through magnetisation transfer imaging which may be a useful prognostic biomarker.

2. AIMS AND OBJECTIVES

2.1. Main study

- To evaluate the accuracy and precision of candidate biomarkers (imaging, clinical, biofluid) in predicting prognosis in mTBI due to impact, blast and sport concussion.
- To identify biomarkers at the time of injury to enable a rapid decision to return to play, work or duty.
- To develop a multifaceted biomarker algorithm to predict prognosis in mTBI.

Biomarkers to be assessed are summarised in Table 2 below, and will include brain imaging (structural, functional, MRI, MEG, EEG), biofluid and hormone biomarkers, visual and vestibular disturbances, cerebral physiology, physical function, mental health, headache markers and cognitive dysfunction.

2.2. Candidate biomarker variability study (nested study 1)

- To assess the variability of candidate biomarkers in healthy controls and mTBI patients to understand if biomarkers are reliable and that any change in a biomarker indicates change over random fluctuation.

2.3. Case-control study (nested study 2)

- To identify novel biomarkers in patients with mTBI compared to healthy controls.
- Exploratory studies will enable mechanistic insights to be gained.

Table 2: List of primary biomarkers by workstream

Workstream	Assessment	Primary biomarker
Global		
	Return to work/duty/play	Yes/No
	Mayo-Portland adaptability inventory	Score
Headache		
	Headache diary	Monthly headache days
Mental health		
	PCL-5 (Post-Traumatic Stress Disorder checklist for DSM-5)	PTSD (Post-traumatic stress disorder) Yes/No
Vestibular		
	Vestibular perceptual thresholds	Score
Cognition		
	Cognitive battery	corrected Global Composite Score
Visual		
	OCT (Optical coherence tomography)	RNFL thickness (Retinal nerve fibre layer thickness)
Imaging		
	MEG (Magnetoencephalography)	Delta/theta waves
	Structural MRI (Magnetic resonance imaging)	FA (fractional anisotropy) / MD (mean diffusivity)
	Functional MRI	Dynamic connectivity (mean dwell time)
	Physiology fMRI	Cerebrovascular reactivity (CVR)
Fluid / hormone		
	Blood	Glial Fibrillary Acidic Protein
	Blood	Cortisol
Cerebral physiology		
	Doppler	Cerebrovascular reactivity (CVR)
	fNIRS (Functional near infrared spectroscopy)	Neurovascular coupling (relative change in oxy- and deoxyhaemoglobin)
	TMS (Transcranial magnetic stimulation)	Cortical silent period
	EEG (Electroencephalogram)	Delta/theta waves
	Physical function	6-Minute Walk Test
	Exercise capacity	Maximal voluntary contraction / muscular endurance

3. STUDY DESIGN AND SETTING

3.1. Overall design

Longitudinal prospective cohort study with nested variability and case-control studies:

Main study: Longitudinal prospective cohort study: 610 participants will undergo assessment of candidate biomarkers (enrolment at injury day <24 hours, +21 days and month 3 according to when patients are identified). Prognostic outcome assessments will follow at 6, 12 and 24 months.

Nested study 1: Candidate biomarker variability study: biomarkers will be repeated over 12 days in cohorts of 20 mTBI patients and 20 healthy controls.

Nested study 2: Observational case-control prospective study: 100 cases of mTBI will be compared to 100 healthy controls to identify novel biomarkers.

3.2. Study setting

- Multi-centre recruitment and clinical assessment through the UK mTBI research network (including Defence Medical Rehabilitation Centre Stanford Hall, University Hospitals Birmingham and further centres)
- Imaging at Centre for Human Brain Health, University of Birmingham; Sir Peter Mansfield Imaging Centre, University of Nottingham; Aston Institute for Health and Neurodevelopment, and Defence Medical Rehabilitation Centre Stanford Hall

3.3. Sub-studies

There will be two control cohorts in this study program:

- Healthy participants (n=20 clinical assessment day and n=20 imaging assessment day as part of the nested variability study)
- Healthy participants (n=100) as part of the nested case-control study

3.4. Assessment of risk

All studies can be considered to involve an element of risk and in accordance with Birmingham Clinical Trials Unit (BCTU) standard operating procedures this study has been risk assessed to clarify any risks relating uniquely to this study beyond that associated with usual care. A Risk Assessment has been conducted and concluded that this study corresponds to the following categorisation: No higher than the risk of standard medical care.

3.5. Selection of participants

3.5.1. Main Study

- Both military and civilian mTBI patients including impact, blast and sports injuries will be recruited. All consecutive potential participants will be approached to take part.

3.5.2. Nested study 1: Candidate biomarker variability study

- Both military and civilian mTBI patients including impact, blast and sports injuries will be recruited. All consecutive potential participants will be approached to take part.
- Both military and civilian healthy controls will be recruited.

3.5.3. Nested study 2: Observational case-control prospective study

- Both military and civilian mTBI patients including impact, blast and sports injuries will be recruited. All consecutive potential participants will be approached to take part.
- Both military and civilian healthy controls will be recruited.

3.6. Recruitment

Participants will be recruited from the study centres and their dependant patient identification centres (PICs).

Recruitment of mTBI patients will be from dedicated concussion/mTBI clinics and emergency departments.

- Birmingham – University Hospitals Birmingham, Concussion service, Trauma service
- Military – Defence Medical Rehabilitation Centre, mTBI team, rehab teams
- University Hospitals Coventry and Warwickshire
- Further centres

Healthy controls will be recruited from study centres, local sports clubs, etc. and online and poster advertisements (see Section 6.1).

4. ELIGIBILITY

4.1. Main Study

4.1.1. Inclusion

- Age ≥18 years & ≤60 years
- mTBI: Acute (<3 months) mTBI (VA/DoD criteria, Table 3 below)

Table 3: Classification of TBI Severity [193]

Criteria	Mild	Moderate	Severe
Structural imaging	Normal	Normal or abnormal	
Loss of Consciousness	0-30 min	>30 min and <24 hours	>24 hours
Alteration of consciousness / mental state*	up to 24 hours	>24 hours; severity based on other criteria	
Post-traumatic amnesia	0-1 day	>1 and <7 days	> 7 days
Glasgow Coma Scale (GCS) (best available score in first 24 hours)*	13-15	9-12	<9

(If a patient meets criteria in more than one category of severity, the higher severity level is assigned)

*Alteration of mental status must be immediately related to the trauma to the head. Typical symptoms would be looking and feeling dazed and uncertain of what is happening, confusion, difficulty thinking clearly or responding appropriately to mental status questions and being unable to describe events immediately before or after the trauma event.

4.1.2. Exclusion

- Prior diagnosis of PTSD or severe mental illness (as defined in section 23.4 Appendix 2)
- Pregnancy
- Prior brain injury (from trauma, stroke or other aetiologies) without full functional and symptomatic recovery
- Inability to comply with study schedule or follow-up*
- Inability to provide informed consent (e.g. due to cognitive impairment)
- Any progressive neurodegenerative or neuroinflammatory condition
- Alcohol use disorder or drug dependence
- Patients with medical conditions that are unstable or untreated

Note that some recruited participants may not be able to take part in the imaging aspects of the study since they may fail MRI suitability screening (e.g. metal/shrapnel from blast injury). This does not exclude them from the wider study, as they can complete all other assessments. However, all participants taking part in the imaging variability and case-control nested studies must pass the MRI screening to be eligible.

4.2. Nested study 1: Candidate biomarker variability study

mTBI cohort eligibility as per main study in Section 4.1. The inclusion and exclusion criteria for the healthy controls (variability) is as below:

4.2.1. Inclusion

- Age ≥18 years & ≤60 years

4.2.2. Exclusion

- Prior diagnosis of PTSD or severe mental illness (as defined by Section 23.4 Appendix 2)
- Pregnancy
- Prior brain injury (from trauma, stroke or other aetiologies) without full functional and symptomatic recovery
- Inability to comply with study schedule or follow-up
- Inability to provide informed consent (e.g. due to cognitive impairment)
- Inability to safely enter the MRI environment (for imaging variability and case-control study)
- Any progressive neurodegenerative or neuroinflammatory condition
- Cardiovascular or cerebrovascular disease or hypertension (no current diagnosis/medication)
- Alcohol use disorder or drug dependence
- Patients with medical conditions that are unstable or untreated
- History of pituitary hormone deficits

4.3. Nested study 2: Observational case-control prospective study

mTBI cohort as per main study in Section 4.1.
Healthy controls as per candidate biomarker variability study in Section 4.2.

4.4. Co-enrolment

Co-enrolment in other observational studies as well as in interventional trials is permitted.

5. IDENTIFICATION OF PARTICIPANTS

5.1. Identification of mTBI patients

Potentially eligible participants will be identified by their clinical care teams in secondary care NHS Trusts including emergency departments as well at MoD treatment facilities.

NHS and military research staff may assist clinicians with screening and use of electronic record searches to help identify patients according to local permissions as appropriate.

Once potential participants are identified according to the inclusion and exclusion criteria, they will initially be approached by a member of the clinical care team (including embedded researchers where appropriate) to see if they would be happy to discuss potential participation in research. If they agree to discuss the study, a member of the research team will then introduce it, and the informed consent process will take place using the online consent form in person or remotely by telephone or video call as appropriate as per section 6.

5.1.1. Participant Identification Centres

At some sites, participants will also be recruited via PICs, and referred to the main recruiting centre. For example, potentially eligible military participants may be identified in military primary care, and rehabilitation services.

5.1.2. Social media by way of patient forums, Facebook, Twitter, etc.

The study will be advertised for the purposes of recruitment using ethically approved material via websites and social media platforms related to head injury, sport and military personnel. Posters in clinical areas and advertisements on charity websites will also be developed for promoting and supporting the study for recruitment.

5.2. Identification of healthy controls

The study will be advertised for the purposes of recruiting healthy controls using ethically approved material via research facilities, websites and social media platforms related to head injury, sport and military personnel and contact details for the research team will be provided to make first contact regarding the study. Once contact is made, the informed consent process can take place as per section 6.

5.3. Screening

Details of all people approached about the study will be recorded on the mTBI-Predict Participant Screening Log, which will be kept in the Investigator Site File and should be available to be sent to the Study Office upon request.

5.4. Study entry points

5.4.1. mTBI patients

mTBI patients may enter the study at any time up until 3 months after their injury, and will have their first assessment at the next timepoint after their injury:

- Within 24 hours of the injury. After consenting, visit 1 procedures should be completed (section 7.5).
- 21 days (-7/+4 days) post-injury. After consenting, visit 3 should be booked.
- 3 months (± 7 days) post-injury After consenting, visit 8 should be booked.

5.4.2. Healthy controls for case-control study

Once healthy controls have given informed consent, a research team member will explain how to use the app and actigraphy equipment, which will be used at home prior to visit 2, and visit 3 should be booked.

5.4.3. Healthy controls for variability study

Once healthy variability controls have given informed consent, a first visit 3 or 4 should be booked.

6. CONSENT AND REGISTRATION

It is the responsibility of the Principal Investigator (PI) to obtain written informed consent for each participant prior to performing any study related procedures. This task can be delegated by the PI to other members of the local research team if local practice allows and this responsibility has been documented in the site delegation log.

Obtaining consent can be undertaken in-person or remotely and will use electronic online consent forms. Paper copies of the PIS and ICF will also be available from the Study Office and will be printed or photocopied onto the headed paper of the local NHS Trust.

If the patient is lacking capacity on initial approach, they will ordinarily be admitted for observation in hospital. For mTBI, capacity should be regained within 24 hours and the research nurse can re-approach the patient for consent.

