Curtin Medical School

Towards the Development of an Integrative, Evidence-based Suite of Indicators for the Prediction of Outcome Following Mild Traumatic Brain Injury

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"*Wisdom is never lost if it is shared*"

Professor Alex Cohen AO

1926 - 2019

Thesis Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person expect where due acknowledgement has been made.

This thesis contains no material which has been accepted for the award of any other degree of diploma in any university.

The research presented and reported in this thesis was conducted in accordance with the National Health and Medical Research Council National Statement on Ethical Conduct in Human Research (2007)- updated March 2014. The proposed research study received human research ethics approval from the Royal Perth Hospital Ethics Committee Approval Numbers 15-062 (ANZCTR:123615000543583) and Royal Perth Hospital Ethics Committee RGS0000003024, Curtin University (HRE2019-0209), Ramsay Health Care (#2009) and St John of God Health Care (#1628).

Signature:

Date: **9th of July 2021**

Abstract

Mild traumatic brain injury (mTBI), also known as concussion, is the most common form of brain injury in Australia and worldwide. mTBI is characterised by rapid, transient changes in neurological function that are accompanied by an array of signs and symptoms, which frequently include headache, dizziness, neck pain, difficulty concentrating and remembering as well as disturbances to mood and sleep. Each mTBI is unique; the clinical presentation of mTBI is known to differ across individuals and instances of mTBI. While most individuals are expected to recover from the signs/symptoms of mTBI within 2 weeks of injury, approximately 10-20% will continue to experience ongoing symptoms beyond this typical recovery timeframe and experience a debilitating condition known as persistent post-concussion symptoms (PPCS). Unfortunately, it is not currently possible to identify which individuals will go on to develop PPCS at time of mTBI diagnosis, nor is there a consensus on how to manage individuals with this condition.

The ability to identify individuals that may be at risk of delayed recovery following mTBI would be of great benefit to clinicians and researchers to improve patient outcomes. A prognostic model could enable the provision of personalised healthcare to patients by facilitating triage to the most appropriate forms of treatment according to their individual needs before symptoms become chronic. Researchers would also benefit from prognostic models, which could be used to enrich clinical trials for evidence-based treatments that aim to prevent or ameliorate other late-stage conditions associated with mTBI, such as chronic traumatic encephalopathy.

Prognostic models for PPCS developed to date tend to be limited to one or a small subset of prognostic factors and are yet to be clinically useful. Given the multifactorial nature of the condition, it is hypothesized that predictive models could be rendered more powerful through the development of a multivariate 'suite' of factors that encompasses the diverse pathobiological underpinnings and symptomatology of mTBI. Consequently, the overarching aim of this PhD project is to contribute to the exploration of established and novel factors that may be used to predict delayed recovery following mTBI.

This thesis begins with a comprehensive review of the literature (Chapter 2) followed by a pilot study, which aimed to identify criteria that may be useful in the development of predictive models (Chapter 3). More specifically, the pilot study investigated an array of demographic, injury-related characteristics, blood-based biomarker, neuropsychological assessment and magnetic resonance imaging (MRI) outcome measures to identify those that were most promising for

incorporation into future studies aiming to develop a multivariate, suite-based approach to predicting PPCS. The most promising indicators identified were neuropsychological measures of immediate memory, delayed memory, and attention, as well as executive functioning, as measured by the *Repeatable Battery for the Assessment of Neuropsychological Status Update (RBANS®) Update* and the *Trails Making Test Form B*, respectively.

Based on this preliminary investigation, a large-scale prospective longitudinal observational cohort study called the *Concussion Recovery Study (CREST)* was established to explore a substantial range of novel and established factors that could potentially serve as predictors for PPCS. Due to the effect of *COVID-19* on participant recruitment, data could not be obtained from a sufficient number of individuals to proceed with analysis. Therefore, a protocol paper describing the research design and procedures for this study is presented as the second chapter of this thesis (Chapter 4).

Advanced neuroimaging techniques have emerged as a particularly promising area of investigation for identifying predictive indicators of PPCS. Quantitative Susceptibility Mapping (QSM) is a novel and increasingly popular post-processing advanced MRI analysis technique that uses magnetic susceptibility as a contrast mechanism. Iron is a major source of susceptibility in the brain, and QSM can be used to investigate a variety of iron-related pathophysiological changes that ensue following mTBI. To date, the applications of QSM within the field of mTBI have been limited to a handful of small-scale investigations, of which only two have correlated findings with ongoing symptoms following mTBI. Chapter 5 of this thesis presents a seminal narrative review of the literature in which QSM has been applied within the field of mTBI.

The narrative review identified that QSM could be used to detect susceptibility changes within specific brain regions, which themselves could potentially be used as biomarkers to predict PPCS. Recent advances in MRI analysis software offer the opportunity to analyse subregions of individual brain areas, which in turn may provide further and increasingly detailed insight into pathological changes following mTBI. The implementation of such analyses may also help identify more specific and precise indicators of PPCS. Accordingly, Chapter 6 of this thesis details an analysis pipeline for generating QSM images and presents an example analysis in which the pipeline was applied to extract mean tissue magnetic susceptibility values for the thalamus, which is increasingly being recognised as an important brain area within the context of mTBI. As noted above, *COVID-19* resulted in a limited amount of available data, specifically, $n = 17$ *CREST* participants. Due to the low numbers of participants that had been enrolled into the study thus far, it was not considered appropriate to address the core hypothesis of this thesis (i.e. the prediction of PPCS), nor was it possible to conduct multiple comparisons for individual thalamic nuclei. However, a novel

investigation was conducted using the developed QSM pipeline to examine differences in the susceptibility of the thalamus between individuals with and without a self-reported history of previous mTBI.

The thesis concludes with a general discussion (Chapter 7), which draws together the various elements of this thesis, notes limitations and describes directions for future investigations.

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It is often said that a PhD is a journey. And like any good journey, it's never about the destination- it's the people you meet along the way that make the experience all the more memorable and rewarding. I have been fortunate enough to have met many incredible souls over the course of my PhD candidature, and I appreciate each and every one that I have had the privilege to share this extraordinary journey with.

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Attribution of Research Outputs

The following tables acknowledge the individuals who made contributions to the published works that are presented in this PhD thesis. In each instance, the contributions of the PhD candidate are listed first, followed by the contributions of the co-authors.

Gozt, A., Licari, M., Halstrom, A., Milbourn, H., Lydiard, S., Black, A., Arendts, G., Macdonald, S., Song, S., MacDonald, E., Vlaskovsky, P., Burrows, S., Bynevelt, M., Pestell, C., Fatovich, D., & Fitzgerald, M., (2020). Towards the development of an integrative, evidence-based suite of indicators for the prediction of outcome following mild traumatic brain injury: Results from a pilot study. *Brain Sciences*, *10*(1), 23. https://doi.org/10.3390/brainsci10010023

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Gozt, A.			10	40	15	65
Licari, M.		10			10	20
Arendts, G.						
Macdonald, S.						
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Bynevelt, M.						
Pestell, C.						
Fatovich, D.						
Fitzgerald, M.						
Halstrom, A.			10			10
Milbourn, H.						
Lydiard, S.						
Black, A.						
Song, S.						
Burrows, S.				5		5
Vlaskovsky, P.						

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Hellewell, S.C.		8	5	13
Thorne, J.				
Thomas, E.				
Buhagiar, F.				
Markovic, S.				
Van Houselt, A.				
Ring, A.		1	1	2
Arendts, G.				
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Gozt, A.		30	5	10	30	75
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Ward, P.G.D.					5	5
Bynevelt, M.					2	2
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Publications, Abstracts and Awards

The following is a list of publications, conference abstracts and presentations to which the candidate has contributed to during the course of their candidature.

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Gozt, A., Licari, M., Halstrom, A., Milbourn, H., Lydiard, S., Black, A., Arendts, G., Macdonald, S., Song, S., MacDonald, E., Vlaskovsky, P., Burrows, S., Bynevelt, M., Pestell, C., Fatovich, D., & Fitzgerald, M., (2020). Towards the development of an integrative, evidence-based suite of indicators for the prediction of outcome following mild traumatic brain injury: Results from a pilot study. *Brain Sciences*, *10*(23). https://doi.org/10.3390/brainsci10010023

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Conference Presentations

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***Gozt, A.,** Thorne, J., Thomas, E., van Houselt, A., Buhagiar, F., Cowen, G., Ring, A., Xu, D., Robinson, S., Licari, M., Bynevelt, M., Pestell, C., Fatovich, D., & Fitzgerald, M. Introducing CREST: The Concussion Recovery Study. *14th International Neurotrauma Society Symposium (INTS 2020).* Melbourne, Australia (2021). Poster.