A Participant Information Sheet (PIS) (appropriate to the study arm that they are being recruited to) will be provided to facilitate the consent process. The PI or delegate will explain the aim of the study, the study procedures, and the anticipated benefits and potential hazards of taking part. They will explain that participation is voluntary and that the participant is free to decide not to take part and may withdraw from the study at any time without this affecting their standard of care. The potential participant will be given sufficient time and opportunity to read the PIS, ask questions, and discuss their participation with others outside of the site research team if required.

If the potential participant then wishes to participate in the study, they will be asked to electronically sign and date the latest version of the Informed Consent Form (ICF). The PI or delegate will then electronically countersign and date the ICF.

All potential participants will be approached to also take part in the candidate biomarker variability study until the required sample size is reached; all potential participants will then be approached to also take part in the case-control study until the required sample size is reached.

A copy of the ICF will be emailed to the participant. Participants will consent to their email address being stored and used for this purpose (they will also consent to their email and/or mobile number being used for data collection app reminders). Should participants wish to do so, they can receive a printed copy of the ICF instead. A copy will be stored in the medical notes and a copy in the Investigator Site File. Once the participant is entered into the study, the participant's study number will be entered on the ICF maintained in the Investigator Site File.

In addition, the participant will understand and acknowledge that a copy of the signed ICF will be transferred electronically to the study team at BCTU for review and storage in the study database.

Details of the informed consent discussions will be recorded in the participant's medical notes. This will include date of discussion, the name of the trial, summary of discussion, version number of the PIS given to participant, version number of ICF signed and date consent received. Where consent is obtained on the same day that study related assessments start, a note should be made in the medical notes of what time consent was obtained and what time procedures started.

At each visit the participant's willingness to continue in the study will be ascertained and documented in the medical notes. Throughout the study the participant will have the opportunity to ask questions about the study. Any new information that may be relevant to the participant's continued participation will be provided. Where new information becomes available which may affect the participants' decision to continue, participants will be given time to consider and if happy to continue they will be re-consented. Re-consent will be documented in the medical notes. The participant's right to withdraw will remain.

Participants will be asked to give consent to allow members of the local research team to access their primary care medical records to gain a complete and detailed medical history at baseline.

Visits throughout the informed consent process and beyond will take place in person at the clinic or participant's home, or by telephone or video call as per local practice where patient and/or public health circumstances dictate. Where visits are in the participant's home, or by telephone or video call, due care will be paid to ensure the participant is in a suitably safe and confidential environment before proceeding.

6.1. Optional consent

Participants will be offered optional consent choices to allow linkage of their data available in NHS routine clinical datasets, including primary care data (e.g. Clinical Practice Research Datalink, The Health Improvement Network, QResearch) and secondary care data (Hospital Episode Statistics) through NHS Digital and other central UK NHS bodies. If participants agree, they will consent to the Study Office sending their name, date of birth and NHS number to the relevant national registry and then for the registry to link this to their data and send information back to the Study Office. The consent will also allow access to other new central UK NHS databases in the future. This will allow us (subject to receipt of additional funding via another grant application) to assess longer-term health and health service usage data without needing further contact with study participants.

6.2. Registration

After eligibility for registration has been confirmed and informed consent has been received, the participant can be registered to the study using the online system. Registration will be provided by BCTU using a secure online system (available at https://mtbi-predict.bctu.bham.ac.uk). Unique log-in usernames and passwords will be provided to those who wish to use the online system and who have been delegated the role of registering participants (both mTBI patients and healthy controls)

into the study as detailed on the mTBI-Predict Site Delegation Log. These unique log-in details must not be shared with other staff and in no circumstances should staff at sites access the system using another person's login details. The online system will be available 24 hours a day, 7 days a week, apart from short periods of scheduled maintenance.

6.3. Registration process

Registration Forms will be provided to investigators and may be used to collate the necessary information prior to registration. All questions and data items on the online Registration Form must be answered before a participant can be registered and a Study Number given.

Following registration, a confirmatory e-mail will be sent to the member of staff who registered the participant and local PI. A link to set up the study app will be sent to the participant (see section 7.5).

The local research team should add the participant to the mTBI-Predict Participant Recruitment and Identification Log which links participants with their Study Number. PIs must maintain this document securely and it must not be submitted to the Study Office. The mTBI-Predict Participant Recruitment and Identification Log should be held in strict confidence.

6.4. Informing the participant's GP and other parties

Participants' GP should be notified that they are in the mTBI-Predict study if they consent to this, using the mTBI-Predict GP Letter relevant to their cohort.

7. STUDY ASSESSMENTS

7.1. mTBI patients

mTBI patients enter the study within 24 hours of injury, ≤day 21, or ≤3 months, at either visit 1, visit 2 or visit 7 respectively. Patients will be approached consecutively until a total of 610 is reached.

mTBI patients enrolled within 24 hours of injury will enter the study at visit 1, with subsequent follow-up at day 21 (-7/+4 days) for visits 2, 3, 4, 5; at 2 months ±7 days (visit 6); and at 3 months ±7 days (visits 7, 8, 9, 10).

All mTBI patients will be followed up at 6 months (visits 11 and 12); at 12 months (visit 13); and at 24 months (visit 14) (all ±14 days).

All visits should be completed within the time window given here. All time points given are post-injury.

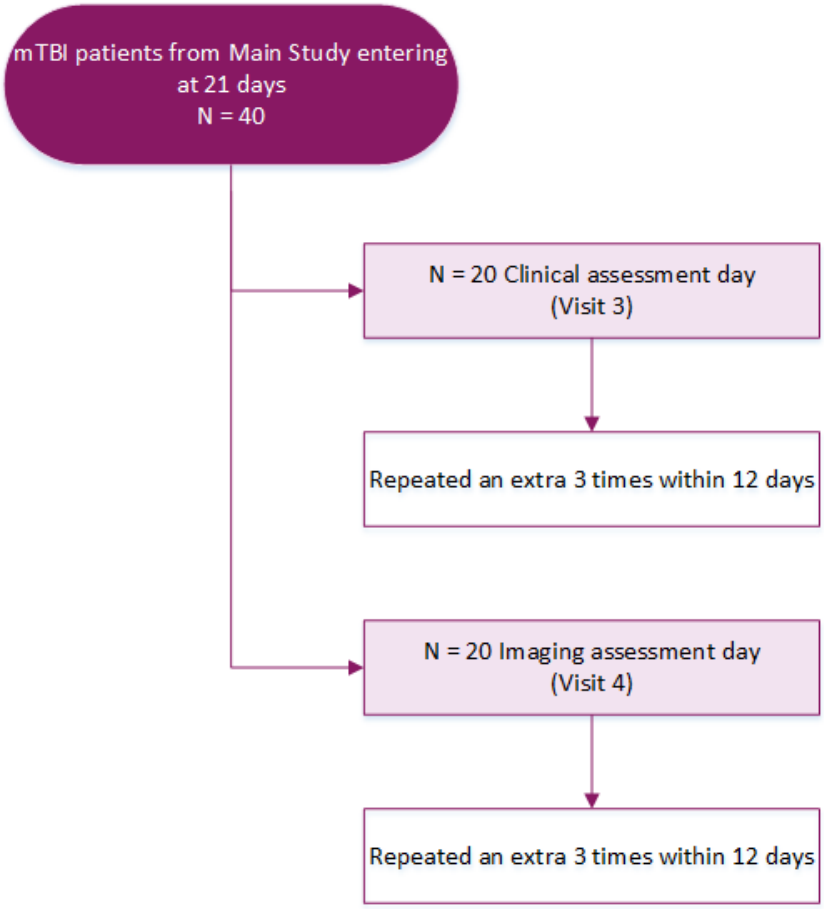
Note: Visit 5 is an additional visit of exploratory imaging and will be discontinued after 50 mTBI patients have completed both it and visit 10.

Visit 12 is an additional visit and will be discontinued after 100 mTBI patients have completed it.

For the variability study (**Figure 1**):

- 20 mTBI patients will repeat visit 3 (clinical) on an extra three occasions within 12 days (4 clinical visits in total).
- 20 further mTBI patients will repeat visit 4 (imaging) on an extra three occasions within 12 days (4 imaging visits in total).

Figure 1: Variability Study for the mTBI patients



7.2. Healthy controls (case-control)

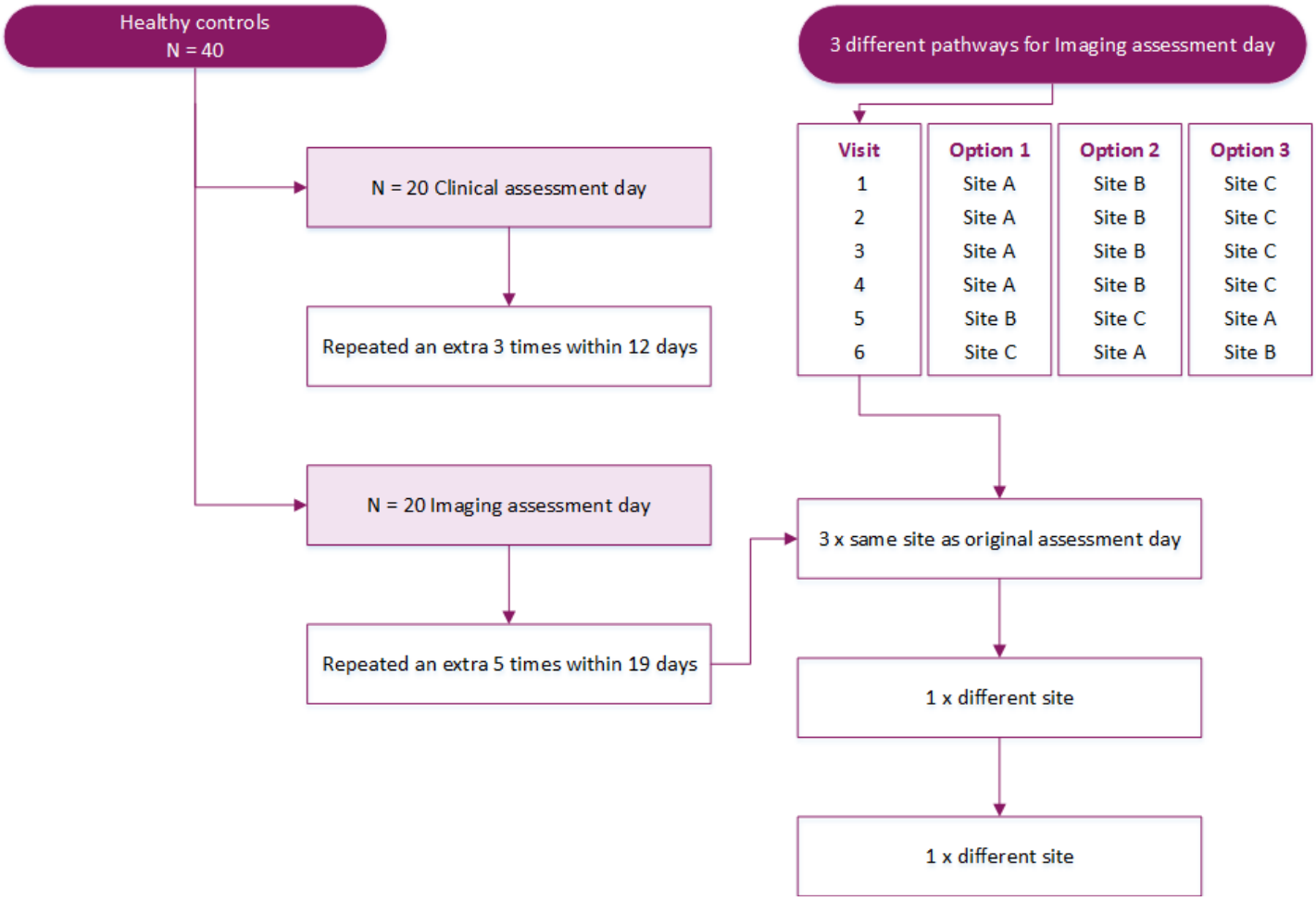
Healthy case-control participants are assessed as per visits 2, 3 and 4. These visits should be completed within a month of enrolment.

7.3. Healthy controls (variability)

For the variability study (**Figure 2**):

- 20 healthy controls will repeat assessments as per visit 3 (clinical) on an extra three occasions within 12 days (4 clinical visits in total)
- 20 healthy controls will repeat imaging assessments as per visit 4 (imaging) on an extra five occasions within 19 days (3 at the original imaging site, 1 at a second site and 1 at a third site; 6 imaging visits in total).

Figure 2: Variability Study for the Healthy Controls



7.4. Study visits

The summary visit schedule for the study is shown in **Table 4** below.

- 24-hour assessment is described in section 7.5
- 21-day assessments are described in section 7.6
- 2-month assessment is described in section 7.7
- 3-month assessments are described in section 7.8
- Follow-up assessments at 6, 12 and 24 months are described in sections 7.9, 7.10 and 7.11

Participants will be recruited to all relevant studies; once sufficient numbers have been recruited for each nested study (described above; see Section 14 for sample sizes) the nested study will be dropped from the protocol.

This will enable mTBI patients to be assessed in the main study (longitudinal prospective study), as well as mTBI patients and healthy controls in the case-control study and mTBI patients and healthy controls in the variability study.

Study visits are:

- 24-Hour Assessment: Visit 1 (mTBI patients identified within 24 hours of injury): this visit is carried out in person and consists of symptom questionnaires (headache, vestibular, concussion and mental health), blood, hair and saliva samples, training in use of the study

app and actigraphy equipment, remote saliva sample collection, and instruction about how to complete the physical function 6-minute walk test as part of the next visit (visit 2).

- 21-Day Assessments: Visits 2-5 (mTBI patients enrolled at 21 days, plus healthy controls for both the case-control and variability studies within a month of enrolment): these visits contain a remote assessment day (visit 2), a clinical assessment day (visit 3), an imaging assessment day (visit 4), and an exploratory imaging assessment day (visit 5). Visits 3-5 do not have to take place in this order.
- 20 mTBI patients and 20 healthy controls will repeat visit 3 on an extra three occasions within 12 days (4 clinical days in total).
- 20 mTBI patients will repeat visit 4 on an extra three occasions within 12 days (4 imaging days in total).
- 20 healthy controls will repeat visit 4 on an extra five occasions within 19 days (4 at original imaging site, 1 at second site, 1 at third site; 6 imaging days in total).
- Visits 5 and 10 will be discontinued after 50 mTBI patients and 50 healthy controls have completed both.

- 2-Month Assessment: Visit 6: all mTBI patients enrolled before or at 21 days will complete a remote assessment at month 2. This will be an abridged version of visit 2.

- 3-Month Assessments: Visits 7-10 (mTBI patients enrolled before or at 3 months) these visits contain a remote assessment day (visit 7), a clinical assessment day (visit 8), an imaging assessment day (visit 9) and an exploratory imaging assessment day (visit 10). Visits 8-10 do not have to take place in this order.
- Visits 5 and 10 will be discontinued after 50 mTBI patients and 50 healthy controls have completed both.
- 6-Month Assessment: Visits 11 & 12 (mTBI participant follow-up): these visits consist of a remote assessment day (visit 11) and an additional clinical assessment day (visit 12).
    - Visit 12 will be discontinued after 100 mTBI patients have completed them.
- 12-Month Assessments: Visit 13 (mTBI participant follow-up): this visit consists of a remote assessment day.
- 24-Month Assessment: Visit 14 (mTBI participant follow-up): this visit consists of a remote assessment day.

Table 4: Summary of mTBI-Predict study visits

Visit	1*	2*	3	4	5	6	7*	8	9	10
Time-point from injury	≤24 hours	Day 21 (assessment window: -7/+4 days)				Month 2 (±7 days)	Month 3 (±7 days)			
Location	Research facility / A&E	Remote	Research Facility	Imaging Centre	University of Nottingham	Remote	Remote	Research Facility	Imaging Centre	University of Nottingham
Eligibility and consent	x	(x)					(x)			
Confirm consent to continue		x	x	x	x	x	x	x	x	x
Primary care records check			x					x		
Clinical assessment day			x					x		
Imaging assessment day				x					x	
Remote assessments		x				x	x			
Daily headache /sleep diary, Actigraphy	Continuous from entry to month 3 visit									
Exploratory imaging					x					x

*Entry points are visits 1, 2, or 7.

Assessments in green are exploratory and will be discontinued after 50 mTBI patients and 50 healthy controls have completed them.

Assessments marked (x) will be done if not already carried out.

Table 4 (cont.): Summary of mTBI-Predict study visits

Visit	11	12	13	14
Time-point from injury	6 months (assessment window: ± 14 days)		12 months (± 14 days)	24 months (± 14 days)
Location	Remote	Research Facility	Remote	Remote
Confirm consent to continue	x	x	x	x
Clinical assessment day		x		
Remote assessments	x		x	x
Daily headache /sleep diary, Actigraphy	Continuous for 1 month prior to visit		Continuous for 1 month prior to visit	Continuous for 1 month prior to visit
Cognition mini-battery	Weekly for 1 month prior to visit		Weekly for 1 month prior to visit	Weekly for 1 month prior to visit

Visits 5 and 10 in green are exploratory and will be discontinued after 50 mTBI patients and 50 healthy controls have completed them.

Visit 12 in yellow is exploratory and will be discontinued after 100 mTBI patients have completed it.

7.5. 24-hour assessment

This visit will occur within 24 hours after injury for mTBI patients and will be conducted in person.

7.5.1. Visit 1

For mTBI patients, visit 1 will occur within 24 hours of injury at a secondary care facility or clinical research facility. Eligibility will be checked, the participant will be given initial information regarding the study, and consent will be taken to begin the study as per sections 5 and 6. Basic medical and demographic to enable enrolment into the study will be collected.

Participants will:

- Receive the study actigraphy device and training in how to use it
- Receive training in how to install and use the study app
- Receive training in collection of remote saliva samples
- Give:
    - Blood sample
    - Urine Sample
    - Hair sample
    - Saliva sample

Between visit 1 and visit 2, mTBI patients will complete remote assessments as below:

- App-based diary, questionnaires and cognitive test.
    - Daily headache and sleep diary
    - R-PSQ (Rivermead Post-Concussion Symptoms Questionnaire)
    - Cognitive mini-battery (8.1.6)
- Days 1-3 twice daily saliva samples
- Days 4-7 once daily saliva samples
- Then once weekly saliva samples until 3-month time-point
- Complete 6-minute walk test once before 21-day visit

Collection of the above saliva samples and completion of the 6-minute walk test will be prompted by the study app. Training and tubes/Royal Mail Safeboxes will be provided for the collection of saliva samples.

7.6. 21-day assessment

The study assessments at the 21-day timepoint will be split into days for remote assessments (visit 2), clinical assessments (visit 3), imaging assessments (visit 4), and additional exploratory imaging assessments (visit 5).

If this is their enrolment point into the study, participants will:

- Receive the study actigraphy device and training in how to use it
- Receive training in how to install and use the study app

7.6.1. Visit 2 - Remote assessments

Participants will complete a range of assessments using the study app. It is not required that all assessments are completed on the same day; the study app will remind the participants to complete assessments and allow them to manage and schedule their completion:

- Return to work/duty/play (yes/no)

- MPAI (Mayo-Portland Adaptability Inventory)
- R-PSQ (Rivermead Post-Concussion Symptoms Questionnaire)
- QoLiBri (Quality of life after brain injury questionnaire)
- Pain Catastrophizing Scale
- PVAQ (Pain Vigilance and Awareness Questionnaire)
- Perceived injustice questionnaire
- Locus of control questionnaire

Symptom diary: headache and sleep symptoms recorded daily for 28 days.

Actigraphy: Participants will be asked to wear the actigraphy device for 2 weeks. During these 2 weeks, participants will be asked to complete a brief set of cognitive tests (5 minutes) daily on weekdays, to monitor sleep and cognition. After 2 weeks participants will not be required to continue with daily cognitive tests but will be asked to continue wearing their actigraph until the 3-month assessments (or for 3 months, for healthy controls). Data will be downloaded by the study app.

Physical function testing: Participants will complete a 6-minute walk test. Upon completion, participants will record their perceived exertion and fatigue via the study app.

Mental Health:

- PCL-5 (PTSD Checklist for DSM-5) (Primary Measure)
- PHQ-9 (Patient Health Questionnaire depression scale)
- GAD-7 (Generalised Anxiety Disorder 7)
- SBQ-R (Suicidal Behaviour Questionnaire-Revised)
- AUDIT (Alcohol Use Disorders Identification Test)

Headache:

- HIT-6 (Headache impact test-6)

Vision:

- BIVSS (Brain Injury Visual Symptom Survey)

Vestibular:

- Balance Vigilance Questionnaire
- VVAS (Visual Vertigo Analogue Scale)
- DHI (Dizziness Handicap Inventory)
- ABC (Activities Balance Confidence)
- BSQ (Body Sensations Questionnaire)

Cognition:

- Participants will undertake the full cognitive battery as per section 8.1.5.

Sleep:

- PSQI (Pittsburgh Sleep Quality Index)
- rMEQ (Reduced Morningness-Eveningness Questionnaire)
- ESS (Epworth Sleepiness Scale)

- FSS (Fatigue Severity Scale)
- ISI (Insomnia Severity Index)
- BQ (Berlin Questionnaire)

Collection of the above assessments will be prompted by the study app.

7.6.2. Visit 3 - Clinical assessment day

Note: 20 mTBI patients and 20 healthy controls will repeat these assessments on three additional occasions in 12 days, as part of the variability nested study (only fluid/hormone biomarker samples, visual assessments, vestibular, cerebral physiology repeated).

Participants will attend a clinical research facility where the following data will be collected.

** these elements of the clinical assessment may be done remotely before the clinical assessment day*

Demographic details*

Physical Health*

Clinical Examination

Mental Health*

Headache*

Glasgow Outcome Scale – Extended (GOS-E)*

Fluid and hormone biomarker samples: Samples will be collected between 0900-1000 hours wherever possible; participants will be fasted from midnight.

- Blood samples
- Saliva samples
- Urine samples

Visual assessments:

To assess retinal and optic nerve structure:

- OCT – Heidelberg OCT, posterior pole, disc volume and RNFL, OCTA and optic nerve head and macula (20°x20°)

To assess visual function:

- Visual acuity – uniocular best corrected logMar
- Visual field assessment – HVF SITA Fast 24:2 (Humphrey Visual Field Swedish Interactive Thresholding Algorithm)
- Colour vision – Colour Assessment and Diagnosis
- Visual reaction and processing time – Eye Movements and Intrinsic Latency
- Contrast sensitivity – Pelli Robson Chart or Acuity Plus
- Pupillometry – NeuroOptics DP2000
- Accommodation – Autorefraction

Vestibular assessments:

- Vestibular history*
- Assessment of walking

- Dix-Hallpike manoeuvre to assess BPPV
- Assessment of eye movements
- Vestibular reflex and perceptual thresholds
- Posturography +EEG/EMG (electromyography)
- Pure tone audiogram
- Vestibular-evoke myogenic potentials and auditory-evoked potentials

Cerebral physiology:

Participants will be assessed using TMS, Doppler, fNIRS and EEG.

Note: Only participants entering the study at 21 days will complete full demographic details.