Local

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Awards

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Media

Sportsfan Radio, 88.3 Southern FM (Melbourne, VIC, Australia). Interview with Mark Seymour on Sports Concussion. November 15th 2020

Diving headlong into study. Feature article in Community Newspaper (Perth, WA, Australia). January 16th 2020

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List of Abbreviations

AD	Axial Diffusivity
ApoE	Apolipoprotein E
AMPA	D-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid
BBB	Blood-Brain Barrier
Ca^{2+}	Calcium ion
CBF	Cerebral Blood Flow
CNS	Central Nervous System
CREST	Concussion Recovery Study
CT	Computerised Tomography
CO_2	Carbon Dioxide
COSMOS	Calculation of Susceptibility through Multiple Orientation Sampling
COVID-19	Corona Virus Disease 2019
DNA	Deoxyribonucleic Acid
DTI	Diffusion Tensor Imaging
dMRI	Diffusion Magnetic Resonance Imaging
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders- 4th Edition
ED	Emergency Department
FA	Fractional Anisotropy
GCS	Glasgow Coma Scale
GFAP	Glial Fibrillary Acid Protein
GM	Grey Matter
GP	General Practitioner
GRE	Gradient Recalled Echo
HARPERELLA	Harmonic Phase Removal using the Laplacian operator
HEIDI	Homogeneity Enabled Incremental Dipole Inversion
ICD-10	International Classification of Diseases-10th
K^+	Potassium ion
LOC	Loss of Consciousness
MBP	Myelin Basic Protein
MEDI	Morphology Enabled Dipole Inversion
MD	Mean Diffusivity
MRI	Magnetic Resonance Imaging
mTBI	Mild Traumatic Brain Injury

MVA	Motor Vehicle Accident
Na^+	Sodium ion
NF	Neurofilament Proteins
NF-L	Neurofilament Protein- Light
NMDA	N-methyl-D-aspartate
NSE	Neuron Specific Enolase
PCSS	Post Concussion Symptom Scale
PDF	Projection onto Dipole Fields
PPCS	Persistent Post-Concussion Symptoms
PPM	Parts per million
PTA	Post-Traumatic Amnesia
PTSD	Post-Traumatic Stress Disorder
qEEG	Quantitative Electroencephalography
QOL	Quality of Life
QSM	Quantitative Susceptibility Mapping
RD	Radial Diffusivity
ROS	Reactive Oxygen Species
RPH	Royal Perth Hospital
RMPCQ	Rivermead Post Concussion Symptoms Questionnaire
S100B	S100 Calcium Binding Protein B
SBDP	Spectrin Break Down Products
SHARP	Sophisticated Harmonic Artifact Reduction for Phase Data
SWI	Susceptibility Weighted Imaging
TBI	Traumatic Brain Injury
VOMS	Vestibular/Ocular Motor Screening Assessment

1 General Introduction

1.1 Preliminary Background and Research Context

Traumatic brain injury (TBI) is a type of acquired brain injury that is commonly defined as a mechanical injury to the head caused by an external force. The incidence of TBIs internationally is estimated between 295 and 369 per 100,000 persons[1,2], although this number is likely conservative as it does not account for instances of TBI where medical attention is not sought. Globally, the most common causes of non-fatal TBI include falls and road traffic accidents[2]. Populations that have been identified as being most at risk of TBI include young males, the elderly and children under the age of one year, although the aetiology of TBI varies across these groups. For adolescents and young adults, the greatest number of TBIs are accounted for by motor vehicle accidents, sports and recreational activities, while falls are the primary cause of TBIs amongst paediatric and geriatric populations[3].

TBI can be typified in a number of different ways and is conventionally categorised according to a 3-point severity spectrum (*mild-moderate-severe*). The Glasgow Coma Scale[4] (GCS) is the most widely implemented clinical scoring system that is used to determine TBI severity[1], with each severity grading being associated with a presence or absence of distinct physiological responses[5]. Falling on the less severe end of the spectrum is mild traumatic brain injury (mTBI).

mTBI, also referred to as concussion, accounts for approximately 80% of all TBIs in Australia and worldwide[6–8]. mTBI is characterised by rapid and transient changes in physiological and cognitive function[9], which are accompanied by a number of signs and symptoms. Commonly, these include headache, dizziness, difficulty concentrating, neck pain, as well as altered mood and sleep disturbances[10]. While mTBI symptoms can be broadly categorised into physical, cognitive, emotional and sleep-related domains[11], the clinical presentation of mTBI can vary greatly between individuals[12] as well as individual instances of mTBI; both are important factors that have hampered efforts to develop reliable prognostic tools and whose importance has only recently been fully acknowledged.

Individuals who have experienced a mTBI are generally anticipated to recover from any signs or symptoms that they may be experiencing within approximately two weeks of their injury[13–16]. However, it is increasingly recognised that mTBI recovery is multifactorial[17], and that this expected recovery trajectory, which has been ascertained based upon research predominantly conducted amongst young athletic populations, may not accurately reflect recovery experiences across age, sex, and socioeconomic status. In fact, it is often reported that 10-20% of individuals who have sustained a mTBI continue to experience mTBI-associated symptoms at least 1 month following injury[18]. When

individuals experience symptoms for 3 or more months post-injury they are said to be experiencing a condition known as *persistent post-concussion symptoms* (PPCS).