7.6.3. Visit 4 - Imaging assessment day

Note: 20 mTBI patients will repeat these assessments on three additional occasions in 12 days, as part of the variability nested study. Additionally, 20 healthy control participants will repeat the imaging assessment day (Visit 3) on five additional occasions in 19 days (3 at original imaging site, 1 at second site, 1 at third site; 6 imaging visits in total).

MEG protocol:

- Resting-state data
- Spatial attention task
- CRT task
- Implicit face processing task

MRI structural protocol:

- T1 anatomical
- T2 anatomical
- T2 FLAIR
- Diffusion weighted imaging
- Susceptibility weighted imaging

MRI functional protocol:

- Resting-state data:
- CRT task

MRI cerebral physiology:

The following measures will be made from macro and microvasculature:

- Baseline CBF
- CVR

7.6.4. Visit 5 – Exploratory imaging assessment day

Participants will undergo OPM-MEG and 7T MRI scans at the University of Nottingham:

- **OPM-MEG:** the same tasks referred to in section 7.6.3 for standard MEG, exploring the increased signal to noise and benefits of greater patient movement during data acquisition.
- **7T MRI:** scan sequences to be performed: multi-echo gradient echo from which SWI, QSM and T2* maps will be derived, MP2RAGE, 4D Phase contrast angiography (measuring blood

flow through vascular tree), Magnetisation Transfer sequence (investigating markers of neuroinflammation).

Visit 5 will be discontinued after 50 mTBI patients and 50 healthy controls have completed this visit.

7.7. 2-month assessment – Visit 6

The study assessment at 2 months (visit 6) will consist of remote assessments as per section 7.6.1.

7.8. 3-month assessment – Visits 7-10

If this is their enrolment point into the study, participants will:

- Receive the study actigraphy device and training in how to use it
- Receive training in how to install and use the study app

The study assessments at 3 months will be split into individual days for remote assessments (visit 7 – outlined in 7.6.1), clinical assessments (visit 8 – outlined in 7.6.2), imaging assessments (visit 9 – outlined in 7.6.3), and exploratory imaging (visit 10 – outlined in 7.6.4).

Note: Only those mTBI patients entering the study at 3 months will complete demographic details and physical health components of the clinical assessment visit (as detailed in 7.6.2).

Visit 10 will be discontinued after 50 mTBI patients and 50 healthy controls have completed the visit.

7.9. 6-month assessment – Visit 11 & 12

The study assessments at 6 months will be split into individual days for remote assessments (visit 11 – outlined in 7.6.1) and clinical assessments (visit 12 – outlined in 7.6.2). One month prior to the visit day, mTBI patients will be reminded to wear their actigraph for 2 weeks and asked to complete daily headache and sleep diaries for 28 days, as detailed in 7.6.1. Over this period, participants will also complete weekly mini-cognition battery assessments. The study app will send reminders to complete these assessments.

Note: Participants will not complete the medical history components of the clinical assessment visit (as detailed in 7.6.2).

Visit 12 will be discontinued after 100 mTBI patients have completed it.

7.10. 12-month assessment – Visits 13

The study assessment at 12 months (visit 13) will consist of remote assessments only as per section 7.6.1. One month prior to the visit day, participants will be asked to complete daily headache and sleep diaries for 28 days, as detailed in 7.6.1. Over this period, participants will also complete weekly mini-cognition battery assessments. The study app will send reminders to complete these assessments.

7.11. 24-month assessment – Visit 14

The study assessment at 24 months (visit 14) will consist of remote assessments only as per section 7.6.1. One month prior to the visit day, participants will be asked to complete daily headache and sleep diaries for 28 days, as detailed in 7.6.1. Over this period, participants will also complete weekly mini-cognition battery assessments. The study app will send reminders to complete these assessments.

7.12. Masking of test results

Results of exploratory tests undertaken specifically for the purposes of the study will not be made available to treating clinicians and participants whilst the study is ongoing and therefore will not influence patient management. Results of tests which have known clinical utility will be disclosed to treating clinicians in a timely manner.

Serving military personnel on the study staff will be blinded to results of tests that have potential to disclose use of drugs prohibited by Queen's Regulations by military personnel enrolled within the study. This stipulation is to prevent a requirement by such staff to report personnel enrolled in the study which would prejudice enrolment.

7.13. Interactions or contraindications

Routine care will continue as normal for all participants: there are no prohibited medications or interventions.

8. STUDY PROCEDURES

8.1. Remote assessments

Participants will complete assessments using a study app installed on a personal device:

8.1.1. Diary assessments

- **Symptom diary:** headache and sleep symptoms recorded daily. In the daily sleep diary (~1 minute to complete), participants indicate the times they went to sleep, woke up, how long it took them to fall asleep, and provide subjective ratings of their sleep quality. In the headache diary (~1 minute to complete), participants indicate presence of headache, severity, duration, features, aura and analgesic use.
- Health resource questionnaire

8.1.2. Actigraphy

- Participants will be given a Garmin device to wear daily at the timepoints specified in section 7. Data will be downloaded by the study app.

8.1.3. Physical function testing

- Participants will complete a 6-minute walk test during scheduled remote assessments at 21 days, 3 months, 6 months, 12 months and 24 months. Participants can choose a convenient location to complete this task (e.g. local park or street), but it should be the same place across all measured time points. The test will be synchronised with the Garmin device and recorded data completed within the app.

Outcome measures obtained from test are:

- Distance travelled
- Perceived exertion (CR10 scale: 1-10)
- Perceived fatigue (CR10 scale: 1-10).

8.1.4. Patient Reported Outcome Measures (PROMs)

The measures listed in 7.6.1 are completed as an app-based questionnaire, including global outcome, mental health, headache, vision, vestibular and sleep questionnaires.

8.1.5. Cognition

All participants will complete a familiarisation session with the full battery of tests with remote support before any formal testing. This will not be recorded as outcome data, but instead allows participants to become familiar with the task instructions and requirements.

Tests will be completed using a computerised cognitive battery on the Cognitron platform (https://www.cognitron.co.uk/). This includes a core set of tests optimised for assessing multiple dimensions of cognition that are likely to be affected in mild to moderate TBI patients, alongside several additional tasks capturing more subtle executive and reasoning problems (marked with *).

- **Immediate & delayed recall:** participants are presented with a sequence of objects to memorise at the start of the battery. They are then presented with an array of objects and must indicate which they have seen before, including foils that are semantically similar. Recognition memory is repeated at the end of the battery, capturing delayed recall.

  NB. Alongside assessing memory, this task allows us to flag poor effort (as occurs with functional cognitive disorder), as most participants will score quite high, though not at ceiling.

- **2D manipulations:** participants are presented with a 6x6 grid containing 6 coloured squares, and 4 possible responses that include the same pattern rotated in space. The task continues for 3 minutes, and the score reflects the number of correct responses.

- **Picture completion:** participants are presented with pictures of scenes on a 5x5 grid with a number of empty cells for them to complete by selecting the right cell from an array that includes rotated cells and distractors. There are 12 trials and scores reflect the total number of errors.

- **Simple Reaction Time:** participants see a target in an unpredictable location on the screen at an unpredictable offset and have to respond as quickly as possible by clicking in the centre of it. The task runs until 60 responses are submitted. Scores reflect the mean reaction time and distance from target, as well as motor accuracy and delay as a function of intertrial spatial distance.

- **Choice Reaction Time:** participants see an arrow in the centre of the screen pointing to the left or the right and have to respond as quickly as possible by clicking on the side of the screen that the arrow is pointing. The task runs until 60 responses are submitted. Scores reflect the mean reaction time for correct responses.

- **Card pairs:** this task includes a set of 12 face-up cards containing paired figures for the participants to memorise. After 7 seconds, the cards turn down and participants have to identify each pair. There are 5 trials and the score reflects the percentage of correct responses.

- **Tower of London*:** participants are presented with two sets of 3 pegs that have 3 coloured blocks on each. They have to calculate the fewest number of moves required for the balls in the first set to be moved into the position displayed by the second, moving only one at a time. There are 10 trials and the score reflects the correct number of responses.

- **Trail Making:** This task includes two blocks. In the first, they see numbers 1-26 on the screen and are asked to click on them in numerical order as fast as they can. In the second block, they see numbers 1-13 and letters A-M and they are asked to alternate between them in numerical and alphabetical order respectively (e.g., 1, A, 2, B, etc.). Scores represent reaction times for each task, as well as a measure of the additional load in the second block as compared to the first one.
- **Paired Associates:** working memory task where participants are presented with a sequence of objects in spatial locations that they must remember. They are then probed with the objects and must indicate the location that they were in. The number of object-location paired associates increases with each correct response until participants give 3 consecutive incorrect responses. The score reflects the maximum number of paired associates they can memorise.
- **Verbal analogies*:** verbal reasoning task where participants are presented with two statements regarding the relationships between pairs of words and must decide if the nature of the relationships are the same. The score reflects the number of correctly solved problems in two minutes.
- **Word definitions:** unusual words are displayed on the screen. The participant must select from amongst four definitions of the words based in part on their experience of the words, but also their knowledge of the relationship between the form and meaning of words. The score reflects the number of correctly identified word definitions.
- **Continuous attention task*:** A series of digits on noisy backgrounds appear briefly on the screen. The outcome measure is detection rate, and this is broken down by time segment, capturing loss of sustained attention to the continuous monitoring of the task. Every 40 seconds, a visual analogue scale captures information about fatigue and motivation, enabling changes in these to be analysed as participants progress through the task.

Interspersed with the tests are brief questions probing participants' perceptions on their performance. This allows metacognitive awareness to be assessed. Metacognitive awareness is relevant to TBI patients, a subset of whom lack insight into their impairments.

8.1.6. Cognitive mini-battery assessment

In addition, we will deploy a very brief (~5 minutes) battery to be used weekly during the first 2 weeks following the 21-day assessments, as well as in 1 month periods at 6, 12 and 24 months, alongside sleep recordings with a wearable device (actigraph) and a daily sleep diary. This mini-battery will consist of the immediate & delayed recall test, simple reaction time test, and continuous attention task (outlined in 8.1.5 above, tasks underlined).

8.2. Clinical procedures

8.2.1. Clinical history

Prior to a first clinical assessment visit, the following will be recorded during a structured interview which may take place remotely:

- **Demographic details:** Age, sex at birth, ethnicity, race, sexuality, socioeconomic deprivation (assessed by postcode), education (highest level reached), functioning (highest ever and last 12 months), civilian or military, litigation activities.

- **Physical Health:** Past medical history, history of TBI (including traumatic/non-traumatic, blast scoring, severity (GCS, VA/DoD), number of days hospitalised, post-traumatic amnesia), menstrual history (if appropriate).
- **Mental Health:** Past psychiatric history, Medication history (current and past), Trauma type (assault vs. non-assault), PCL-5 (if >50, complete MINI Diagnostic Interview for ICD-10), Social and Occupational Functioning Assessment Scale (SOFAS), Alcohol/substance use
- **Headache:** Headache history obtained by structured clinical interview. Headache phenotype (according to criteria from the International Headache Society) will be assessed.
- **Glasgow Outcome Scale –** Extended (GOS-E)*: A questionnaire which categorises the outcomes of patients after TBI.
- **Vestibular:** Structured history to include symptoms scales of dizziness, vertigo and imbalance.

8.2.2. Clinical examination

The following will be recorded during clinical examination at the clinical assessment visit:

- **Height:** will be measured to the nearest 0.1 cm with a rigid stadiometer.
- **Body mass**: will be measured in light indoor clothing to the nearest 0.1 kg.
- **Waist circumference**: will be recorded to the nearest 0.1 cm at the mid-point between the lower costal margin and the level of the anterior superior iliac crest.
- **Hip circumference**: will be recorded to the nearest 0.1cm, from the widest point of the hips and the maximum protrusion of the gluteal muscles.
- **Brachial blood pressure:** will be measured as recommended by the British Hypertension Society (http://www.bhsoc.org/how_to_measure_blood_pressure.stm) three times in the sitting position using standardised Welsh Allyn or Dinamap blood pressure monitors. The average of the second and third blood pressure readings will be recorded.

8.2.3. Biofluid sampling

Samples will be collected between 0900-1000 hours wherever possible; participants will attend having fasted from midnight. Participants will give samples consisting of:

- **Blood samples:** collected via peripheral vein as per normal clinical practice. 30ml total will be collected; 6ml in an EDTA tube for plasma and 6ml in a serum separator tube for serum for the local NHS pathology lab to analyse as per local practice, and 12ml in 2 EDTA tubes for plasma and 6ml in a serum separator tube for serum to be processed before being stored for future analysis at UoB.
- **Saliva samples:** as per normal clinical practice.
- **Hair samples:** as per normal clinical practice from crown of head if possible.
- **Urine samples:** urine sample as per normal clinical practice.

8.2.3.1. Laboratory procedures

Full details of sample processing will be described in a separate study laboratory manual. It will be the responsibility of the local PI to maintain a sample log, recording samples collected, stored, and sent. All samples will be collected and then either analysed locally where necessary or processed and then sent to the University of Birmingham (UoB) for storage and analysis.

8.3. Visual assessments

Visual assessment acquisition will be specified in the visual assessment study manual.

To assess retinal and optic nerve structure:

- **OCT:** Heidelberg OCT will be performed including posterior pole, disc volume and RNFL scans and OCTA will be performed including optic nerve head and macula (20°x20°) scans.

To assess visual function:

- **Visual acuity:** uniocular best corrected log of the minimum angle of resolution (logMar), will be recorded using a chart or Acuity Plus.
- **Visual field assessment:** automated perimetry (Humphrey 24-2 SITA fast central threshold) will be performed to measure visual field sensitivity.
- **Colour vision:** Colour Assessment and Diagnosis.
- **Visual reaction and processing time:** Eye Movements and Intrinsic Latency.
- **Contrast sensitivity:** Pelli Robson chart or Acuity Plus.
- **Pupilometry:** NeuroOptics DP2000.
- **Accommodation:** Autorefraction

8.4. Vestibular assessments

Vestibular assessment acquisition will be specified in the vestibular assessment study manual.

- **Hallpike manoeuvre** for BPPV including assessing for nystagmus
- **Assessment of walking:** Romberg test with eyes closed over 20 seconds, tandem walking errors over 10 steps, tandem standing with eyes open over 20 seconds and eyes closed over 20 seconds.
- **Posturography +EEG/EMG (sway test):** Measurements of balance will be recorded whilst the participant is on a platform with varying conditions.
- **Rotating chair (stopping response):** Assessment of vestibular reflex and perceptual thresholds**.** Eye-tracking goggles will be worn to record eye movements whilst the participant is moved on a rotational chair.
- **Assessment of eye movements:** Eye-tracking goggles will be worn to record eye movements whilst the participant adopts different positions provoking changes in eye movements, alongside assessment of eye tracking during smooth pursuit, saccades and anti-saccades.
- **Acoustic reflex amplitude and latency, hearing level (dB):** Required to screen primarily for conductive hearing loss.
- **Vestibular-evoked myogenic potentials and auditory-evoked potentials:** To assess otolithic pathways. Visual vertical assessment (computer-based) as additional assessment of otolithic function.

8.5. Cerebral physiology assessments

Cerebral physiology assessment acquisition will be specified in the cerebral physiology assessment study manual.

- **TMS task:** We will measure the cortical silent period from the right first dorsal interosseous (FDI), participants will maintain tonic muscle activation of the right FDI at 10% maximal voluntary contraction for 5 s per trial, while TMS is delivered to the left M1 at the intensity that evoked a 1 mV MEP in right FDI at rest for 15 trials.
- **Cerebrovascular responsiveness testing** TCD: Blood velocity through the middle (MCAv) and posterior (PCAv) cerebral artery will be measured, as a proxy of blood flow, using TCD ultrasound. 2MHz TCD probes will be used to insonate these arteries via the temporal window by a trained ultrasound sonographer and fixed in place for the duration of the battery using an adjustable headset. These measures will be collected continuously throughout the battery to track changes across all rest periods and tasks.
- **fNIRS+EEG:** Cortical haemodynamics and the underlying neural activity will be assessed simultaneously during a cognitive challenge task using a commercially available combined fNIRS and EEG system to identify detectable multivariate features. In addition, a resting state EEG assessment will be collected via the same set up to assess if any changes in the ongoing oscillatory activity. In particular, we will be focusing on EEG slowing, which manifests as an increase in delta and theta frequency power and decrease in alpha frequency power.
- **NVC:** Neurovascular function within specific regions of the frontal cortex in response to Attention Network and choice reaction time tasks will be assessed using the combined fNIRS and EEG system.
- **CVR:** CVR is assessed by the continuous measurement and calculation of MCAv/PCAv differences in response to changes in circulating carbon dioxide, induced through the inhalation of a gas mixture containing elevated CO_2 levels and via hyperventilation of ambient room air. In addition to assessment of CVR via TCD, shear-dependent vasodilatory responses of the internal carotid artery (ICA) will be assessed by Duplex ultrasound alongside the more established measures of CVR outlined above. The protocol for exposure to CO_2 will consist of 4-minutes of continuous inhalation of a mixture containing 5% CO_2 in air, as per the guidelines [51, 52] to ensure a steady-state response in MCAv/PCAv and ICA vasodilatory responses. Following the 4-min CO_2 inhalation procedure, participants will complete guided mild hyperventilation for 2 minutes to reduce end-tidal CO_2 by 10 mmHg. The 4-min CO_2 inhalation part of the protocol will also be part of the cerebral physiology MRI session (see below).
- **CA:** This task will assess CA via the relationship between changes in MCAv/PCAv induced by transient alterations in beat-by-beat blood pressure. CA will be assessed by transfer function analysis of MCAv/PCAv over repeated squat-stand cycles at two different frequencies (0.05 Hz and 0.10 Hz), which have been shown to provide reliable and robust measures of coherence (correlation index), phase (timing buffer) and gain (amplitude buffer) [54].
- **Exercise task:** In this task, participants will complete 3 maximal voluntary contractions, followed by a 5-minute maximal endurance rhythmic hand grip exercise task (contraction every second, with the aim to generate maximal cumulative force over the 5-minute period) using a hand grip dynamometer [194]. Throughout the task, TCD and fNIRS measures will be

obtained to determine changes in cerebral haemodynamics, alongside ratings of perceived physical exertion and fatigue [194].

8.6. Imaging assessments

Participants will undergo MEG, MRI and fMRI scans.

- **MEG:** Participants will undergo a MEG scan and undertake the following tasks:

  Resting-state data: This task allows for identifying the resting-state networks. In the fMRI data this will be reflected in the correlation structure of the BOLD signal in different regions. In the MEG data the network dynamics will be reflected in how oscillatory activity interacts between different regions in the delta, alpha and beta band.

  Spatial attention task: This task probes the neuronal activity associated with the allocation of spatial attention. The allocation of attention will be reflected in the modulation of alpha and gamma activity in posterior regions and their interaction with the dorsal attention network exercising the top-down control. The BOLD-fMRI signal will likewise be modulated in posterior visual regions as well as in the dorsal attention network.

  CRT task: This is a simple sustained attention task that taps into processing speed deficits. In fMRI, the CRT captures the engagement of bilateral sensory, motor, and superior parietal regions, as well as the thalami (amongst others).

  Implicit face processing task: Participants will complete an implicit face processing task in which emotional stimuli are comprised of happy, angry, neutral faces.

- **MRI:** Participants will undergo 3T MRI scans, including structural (T1, FLAIR, DTI) and functional MRI (resting state, CRT task as described above)
- **MRI physiology:** This scan will include baseline perfusion and cerebrovascular reactivity measures. These will be used to provide complementary measures of CBF and CVR to those which are acquired with Doppler. These measures will interrogate the macro- and micro-vasculature with regional specificity using arterial spin labelling, Phase contrast angiograph (PCA) and BOLD-CVR.

8.6.1. Exploratory Imaging assessments

Participants will undergo OPM-MEG and 7T MRI scans at the University of Nottingham:

- **OPM-MEG:** same tasks as above for standard MEG, exploring the potential for increased signal to noise and reduced movement artefacts during data acquisition using OPM-MEG.
- **7T MRI:** scan sequences to be performed: multi-echo gradient echo from which SWI, QSM and T2* maps will be derived, T1 weighted anatomical, T2 weighted anatomical, Angiography, 4D Phase contrast angiography (measures blood flow through vascular tree), and Magnetisation Transfer sequence (investigate markers of neuroinflammation).

8.7. Withdrawal and changes in levels of participation

Informed consent is defined as the process of learning the key facts about a clinical study before deciding whether or not to participate. It is a continuous and dynamic process and participants should be asked about their ongoing willingness to continue participation at all visits. Participants should be aware from the beginning that they can freely withdraw (cease to participate) from the study at any time. A participant may wish to cease to participate in a *particular* aspect of the study.

Participants found to be ineligible post-registration should be followed up according to all study processes and will still have their data analysed unless they explicitly change their level of participation.

The changes in levels of participation within the study are categorised in the following ways:

No in-person assessment: The participant would no longer like to attend in person follow-up but is willing to be followed up remotely in accordance with the schedule of assessments and if applicable using any central UK NHS bodies for long-term outcomes (i.e., the participant has agreed that data can be collected and used in the study analysis).

No study related follow-up: The participant does not wish to take further part in study-specific follow-up but is willing to be followed up at standard clinic visits and if applicable using any central UK NHS bodies for long-term outcomes (i.e., the participant has agreed that data can be collected at standard clinic visits and used in the study analysis, including data collected as part of long-term outcomes).

No further data collection: The participant is not willing to be followed up in any way for the purposes of the study AND does not wish for any further data to be collected (i.e. only data collected prior to any changes of levels in participation can be used in the study analysis).

The details of changes of levels in participation within the study (date, reason and category of status change) should be clearly documented in the source documents and will be recorded on the study database using a Change of Status form.

9. OUTCOME MEASURES

9.1. Main study outcomes

- Primary outcome: Ability of candidate biomarkers to predict full return to play, work or duty at 6 months post-injury.
- Secondary outcomes: Ability of candidate biomarkers to predict global function, persistent PTH, cognitive dysfunction, depression, PTSD, vestibular disturbances and physical function at 6 months post-injury and beyond.
- Exploratory outcomes: Accuracy of a multifaceted computer modelled biomarker algorithm to predict sequelae of mTBI (full return to play, work or duty, persistent PTH, cognitive dysfunction, depression, PTSD, vestibular disturbances, and physical function).

9.2. Variability study outcomes

The primary outcome is the variability of candidate biomarkers for each workstream.

The secondary outcome is the variability of candidate brain imaging biomarkers between imaging centres (University of Birmingham, Aston University and University of Nottingham). For reasons of capacity as well as real world applicability, scanning in the main study will be split across the different imaging centres.

9.3. Case-control outcomes

The primary outcome is identification of novel candidate biomarkers.

The exploratory outcome is to gain mechanistic insights into the candidate biomarkers.

10. ADVERSE EVENT REPORTING

There is no reason to anticipate any safety concerns arising as a result of any of these diagnostic tests and as such monitoring of adverse events (AEs) to assess the study's safety is not required. Any withdrawals from the study due to intolerability of any of the tests will be regularly reviewed by the Study Management Group (SMG) and the Study Steering Committee (SSC).

11. DATA HANDLING AND RECORD KEEPING

11.1. Source data

Source data is defined as all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical study necessary for the reconstruction and evaluation of the study. To allow for the accurate reconstruction of the study and clinical management of participants, source data will be accessible and maintained.