Research into PPCS is in a relative state of infancy and much remains to be elucidated about the condition. Unfortunately, it is not possible to identify which individuals will experience delayed recovery at the time of mTBI diagnosis at present, nor is there an agreement on how to manage individuals with this complex and often debilitating condition. Given the relative frequency with which mTBI occurs, having the capacity to predict who will go on to develop PPCS would be of great benefit to clinicians and researchers alike. From a clinical standpoint, prognostic models could aid clinical decision making and help better manage patient expectations regarding their recovery. Importantly, they would also assist in delivering personalised healthcare by facilitating triage to the most pertinent forms of treatment based upon each individual's unique needs, which in turn could help improve patient outcomes. Prognostic models would also be of benefit to researchers, who could apply them to enhance clinical trials aiming to develop treatments or therapies to prevent or ameliorate the effects of PPCS or other late-stage conditions associated with mTBI, such as chronic traumatic encephalopathy[19–22], Alzheimer's disease[23–25], and mild cognitive impairment[24].

Prognostic models for PPCS that have been developed to date tend to be limited to one or a small subset of prognostic factors, and their predictive power has not yet been shown to be clinically useful[26,27]. Predictive models could be rendered more powerful through the development of a multivariate suite of factors that encompass/incorporate the diverse pathobiological underpinnings and symptomatology of mTBI.

1.2 Aims

The overarching aim of this thesis was to contribute to the exploration of factors that may be used to predict delayed recovery following mTBI. More specifically, it addresses the following three aims:

i) To conduct a pilot study to investigate an array of demographics, injury-related characteristics, blood-based biomarker, neuropsychological assessment, and magnetic resonance imaging (MRI) outcome measures, and identify those that were most promising so that they may be incorporated into future studies aiming to develop a multivariate, suite-based approach to predicting PPCS (**Chapter 3**).

ii) To initiate a large-scale prospective longitudinal observational cohort study designed to develop a multivariate approach to predicting PPCS (**Chapter 4**).

iii) Explore in greater detail the predictive utility of Quantitative Susceptibility Mapping (QSM), a novel advanced MRI analysis technique (**Chapters 5 and 6**).

1.3 Thesis Format and Composition

This doctoral thesis is presented as two series encompassing four studies, each of which contribute to better understanding the prediction of outcome following mTBI. *Series One* is comprised of one research and one protocol paper, while *Series Two* constitutes a narrative review and a data chapter. The work undertaken within the various studies of this thesis is briefly outlined below.

Chapter 2 comprises a review of the literature for mTBI as well as established and emerging factors that may be useful in predicting outcome following injury. Commencing with a short overview of mTBI, it's prevalence, aetiology and societal impact, the review then briefly covers the symptoms of mTBI, the emergent concept of mTBI clinical profiles, current diagnostic criteria as well as the ways in which mTBI can be assessed. The concept of PPCS, the crux of this thesis, is then introduced. This is followed by a comprehensive outline of the current evidence for an array of pre-, peri-, and post-injury factors. This includes an overview of the pathophysiological underpinnings of mTBI, with a special focus on secondary degeneration mechanisms that lead to the release of potentially predictive biomarkers or alterations in parameters detectable by advanced neuroimaging. A substantial degree of detail is provided as this information has helped to inform the study design of subsequent observational cohort studies reported in this thesis. In so doing, the review also identifies gaps in the research which need to be explored to enable a more comprehensive and holistic approach to predicting persisting symptoms following mTBI.

Chapter 3 details the findings of a pilot study that was conceived with the aim of investigating the utility of blood-based biomarkers, neuropsychological tests, and MRI as predictive indicators of PPCS following injury. The aims of this pilot study were two-fold. Firstly, it endeavoured to evaluate the potential to create a multivariate prognostic model for PPCS that incorporated demographic, injury-related characteristics, neuropsychological, blood-based biomarker, and neuroimaging outcomes. Secondly, it aimed to contribute data for the blood-based biomarker and MRI outcomes, for which there was relatively limited literature regarding differences between mTBI and healthy controls at the time. The pilot study addressed these two aims with the intention that the outcomes could be validated in a larger scale study designed to identify a suite of outcome measures that could serve to predict PPCS. Chapter 3 has been published in the journal *Brain Sciences* and is presented within this thesis.

Chapter 4 is a protocol paper for the *CREST Concussion Recovery Study (CREST)*. This prospective, observational cohort study was conceived as the next step following on from the pilot study that was reported in Chapter 3. Building upon insight that was gained from the pilot study, the *CREST* research project has been considerably expanded to include a broader range of outcome measures across the domains of *i)* demographics, *ii)* injury characteristics, *iii)* relevant aspects of

medical history, *iv)* personality, *v)* blood-based biomarkers, *vi)* cognition, *vii)* exercise tolerance, and *viii)* neuroimaging. Chapter 4 has been published in *BMJ Open* and is presented within this thesis.