Some data variables may be entered directly onto the eCRF, these are clearly identified and detailed in **Table 5**: Source data in

Source data is kept as part of the participants' medical notes generated and maintained at site. In addition, for this study, brain imaging (MRI and MEG) is performed; the source data will be the original output files directly uploaded to the UoB Research Data Store. Similarly, vestibular and physiology workstreams will include the collection of continuously measured electronic data which will be uploaded and stored within the UoB Research Data Store.

Table 5: Source data in mTBI-Predict

Data	**Source**
Participant Reported Outcomes	The original participant eCRF completed via the app and imported directly into the study database is the source.
Lab results	The original lab report (which may be electronic) is the source and will be kept and maintained in line with normal local practice. Information will be transcribed onto the eCRF.
Imaging	The source is the original pseudonymised electronic imaging output file uploaded to the Study Office (via UoB Research Data Store). When data is then interpreted, the eCRF onto which it is transcribed becomes the source.
Vestibular & Cerebral Physiology	The source is continuously collected raw data files acquired through either Labchart, Spike or native software, uploaded to the Study Office (via UoB Research Data Store). These data include outputs from TCD, Duplex Doppler, NIRS, EEG, EMG, eye movement monitoring and movement/postural data from rotating chair and posturography. Processed and analysed data will be imported into the eCRF in CSV format.

Clinical event data	The original clinical annotation is the source document. This may be found on clinical correspondence, or electronic or paper participant records. Clinical events reported by the participant, either in or out of clinic (e.g., phone calls), must be documented in the source documents.
OCT data	The source is the original electronic (CSV format) output file. A copy of this data will be uploaded to the Study Office (via UoB Research Data Store).
Recruitment	The original record of registration is the source. It is held on BCTU servers as part of registration and data entry system.
Withdrawal	Where a participant expresses a wish to withdraw, the conversation must be recorded in the source documents.

11.2. Case Report Form completion

An electronic Case Report Form (CRF) system will be used for mTBI-Predict. The CRFs will include (but will NOT be limited to) the following Forms (see

Table 6: Case report forms in mTBI-Predict).

Table 6: Case report forms in mTBI-Predict

Form Name	**Schedule for submission**
Consent and Registration CRF	At the point of registration
Baseline CRFs	At the point of registration
Follow-up CRFs	Following follow-up visit
Participant completed outcome measures	At the time of completion
Brain imaging, cerebral physiology, hormone/steroid biomarker results CRFs	After completion of raw data analysis
Change of status CRF	After the point of reduced participation or death is discovered by the site research team

Data should be submitted within four weeks of their submission schedule according to table 6.

In all cases it remains the responsibility of the PI to ensure that the CRF has been completed correctly and that the data are accurate. This will be evidenced by the signature of the PI or delegate. The Site Delegation Log will identify all those personnel with responsibilities for data collection.

The delegated staff completing the CRF should ensure the accuracy, completeness and timeliness of the data reported. This will be evidenced by signing and dating the CRF.

Data reported on each CRF will be consistent with the source data and any discrepancies will be explained. All missing and ambiguous data will be queried. Staff delegated to complete CRFs will be trained to adhere to the mTBI-Predict working instructions.

The following guidance applies to data and partial data:

- Only CRFs provided by the Study Office should be used.
- Time format – all times should be in accordance with the 24-hour clock
- Rounding conventions – rounding should be to the nearest whole number: If the number you are rounding is followed by 5, 6, 7, 8, or 9, round the number up. **Example**: 3.8 rounded to the nearest whole number is 4. If the number you are rounding is followed by 1, 2, 3 or 4, round the number down. **Example**: 3.4 rounded to the nearest whole number is 3
- Study-specific interpretation of data fields – where guidance is needed additional information will be supplied
- Entry requirements for concomitant medications (generic or brand names) – generic names should be used where possible
- Missing/incomplete data – should be clearly indicated in notes fields – all blank fields will be queried by the Study Office
- Protocol and GCP non-compliances should be reported to the Study Office on discovery.

11.3. Participant reported outcomes

Participant reported outcomes will be completed online via a bespoke app and data periodically imported automatically into the study database. This is a downloaded standalone app which can run on almost all smart phone or tablet devices. It will target assessments to participants via push notifications with text or email (dependent on participant preference) to be used as a backup reminder system if not completed.

11.4. Data management

Processes will be employed to facilitate the accuracy and completeness of the data included in the final report. These processes will be detailed in the study specific Data Management Plan and include the processes of data entry and data queries.

Data entry will be completed by sites via a bespoke Laravel BCTU study database. The data capture system will conduct automatic range checks for specific data values to ensure high levels of data quality. Queries will be raised using data clarification forms (DCFs) via the study database, with the expectation that these queries will be completed by the site within 30 days of receipt. Overdue data entry and data queries will be requested on a monthly basis.

11.5. Self-evident corrections

No self-evident corrections will be permitted.

11.6. Data security

11.6.1. BCTU

UoB has policies in place, which are designed to protect the security, accuracy, integrity and confidentiality of Personal Data. The study will be registered with the Data Protection Officer at UoB and will hold data in accordance with the Data Protection Act (2018 and subsequent amendments).

The Study Office has arrangements in place for the secure storage and processing of the study data which comply with UoB policies.

The Study Database System incorporates the following security countermeasures:

Physical security measures: restricted access to the building, supervised onsite repairs and storages of back-up tapes/disks are stored in a fire-proof safe.

Logical measures for access control and privilege management: including restricted accessibility, access-controlled servers, separate controls of non-identifiable data.

Network security measures: including site firewalls, antivirus software and separate secure network protected hosting.

System management: the system will be developed by the Programming Team at the Study Office and will be implemented and maintained by the Programming Team.

System design: the system will comprise of a database and a data entry application with firewalls, restricted access, encryption and role-based security controls.

Operational processes: the data will be processed and stored within BCTU.

System audit: The system will benefit from the following internal/external audit arrangements:

- Internal audit of the system
- Periodic IT risk assessment

Data Protection Registration: UoB's Data Protection Registration number is Z6195856.

11.6.2. BEAR Research Data Store

The working data for all workstreams needing extensive and/or collaborative analysis (e.g. brain imaging) will be stored on UoB storage (BEAR Research Data Store), accessed via secured network shares mounted on the computers of the relevant workstreams leads and researchers, who have been given specific access to the data. Backups are made overnight from the Research Data Store and any files that are created or changed that day will be backed up. Backups are also copied to a second location for disaster recovery purposes. Access to the data is restricted to those who have been granted access to the project by the Trial Office and they must have a UoB username and password, which can be provided for collaborating researchers. Data can only be accessed off campus through use of the 2-factor authentication remote access service and a log of all activities will be monitored.

11.6.3. Cognitron

H2 Cognitive Designs will design and maintain the study app. The data collected using the app will be processed and stored in a database hosted within Amazon Web Services within the EU-west-2 region (London). Data within this database are accessible to the research team only, under network and firewall control via 2-factor authentication and encrypted at rest. It will only be connected to participants via trial number; it will collect no other identifiers. H2 Cognitive Designs make daily and weekly backups of this database for assurance and fault recovery. This backup is stored securely under 2-factor authentication within the Amazon Web Services EU-west-1/2 region (Dublin / London). Systems are hardened to at least or exceeding the specifications outlined in NIST-800-700/123 and UK Gov MCSS – Cyber Essentials. Data and information security procedures are developed to the specification required of ISO27001. App data will be imported into the study database regularly and after the last participant assessment is completed all final data will be imported into the study database and the app will be discontinued; participants will be instructed to delete the app once their follow-up is complete.

11.7. Archiving

It is the responsibility of the PI to ensure all essential study documentation and source documents (e.g., signed ICFs, Investigator Site Files, participants' hospital notes) at their site are securely retained for at least 10 years. Archiving will be authorised by BCTU on behalf of UoB following submission of the end of study report. No documents should be destroyed without prior approval from the BCTU Director or their delegate.

The Study Master File will be stored at BCTU for at least 3 years after the end of the study. Long-term offsite data archiving facilities will be considered for storage after this time; data will be stored securely and confidentially for at least 10 years. BCTU has standard processes for both hard copy and computer database legacy archiving.

Once analysis is complete, data which is stored on the BEAR RDS will be archived in the secure UoB BEAR Archive in accordance with UoB Standard Operating Procedures. It will be archived for at least 10 years and will only be accessible upon an application for restoration by an appropriate person such as the Chief Investigator (CI), relevant workstreams lead or Study Office.

12. QUALITY CONTROL AND QUALITY ASSURANCE

12.1. Site set-up and initiation

All PIs will be asked to sign the necessary agreements including protocol, clinical trial site agreement and site delegation log, and supply a current signed CV and GCP certificate. All members of the site research team are required to sign the Site delegation log, which details which tasks have been delegated to them by the PI. The Site delegation log should be kept up to date by the PI. It is the PI's responsibility to inform the Study Office of any changes in the site research team.

Prior to commencing recruitment, each recruiting site will undergo a process of site initiation, either face to face or remotely, at which key members of the site research team are required to attend, covering aspects of the study design, protocol procedures, collection and reporting of data and record keeping. Sites will be provided with an Investigator Site File containing essential documentation, instructions, and other documentation required for the conduct of the study.

12.2. Monitoring

The central and on-site monitoring requirements for this study have been developed in conjunction with the study specific risk assessment and are documented in the study specific monitoring plan.

12.3. On-site monitoring

For this study, all sites will be monitored in accordance with the study risk assessment and monitoring plan. Any monitoring activities will be reported to the Study Office and any issues noted will be followed up to resolution. Additional on-site monitoring visits may be triggered. PIs and site research teams will allow the mTBI-Predict study staff access to source documents as requested. The monitoring will be conducted by BCTU/UoB staff.

12.4. Central monitoring

The Study Office will check received ICFs and CRFs for compliance with the protocol, data consistency, missing data and timing at a frequency and intensity determined by the Data

Management Plan. Sites will be sent DCFs requesting missing data or clarification of inconsistencies or discrepancies.

12.5. Audit and inspection

The Investigator will permit study-related monitoring, audits, ethical review, and regulatory inspection(s) at their site and provide direct access to source data/documents. The investigator will comply with these visits and any required follow-up. Sites are also requested to notify the Study Office of any relevant inspections or local audits.

12.6. Notification of Serious Breaches

The sponsor is responsible for notifying the REC of any serious breach of the conditions and principles of GCP in connection with that study or of the protocol relating to that study. Sites are therefore requested to notify the Study Office of any suspected study-related serious breach of GCP and/or the study protocol as soon as they become aware of them. Where the Study Office is investigating whether or not a serious breach has occurred, sites are also requested to co-operate with the Study Office in providing sufficient information to report the breach to the REC where required and in undertaking any corrective and/or preventive action.

Sites may be suspended from further recruitment in the event of serious and persistent non-compliance with the protocol and/or GCP, and/or poor recruitment.

13. END OF STUDY DEFINITION

The end of study will be 90 days after the date of the last data capture including resolution of biomarker analyses and data clarifications. This will allow sufficient time for the completion of protocol procedures, data collection and input, and data cleaning. The Study Office will notify the REC and the Sponsor within 90 days of the end of study. Where the study has terminated early, the Study Office will notify the REC within 15 days of the end of study. The Study Office will provide the REC and the Sponsor with a summary of the clinical study report within 12 months of the end of study.

14. STATISTICAL CONSIDERATIONS

14.1. Variability studies

We will recruit 20 healthy controls and 20 mTBI patients to both the clinical and imaging assessments days of the study at the 21-day visit (in total 40 healthy controls and 40 mTBI patients). Each participant will then undergo an additional three (or five in the case of imaging for healthy controls) measurements of each biomarker over a 12-day (or 19-day for imaging) period. For some biomarkers (e.g. fluid biomarkers) variability will be assessed by analysis of samples in quadruplicate.