Chapter 5 comprises the first narrative review that reports on the emerging applications for QSM in the detection of TBI pathology and prediction of outcome following injury. The review commences with a brief overview of TBI, the role of iron in the brain and how brain iron can be imaged *in vivo* by harnessing the properties of magnetic susceptibility. Most importantly, the review also summarises the literature that has been reported to date in which QSM has been used to investigate pathophysiological changes and outcome following TBI. This work featured in Chapter 5 has been published in the journal *Neuroscience* and is presented within this thesis.

Chapter 6 presents the data analysis pipeline for QSM data that is being acquired for the *CREST* research project. Due to *COVID-19* related disruptions to recruitment, it was not possible to examine the core hypothesis of this thesis regarding PPCS prediction using QSM data collected as part of *CREST*. As an alternative, an example analysis assessing the relationship between self-reported history of previous mTBI and the formation of calcium deposits within the thalamus, a grey matter (GM) structure located deep within the brain, was conducted using the developed pipeline. Briefly, available QSM data were processed using the MEDI toolbox, and brain parcellation was performed using FreeSurfer software to extract the region of interest. Relative differences in mean tissue magnetic susceptibility within the thalami were examined between individuals who had recently sustained concussion and self-reported a history or no history of previous mTBI. The results of this investigation are also reported in Chapter 6.

Chapter 7 consists of a general discussion in which the findings of this thesis are reviewed and integrated within context of the relevant literature and suggests directions for future investigations. The chapter closes with the final conclusions for this thesis. A complete list of all references cited within the thesis is provided at the end of this chapter. Note that this list does not include the references cited in the published papers presented in this thesis, as these are provided within the chapters where they are presented.

2 Literature Review

2.1 Mild Traumatic Brain Injury

mTBI is a type of acquired brain injury that is caused by direct impact to the head or through impulsive forces to other regions of the body that result in an abrupt acceleration/deceleration of the craniocervical complex, and which is characterised by a rapid onset of transient and reversible changes in neurological function[28]. The term *concussion* is often used interchangeably with mTBI, particularly within the context of sports medicine. It is acknowledged that there are no standardised definitions of either of these terms at present, and the term mTBI has been used preferentially for the purposes of this thesis.

2.2 Prevalence, Aetiology and Societal Impact

Determining the prevalence rates of mTBI is a difficult and complicated task. This is because not all affected individuals seek clinical attention for their injury[29,30], and for those who do, there is often considerable variation in the documentation of mTBI diagnosis[31–33]. Nevertheless, a study commissioned by the World Health Organization (WHO) estimates the global incidence of hospital-treated mTBI to be between 100-300/1000 individuals of the population[31]. Precise data regarding the incidence rate of mTBI in Australia is currently lacking. A report by the Australian Institute of Health and Welfare (2007)[34] found the hospitalisation rate for TBI to be 107 per 100 000 population, however, the incidence rate of TBI may be significantly higher. Work by Feigin and colleagues[8] found the incidence rates for TBI in New Zealand are 790 cases per 100,000 person years. Taking this into consideration alongside the findings that indicate mTBI accounts for approximately 85% of all TBI, it can thus be estimated that there are 170,000 cases of mTBI in Australia per annum[35].

In Australia, the individuals most at risk of mTBI are males between 15-24 years of age[36], however, children under the age of one[37,38] and the elderly (>75 years)[34] have also been identified as at-risk populations. mTBI can result from a number of different causes that have been found to vary according to age groups. For adolescents and adults, the majority of mTBIs are accounted for by sports and recreation-related injuries, and motor vehicle accidents (MVAs)[37], whereas the primary cause of mTBI amongst infants and the elderly are falls[32,34,39].

Whilst it is difficult to quantify its economic and social impacts, mTBI is increasingly being recognised as an emergent public health issue[40]. In Australia, the annual health burden associated with mTBI has been estimated to cost around AU$50 million[41]. Beyond this, there is also a substantial cost associated with lost productivity at work and burden that can be placed upon the families,

friends and carers of individuals with mTBI as well as decreased quality of life for the affected individuals themselves, which have traditionally been overlooked since they are much harder to quantify[42]. mTBI (and in particular, repeated mTBI) has been associated with issues such as second impact syndrome[43] and long-term neurodegenerative conditions such chronic traumatic encephalopathy[19–22,44,45], mild cognitive impairment[24], Alzheimer's disease[23–25,46] and Parkinson's disease[47].

2.3 Pathophysiological Underpinnings of mTBI

Given that studies examining the presence and extent of the pathophysiological changes that follow mTBI are currently lacking in some areas, a comprehensive understanding of the pathobiology of mTBI is yet to be established[48,49]. Nevertheless, it is believed that the biological events and changes that occur following mTBI parallel those of more severe TBI. The subsequent section of this literature review begins with a brief overview of the pathophysiological conceptualisation of TBI. Following this, the key pathophysiological events of TBI are described, and findings from clinical mTBI are discussed where possible. However, in instances where the pathophysiological underpinnings of mTBI are poorly understood, especially for human mTBI, findings from the general TBI and/or pre-clinical literature are referenced, and the distinctions noted.

TBI can be classified according to several classification systems[9]. However, in preclinical and clinical research it is typically conceptualised as consisting of two distinct events: a *primary* or *mechanistic injury*, and a complex self-propagating biochemical injury cascade that follows, which is commonly referred to as *secondary injury*[50,51]. More specifically, *primary injury* refers to the physical injury that results from the transfer of kinetic energy occurring at the time of trauma, which causes the brain to be displaced within the skull and damages various components of brain tissue[51,52]. Primary injury can be further classified as *focal*, when resulting from mechanical impact with the skull (e.g. contusion and/or haematoma), or *diffuse*, when resulting from shear forces that have been initiated by rapid changes in inertia at time of injury (e.g. axonal strain, compromised axolemma)[53]. Focal pathology is generally a rare occurrence in mTBI, thus diffuse injury is thought to be the primary pathologic mechanism underpinning the neurophysiological and neurological dysfunction that characterise this condition. *Secondary injury* or *secondary degeneration*, on the other hand, refers to the series of intra- and extra-cellular pathophysiological processes that are initiated by primary injury and arise over the course of hours to several weeks following TBI[54].

The pathophysiology of mTBI is thought to be complex and involve a number of dynamic processes, many of which occur simultaneously and interplay with each other. For these reasons,

mTBI pathophysiology has been conceptualised as a multidimensional molecular cascade[28,55–58]. The main pathophysiological events characterising this cascade are deleterious alterations in ionic gradients of neural cells and neurotransmission (glutamate excitotoxicity), abnormal energy metabolism, diminished cerebral blood flow and exacerbated levels of oxidative stress[59]. More recently, blood-brain barrier breakdown, neuroinflammation, myelin changes and diffuse axonal injury have also been identified as additional mechanisms that are believed to play an important role in TBI pathology. Understanding these individual mechanisms is necessary, not only to better understand the consequences of TBI and identify potential therapeutic approaches, but also to help identify and pursue blood-based and neuroimaging biomarkers that can be used to diagnose mTBI and predict outcome after injury.

2.3.1 Glutamate Excitotoxicity and Calcium Influx

In mTBI, the mechanical forces associated with the primary injury cause damage to the bilipid membrane of neuronal cells, resulting in immediate dysregulated ionic homeostasis and depolarisation[56,60]. More specifically, mechanoporation of the axolemma and neuronal plasmalemma causes a rapid efflux of intracellular potassium (K^+) ions *via* voltage-gated channels[61], which in turn propagate a feedback loop where increasingly more voltage-gated channels are opened, additional K^+ is released, and further depolarisation occurs[56].

The initial perturbation of the K^+ ionic gradient subsequently causes an indiscriminate release of excitatory amino acids, primarily glutamate. This occurs through an number of mechanisms including excessive release from the presynaptic terminals of neurons, as well as extravasation from damaged neuronal cells and alterations to reuptake mechanisms in astrocytes[62,63]. In fact, extracellular concentrations of glutamate have been observed to increase up to 50-fold following injury in a rodent model of mTBI[64]. The increased presence of glutamate subsequently results in the opening of more ligand-gated K^+ channels and exacerbates potassium efflux in a severity-dependent manner[64–67]. Excitatory amino acids are known to stimulate a number of different receptors and channels, including D-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA), kainite, and N-methyl-D-aspartate (NMDA)[67]. The binding of glutamate to NMDA receptors is particularly significant in mTBI because it also induces the opening of voltage-gated calcium (Ca^{2+}) channels. This opening of channels permits an unrestricted flow of Ca^{2+} into the cell and triggers Ca^{2+}-dependent Ca^{2+} release from intracellular stores, both of which dramatically elevate the concentration of cytosolic Ca^{2+}[57,68]. Sustained, excessive influx of Ca^{2+} is detrimental to neuronal cellular functioning for several reasons. Firstly, it activates mitochondrial Ca^{2+} sequestration, which can lead to metabolic dysfunction and ultimate energy failure[59]. Secondly, intracellular accumulation of Ca^{2+} impairs axonal transport and causes microtubule disassembly and

neurofilament compaction, the latter two of which result in swelling and axotomy[57]. Lastly, abnormally high levels of intracellular Ca^{2+} are known to evoke cellular damage through the activation of deoxyribonucleic acid (DNA) degrading endonucleases, phospholipases (e.g. calcineurin), proteases (e.g. calpains and caspases), transcription factors (e.g. c-Fos, c-Jun and c-Myc)[54], the production of reactive oxygen species (ROS: e.g. hydrogen peroxide, peroxynitrate, and superoxides) and other free radicals (e.g. nitric oxide synthase)[52,60,69], as well as through the initiation of cell death pathways[70].