For each of the biomarkers under investigation (see Appendix 1), we will estimate the variability at the within-individual and the between-individual level. For example, for the 20 healthy controls or mTBI patients, if the analytical standard deviation was 0.5, the within-individual standard deviation was 1.0 and the between-individual standard deviation was 2.3, approximate confidence intervals for these estimates would be (0.45, 0.56), (0.79, 1.19) and (1.56, 3.05), respectively. These values would yield an ICC of approximately 80% with a 95% confidence interval ranging from 65% to 89%.

All confidence interval estimates have been calculated using simulation. We will not reassess at the 3-month time point as we will assume that variability at 21 days will be greater than at 3 months.

We will use multilevel models to estimate the variability at each level, with log transformation of the biomarker outcomes if necessary, for the healthy and mTBI patients combined and separately. We will express the results from this stage of the study as standard deviations, coefficients of variation, ICCs, reference change values and other relevant variability estimates along with 95% confidence intervals as appropriate. Within the imaging variability study, we will also investigate the variability between centres (University of Birmingham, Aston University, and University of Nottingham) using the same method. For this purpose, the 20 healthy participants will have three additional measurements at the centre used at their 21-day visit, alongside additional imaging visits at the two alternative imaging centres (five total variability visits).

14.2. Case-control study

The case-control study seeks to estimate the difference in biomarker values between mTBI patients and healthy controls. We aim to recruit 100 healthy controls and 100 mTBI patients for this nested study. For example, with this sample size we would have more than 90% power to detect a difference for a biomarker with a mean value of 2 for the controls and a mean value of 3.5 for the mTBI patients (mean difference is 1.5), assuming a standard deviation of 3 units. Alternatively, with a binary outcome, such as headache presence, we would again have more than 90% power with the planned number of individuals if the percentage with headache in the healthy participants was 3% and the odds ratio comparing mTBI and healthy was 4.

We will estimate the difference between the healthy and mTBI patients for each of the biomarkers using linear regression for the continuous biomarkers, with transformation of the biomarker values if appropriate, and logistic regression for the binary outcomes. The regression models used for these analyses will be adjusted for any known clinical confounders as appropriate.

14.3. Reduction of candidate biomarkers

To move to the next stage of evaluation, where a prognostic model will be produced, it will be necessary to reduce the number of candidate biomarkers. To do this we aim to use a modified Delphi approach, where we will survey all clinical study co-applicants in addition to clinical experts independent of the study. In the first round of the Delphi process we will ask for judgements on the prognostic capability of the investigated biomarkers, and we will progress through rounds until we reach consensus. Delphi participants will be provided with information from the variability and case-control studies to inform their decisions as necessary.

14.4. Development of a prognostic model

We aim to develop multivariable statistical models that can predict sequelae of mTBI using the investigated biomarkers. The sequelae of mTBI being examined are headache, vestibular dysfunction, mental health (depression, PTSD), cognition, global function and full return to work, play or duty. These clinical outcomes are measured via mean monthly headache days, vestibular disturbance questionnaire, self-reported PHQ-9, self-reported PCL-5, corrected global composite score, and MPAI, respectively. Global composite score and MPAI are continuous outcomes; monthly headache days is a count outcome; and clinical thresholds to PHQ-9 and PCL5 will categorise patients into those with and without depression and PTSD respectively (binary outcomes). Each of these outcomes will be measured at 6-, 12- and 24-months post-injury.

Whilst absolute values of biomarkers will be valuable all biomarkers are measured on multiple occasions, as such characteristics such as the rate of change of biomarkers and time until normalisation will also be considered as potentially important data in their own right.

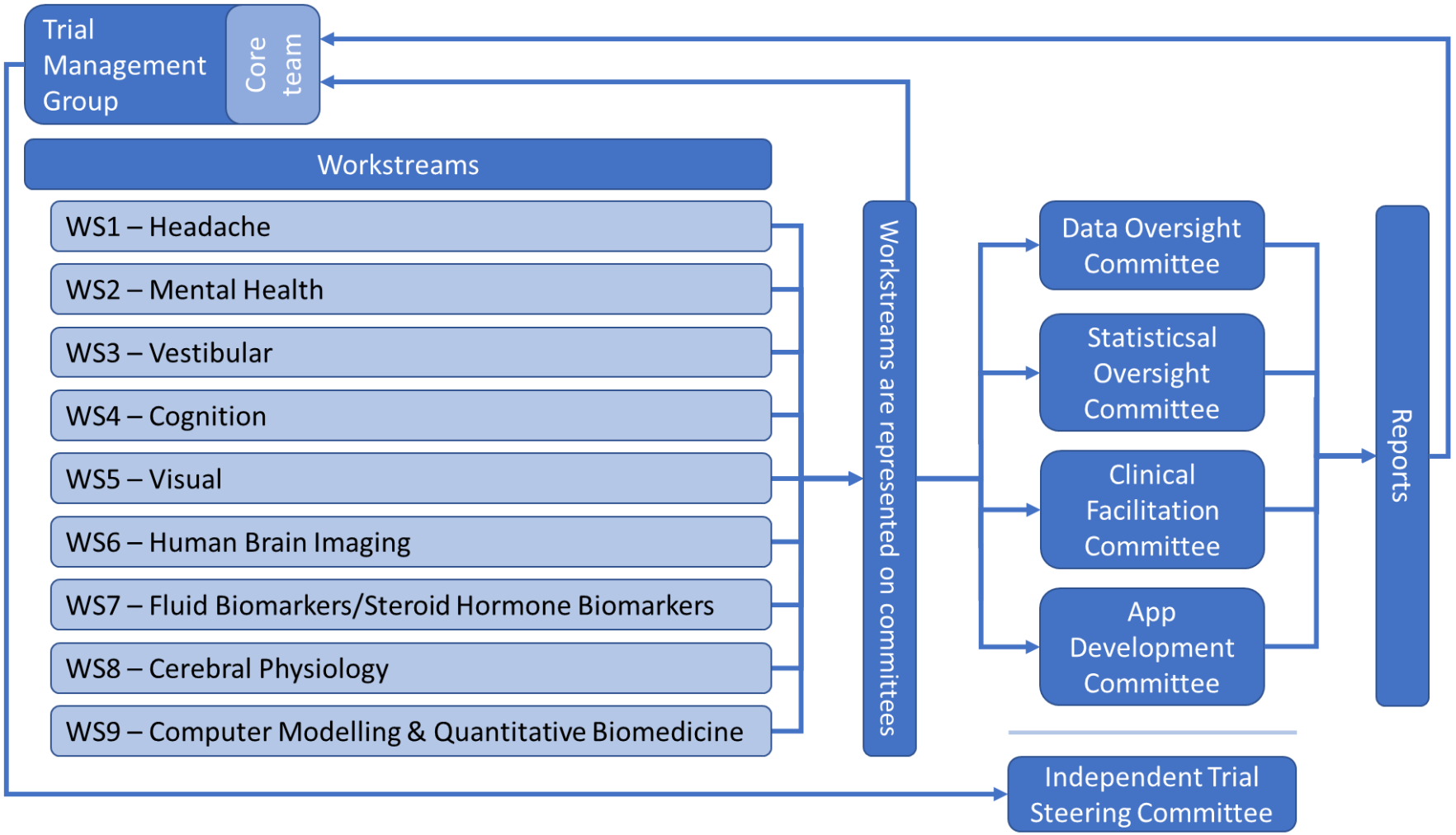
We aim to recruit 610 participants to follow-up for the prognostic modelling phase of the project. At 6 months, for each of the headache, depression and PTSD outcomes, we will have sufficient sample size to investigate a model with 10 candidate parameters; for the global composite score and MPAI outcomes, we will have sufficient sample size to investigate a model with 44 parameters. Sample size calculations were based on methods proposed by Riley [195, 196]. This will allow for 10% drop out which will be reviewed, and the analysis plan modified accordingly.

Depending on the type of outcome, various types of statistical modelling approaches will be used. For example, continuous, binary and count outcomes will be modelled using linear, logistic and Poisson regressions respectively. We will build multivariable models that can predict clinical outcomes primarily at 6 months (secondarily, we may investigate outcomes at 12 and 24 months), based on 21-day biomarker data. We will also examine how changes to these biomarkers from 21 days to three months affect the clinical outcome. Models will be developed in line with best practice, using multiple imputation, backwards selection, and multivariable fractional polynomials in the development, as appropriate. We will internally validate the models produced using bootstrapping techniques and allow for optimism by applying a uniform shrinkage factor. Any multivariable models produced will be checked for compliance to model assumptions. Model performance will be assessed in terms of prediction and calibration, for example C-index for the logistic models.

14.5. Relationships with collaborating consortia

We will seek to undertake exploratory analysis of the data with related international consortia where possible or appropriate. Furthermore, we will seek to cross-validate candidate biomarkers within the related mTBI populations of collaborating consortia.

15. STUDY ORGANISATIONAL STRUCTURE



15.1. Sponsor

The Sponsor for this study is University of Birmingham (UoB).

15.2. Coordinating centre

The study coordinating centre (Study Office) is Birmingham Clinical Trials Unit (BCTU), based at UoB.

15.3. Study Management Group (SMG)

The core SMG comprises individuals responsible for the day-to-day management of the study: the CI, lead statistician, trial team leader, study manager, programme manager, and lead clinical researchers. The role of the group is to monitor all aspects of the conduct and progress of the study, ensure that the protocol is adhered to and take appropriate action to safeguard participants and the quality of the study itself. The SMG will meet sufficiently frequently to fulfil its function.

An extended SMG including Workstream leads and other relevant co-investigators will meet as required to review progress, troubleshoot and plan strategically.

15.4. Study Steering Committee (SSC)

A SSC, comprising independent and non-independent members, will be established for the mTBI-Predict study and will meet as required depending on the needs of the study. Membership and duties/responsibilities are outlined in the SSC Charter. In summary, the role of the SSC is to provide oversight of the study. The SSC will monitor study progress and conduct and provide advice on scientific credibility. The SSC will operate in accordance with a study specific SSC Charter.

15.5. Data Monitoring Committee (DMC)

As there is no intervention in mTBI-Predict, it is considered that a DMC is not required.

15.6. Chief Data Officer

A Chief Data Officer will work with each workstream and BCTU to ensure the quality of the data for the entire programme. The Chief Data Officer responsibilities will include:

- Developing an overarching strategy including design and description of standards for the data architecture within the programme.
- Provision of a coordinated view to harmonise the data from each workstream, whilst ensuring data integrity.
- Ensure all data are optimised to support the research objectives for patient benefit.

15.7. App oversight committee

The app oversight committee will oversee the configuration of the web and mobile App that will be used to collect cognitive, participant reported, and actigraphy data during the course of the project. Work spans from definition of functionality and scheduling to oversight of development work as undertaken by H2 Cognitive Designs, and piloting of the App once it is available.

15.8. Statistical oversight committee

The statistical oversight committee will oversee the overall study analysis, readiness for analysis of the various data streams, and coordinate analyses involving cross-worksteam input.

15.9. Data oversight committee

The data oversight committee will work with the Chief Data Officer to establish and refine data policy, providing strategic direction for the project's data governance, including upload/download protocols, structure and information content. The committee will liaise with workstreams leads to ensure understanding of needs and requirements to allow collaborative research and standardisation of data collection to enable consistent and accurate data. The committee will review data at milestones to ensure consistency and inform adjustments to data policy.

15.10. Clinical facilitation committee

The clinical facilitation committee will work to monitor and refine recruitment and clinical study assessments, reporting to the study management group on conduct and progress.