2.3.2 Changes in Cerebral Glucose Metabolism

Following the disruption of ionic gradients, membrane-bound Na^+-K^+ pumps are activated in an attempt to restore cellular homeostasis and normal membrane potential[59,71]. These pumps, which are dependent on adenosine triphosphate under typical conditions, are shifted into overdrive following injury, and elevated levels of glucose are subsequently required to meet the dramatic increase in energy demand. This induces a transient state of hyperglycolysis, which may last between 30 minutes to 4 hours, and results in a relative depletion of intracellular energy and increase in adenosine diphosphate[56,72]. Disruption of the tricarboxylic acid cycle also results in the concurrent extracellular accumulation of lactate, a by-product of the anaerobic energy production required for hyperglycolysis, which is thought to lead to local acidosis resulting in breakdown of the blood-brain barrier (BBB)[73,74].

Subsequent to this initial period of hyperglycolysis and metabolic uncoupling, cerebral glucose metabolic rates enter a state of impaired metabolism[56]. Typically commencing within 24 hours of injury[72], this period of metabolic depression has been observed to last 5 days in animal studies of mTBI[75]. The extent to which hypometabolic states persist appear to be influenced by age, with global cortical metabolic rates having been observed to return to homeostasis sooner in younger animals relative to older animals (i.e. 3 days vs 10 days, respectively)[76].

2.3.3 Changes in Cerebral Blood Flow

Concomitant with changes in ionic homeostasis and cerebral glucose metabolism, acute decreased cerebral blood flow (CBF) is another pathophysiological hallmark of mTBI. Whilst the decrease in CBF may not be severe enough to reach ischemic levels, it is sufficient to contribute to a disparity between glucose supply and demand[59], and may be related to the onset and persistent of mTBI symptoms. Decreased CBF can ensue from alterations to three distinct mechanisms that are responsible for maintaining CBF homeostasis. More specifically, these are neurovascular coupling, cerebral vasoreactivity and cerebral autoregulation[77].

CBF is largely determined by the functional activity of the brain, and neurovascular coupling is the mechanism responsible for preferentially directing blood flow towards brain regions that are currently experiencing heightened neuronal activity and thus, increased metabolic demand[77]. The intricacies of neurovascular coupling are yet to be fully understood. However, candidate mechanisms thought to underpin the phenomenon that have been identified thus far include activity-related ion content shifts, changes in energy substrates, and the release of neurotransmitters that known to affect vasomotor tone, such as acetylcholine[78]. Furthermore, astrocytes are suspected of playing a role in regulating neurovascular coupling, given their direct contact with endothelial cells and ability to secrete vasodilatory substances such as adenosine, cyclooxygenase-2 metabolites, epoxyeicosatrienoic acid and nitric oxide[79]. Although investigations into changes in neurovascular coupling following mTBI are only just emerging, it is speculated that mTBI may compromise smooth muscle responsiveness[80] and endothelial function, particularly in plial vasculature[81], which in turn could result in decreased CBF.

CBF is also highly sensitive to changes in arterial levels of CO_2[77]. Cerebral vasoreactivity collectively refers to the vasodilation and vasoconstriction processes that occur in response to changing CO_2 concentrations, which are essential for maintaining a constant flow of oxygenated blood to the brain. While the mechanisms of action are yet to be elucidated in their entirety, these cerebrovascular responses to altered levels of CO_2 are thought to be predominately mediated by extracellular pH levels and their subsequent activation of ion channels, particularly K^+ ion channels, located in the vascular smooth muscle[77]. Thus, and as is the case for neurovascular coupling, aberrant endothelial functioning and smooth muscle responsiveness are both thought to be key factors in regulating cerebral vasoreactivity[77]. Disruptions in cerebral vasoreactivity have been observed to occur shortly after sports-related concussion[82], and within days of insult amongst animals that have been subject to mild cortical impact injury[83].

Cerebral autoregulation refers to the brain's ability to maintain constant CBF despite fluctuations in systemic blood pressure, and therefore perfusion pressure, that occur as part of everyday functioning[77,82,84]. More specifically, cerebral arteries are able to maintain stable cerebral perfusion by relaxing when blood pressure decreases and constrict when blood pressure increases[85]. This phenomenon is largely believed to be mediated by interplay between the cholinergic, myogenic and sympathetic autonomic nervous systems[43,86], but may also be influenced by variables such as CO_2 concentration and cardiac output[87]. Cerebral autoregulation is thought to be compromised following TBI when haemorrhaging is present due to the resulting lowering of intracranial blood pressure. Although haemorrhaging is not a typical occurrence in mTBI, it has been observed that approximately 30% of mTBI patients have lost or impaired cerebral autoregulation within 48 hours of sustaining their injury[88], and these changes may persist up to 14 days[89].