15.11. Finance

The research costs of the study are funded by the UK Ministry of Defence awarded to Professor Alex Sinclair, University of Birmingham. Additional costs such as service support costs associated with the study, e.g. gaining consent, are estimated in the Schedule of Events Cost Attribution Template. These costs should be met by accessing the Trust's Support for Science budget via the Local Comprehensive Research Network.

16. ETHICAL CONSIDERATIONS

The study will be conducted in accordance with the UK Policy Framework for Health and Social Care Research and applicable UK Acts of Parliament and Statutory Instruments (and relevant subsequent amendments), which include Data Protection Act 2018 and Human Tissue Act 2004 and the Principles of GCP as set out in the UK Statutory Instrument (2004/1031; and subsequent amendments). The protocol will be submitted to and approved by the REC prior to the study's start.

Before any participants are enrolled into the study, the PI at each site is required to obtain the necessary local approval.

It is the responsibility of the PI to ensure that all subsequent amendments gain the necessary local approval. This does not affect the individual clinicians' responsibility to take immediate action if thought necessary to protect the health and interest of individual participants.

17. DATA PROTECTION AND CONFIDENTIALITY

Personal data and sensitive personal data recorded electronically and on all documents will be regarded as strictly confidential and will be handled and stored in accordance with the Data Protection Act 2018 (and subsequent amendments).

Participants will only be identified by their unique study identification number and initials on CRFs and on any correspondence with the Study Office.

For all participants, full name, full date of birth, sex at birth, and NHS number will be collected on the Registration CRF. The participant's full name will also be collected on the participant consent forms in addition to their email address and/or mobile number.

Other personal data categories that will be collected and analysed include health information and medical history.

Participants will acknowledge the electronic transfer and storage of their ICF to the Study Office. This will be used to perform central monitoring of the consent process. Participants will acknowledge the transfer of their personal data for the purpose of processing for medical research to collaborating institutions. Participants will acknowledge the transfer of their personal data to H2 Cognitive Designs who will be processing data collected by the app on behalf of the study.

In the case of specific issues and/or queries from the regulatory authorities, it will be necessary to have access to the complete study records. Representatives of the mTBI-Predict study team and sponsor may be required to have access to participants' notes for quality assurance purposes, but participants should be reassured that their confidentiality will be respected at all times. The Study Office will maintain the confidentiality of all participant data and will not disclose information by which participants may be identified to any third party.

18. FINANCIAL AND OTHER COMPETING INTERESTS

There are no financial or other competing interests related to the results of this study. Members of the SSC are required to provide declarations on potential competing interests as part of their membership of the committees. Authors are similarly required to provide declarations at the time of submission to publishers.

19. INSURANCE AND INDEMNITY

UoB has in place Clinical Trials indemnity coverage for this study which provides cover to UoB for harm which comes about through the University's, or its staff's, negligence in relation to the design or management of the study and may alternatively, and at UoB's discretion, provide cover for non-negligent harm to participants.

With respect to the conduct of the study at Site and other clinical care of the patient, responsibility for the care of the patients remains with the NHS organisation responsible for the Clinical Site and is therefore indemnified through the NHS Litigation Authority.

20. POST-STUDY CARE

Participants will continue to receive standard care during and after participation in mTBI-Predict.

21. ACCESS TO FINAL DATASET

The final dataset will be available to members of the Study Management and co-applicant group who need access to the data to undertake the final analyses.

Requests for data generated during this study will be considered by BCTU. Data will typically be available six months after the primary publication unless it is not possible to share the data (for example: the study results are to be used as part of a regulatory submission, the release of the data is subject to the approval of a third party who withholds their consent, or BCTU is not the controller of the data).

Only scientifically sound proposals from appropriately qualified Research Groups will be considered for data sharing. The request will be reviewed by the BCTU Data Sharing Committee in discussion

with the CI and, where appropriate (or in the absence of the CI) any of the following: the Sponsor, the SMG, and the SSC.

A formal Data Sharing Agreement may be required between respective organisations once release of the data is approved and before data can be released. Data will be fully de-identified (anonymised) unless the agreement covers transfer of participant identifiable information. Any data transfer will use a secure and encrypted method.

22. PUBLICATION PLAN

Results of this study will be submitted for publication in a peer reviewed journal. The manuscript will be prepared by the CI and authorship will be determined by the mTBI-Predict study publication policy.

Any secondary publications and presentations prepared by Investigators must be reviewed and approved by the SMG prior to wider circulation. Manuscripts should be submitted to the SMG in a timely fashion and well in advance of being submitted for publication to allow time for review and resolution of any outstanding issues.

Authors must acknowledge that the study was performed with the support of the funders and BCTU.

23. APPENDICES

23.1. Appendix 1: Complete list of biomarkers

General	R-PSQ	Score
	MPAI	Score
	QoLiBri	Score
	Pain Catastrophizing Scale	Score
	PVAC	Score
	Perceived Injustice Questionnaire	Score
	Locus of Control Questionnaire	Score
	6-minute walk test	Distance
	6-minute walk test	Perceived Exertion
	6-minute walk test	Perceived fatigue
	GOS-E	Score
Headache		
	Headache phenotype	ICHD phenotype
	Headache diary	Headache presence yes/no
	Headache diary	Monthly headache incidence
	Headache diary	Incidence of moderate to severe intensity headache
	Headache diary	Headache pain intensity 0-timepoint
	Headache diary	Headache pain intensity over time
	Headache diary	Headache days per month from 0-timepoint
	HIT-6 (Headache impact test – 6)	Score
Mental health		
	SOFAS	Score
	PCL-5	PTSD Yes/No
	MINI DSM-5	PTSD Yes/No
	PHQ-9	Score
	GAD-7	Score
	SBQ-R	Score
	AUDIT	Score
Vestibular		
	Hallpike Manoeuvre	BPPV
	Balance Vigilance Questionnaire	Score
	VVQ	Score
	DHI	Score
	ABC	Score
	BSQ	Score
	Rotating Chair (Yaw)	Acceleration in degrees, tilt in degrees
	Rotating Chair (perception and VOR)	Amplitude (volts), Latency (milliseconds)
	Posturography + EEG-EMG	Magnitude of sway (milimetres, volts (EMG))
	Eye movements	Smooth pursuit
	Eye movements	Saccades
	Eye movements	Anti-saccades

	Acoustic reflex	Amplitude (volts), Latency (millisecond)
	Hearing Level	dB
	Vestibular-evoked myogenic potentials	Amplitude (volts), Latency (millisecond)
	Auditory-evoked potentials	Volts
Cognition		
	PSQI	Score
	rMEQ	Score
	ESS	Score
	FSS	Score
	ISI	Score
	BQ	Score
	Cognitive battery	Correct Global composite score
	Actigraphy	Daily level of activity
	Cognitive battery	Immediate & delayed recall
	Cognitive battery	2D manipulations
	Cognitive battery	Picture completion
	Cognitive battery	Simple Reaction Time
	Cognitive battery	Choice Reaction Time
	Cognitive battery	Card pairs
	Cognitive battery	Tower of London
	Cognitive battery	Trail Making
	Cognitive battery	Paired Associates
	Cognitive battery	Verbal analogies
	Cognitive battery	Word definitions
	Cognitive battery	Continuous attention task
Visual		
	OCT	Macular ganglion cell layer thickness (global)
	OCT	Macular ganglion cell layer thickness (sectoral)
	OCT	Optic disc volume
	OCT	RNFL thickness (global)
	OCT	RNFL thickness (sectoral)
	OCTA	Optic nerve head peripapillary flow indices (e.g superficial vascular plexus, skeletal fractal dimension)
	OCTA	Macular flow indices (e.g superficial vascular plexus, skeletal fractal dimension)
	Visual acuity	Uniocular best corrected logMAR
	Visual field assessment	HVF SITA Fast 24:2, Mean Deviation
	Visual field assessment	HVF SITA Fast 24:2, Pattern Standard Deviation
	Colour vision	Protan/deutan CAD score
	Colour vision	Tritan CAD score
	Visual reaction and processing time	Eye Movements and Intrinsic Latency

	Visual reaction and processing time	Functional reaction and response times
	Contrast sensitivity	Uniocular best corrected Pelli Robson contrast sensitivity or Acuity Plus
	Pupillometry	Constriction latency
	Pupillometry	Constriction velocity
	Pupillometry	75% recovery time
	Autorefraction	Accommodation
	BIVSS (Brain Injury Vision Symptom Survey)	Score
Imaging	MEG	Delta/theta
	MEG	Alpha/beta
	MEG	Gamma
	Structural MRI	FA (fractional anisotropy)
	Structural MRI	MD (mean diffusivity)
	Structural MRI	AD (axial diffusivity)
	Structural MRI	RD (radial diffusivity)
	Functional MRI	Dynamic connectivity
		(mean dwell time)
	Functional MRI	Dynamic connectivity
		(FT - fraction of time spent per state)
	Functional MRI	Dynamic connectivity
		(NT - number of transitions between states)
	Physiology fMRI	Cerebrovascular reactivity (CVR)
	Physiology fMRI	Perfusion
	Physiology fMRI	Transit time
	Physiology fMRI	Blood flow in vessels
	7T MRI	Microvascular bleeds (number-SWI)
	7T MRI	Blood flow across vascular tree (PCA)
	7T MRI	Blood oxygen concentration in arteries and veins (QSM)
	7T MRI	Neuroinflamation biomarkers (MT)
	7T MRI	Grey matter volume
Fluid biomarkers		
	Blood	NFL
	Blood	T-tau
	Blood	Glial Fibrillary Acidic Protein
	Blood	NSE
	Blood	UCL-L1
	Blood	S100B
	Blood	IL6
	Blood	TNF-a
	Blood	IL-2
	Blood	IFN-γ
	Blood	IL-1β
	Blood	CRP

	Blood	BDNF
	Blood	CGRP (headache)
	Blood	PACAP (headache)
	Blood	Micro-RNA
	Blood	Untargeted metabolomics
	Blood	Albumin
	Blood	Platelets
	Saliva	Cortisol
Hormone biomarkers		
	Blood	Cortisol
	Blood	Cortisone
	Blood	FBC
	Blood	HbA1c
	Blood	Vitamin D
	Blood	Copeptin
	Blood	Melatonin
	Hair	Cortisol
	Saliva	Cortisol
	Saliva	Melatonin
	Spot urine	Cortisol
Cerebral physiology		
	Doppler	CO_2 vasoreactivity (TCD-derived CBF-CO_2 reactivity)
	Doppler	Cerebrovascular reactivity (shear-dependent blood flow change)
	Doppler	Cerebral autoregulation (gain, phase, coherence metrics)
	fNIRS	Neurovascular coupling (relative change in oxy- & deoxyhaemoglogin)
	fNIRS	Neurovascular coupling (changes in Oxygen Saturation)
	fNIRS	CO_2 vasoreactivity (relative change in oxy- & deoxyhaemoglogin)
	fNIRS	Functional connectivity (sensor/signal correlation)
	TMS	Cortical silent period
	Exercise capacity	Maximal voluntary contraction
	Exercise capacity	Force
	Exercise capacity	Perceived exertion
	Exercise capacity	Perceived fatigue
	Exercise capacity	Cerebral haemodynamics (relative change in oxy- & deoxyhaemoglogin)
	EEG	Delta/theta
	EEG	Alpha/beta
	EEG	Gamma

23.2. Appendix 2: Mental Health Exclusion Criteria

One exclusion criterion is prior diagnosis of PTSD or severe mental illness. Severe mental illness is defined by the following ICD-10 codes:

- F20 – Schizophrenia
- F22 – Delusional disorders
- F25 – Schizoaffective disorders
- F28 – Other psychotic disorder not due to a substance or known physiological condition
- F29 – Unspecified psychosis
- F31 – Bipolar disorder

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