2.3.4 Oxidative Stress

Oxidative stress is a metabolic state that occurs when the number of ROS within a cellular environment outnumber the availability of enzymatic and non-enzymatic antioxidants (e.g. ascorbic acid, catalase, glutathione, manganese superoxide dismutase, and glutathione peroxidase) that are capable of neutralising their deleterious effects[50,90–93]. Following TBI, there is an increase in the production of both ROS and reactive nitrogen species, which occurs as a result of excessive accumulation of intracellular Ca^{2+}[94] as well as exhaustion of endogenous antioxidant reserves[50]. Excessive ROS production has been observed to induce numerous forms of damage, including lipid peroxidation of cellular and avascular structures, protein oxidation, aberrant DNA cleavage and inhibition of the mitochondrial transport chain[95–97]. Since these mechanisms collectively can contribute to immediate cell death[50], ROS are thought to mediate neurotrauma-induced secondary degeneration[98]. Overall, oxidative stress is believed to be a driver of inflammatory processes and cell apoptotic programmes[50].

As an organ, the brain is especially vulnerable to the detrimental effects of oxidative stress for several reasons. Firstly, it is largely comprised of myelin, a substance with a high polyunsaturated fatty acid content. Polyunsaturated fatty acids are known to be highly susceptible to free radical attack due to their double-bond chemical structure, which permit ROS to readily remove hydrogen ions[99]. Secondly, the brain's high functional demand for oxygen inherently increases the rate at which ROS can be generated[100,101]. Thirdly, the brain is known to contain relatively high levels of iron, which is known to actively participate in redox reactions that can catalyse the generation of ROS[102–106].

While oxidative stress and its consequences have been extensively studied in animal models of TBI of all severities, there is currently a dearth of studies investigating this phenomenon in humans. Nevertheless, it is recognised that there is an intimate relationship between oxidative stress and TBI more generally, and several human trials evaluating the use of antioxidant-based nutritional supplements including Omega-3 Fatty Acid Docosahexaenoic acid (DHA), resveratrol, and melatonin in mTBI management are currently underway, or pending publication of results[107].

2.3.5 Blood-Brain Barrier Breakdown

The blood-brain barrier (BBB) is a highly specialised vascular interface that separates circulating blood from neuroparenchymal extracellular fluid[108]. The BBB acts as a diffusion barrier that regulates the passage of molecules between blood and the brain parenchyma[109,110]; a function that is primarily facilitated by specialised cerebral microvascular endothelial cells that make up the BBB and the tight junctions between them[109,111,112]. Glial cells are also known play a supporting role in maintaining BBB integrity and permeability, with astrocytes, microglia and pericytes all being

integral to its formation, maintenance, and contributing to aspects of molecular transport selectivity and specificity[112–114]. Recently, the intimate relationships and dynamic interactions between these different types of cells has been acknowledged with the coining of the term *neurogliovascular unit*[115–117] (see Figure 1).

TBI is known to induce a number of processes that disrupt the structure and functioning of the BBB; some of which are instigated immediately after injury (i.e. hours) while others have a delayed presentation (e.g. 3-7 days)[114,118]. Regardless of when they occur, all can culminate in increased permeability of the BBB, which is often denoted by the terms *BBB breakdown* or *BBB dysfunction* in the literature.

BBB breakdown can arise as both a direct result of the TBI event itself (i.e. *primary BBB breakdown*) and as a consequence of the numerous other pathophysiological processes that occur in response to TBI (i.e. *secondary BBB breakdown*)[118]. Briefly, in the former instance, the traumatic impact of TBI inflicts immediate damage to the cerebral vasculature, which results in dysfunction of the tight junction complexes and compromises the integrity of the basement membrane of the neurogliovascular unit and subsequently leads to increased paracellular permeability of the BBB[114,119]. In addition, it has also been suggested that TBI may result in BBB dysfunction by way of inducing changes in the activity and/or expression of BBB-associated transporters that are responsible for mediating paracellular permeability[114]. On the other hand, BBB breakdown can arise secondarily from post-TBI pathophysiological events such as abnormal neurotransmitter activity, astrocytic dysfunction, inflammation-related mechanisms, metabolic disturbance[118] and oxidative stress[120]. While each of these individual processes contribute to the breakdown of the BBB *via* their own unique mechanisms, they also known to collectively alter the normal functional interactions between the cerebrovascular endothelium and supporting glial cells more broadly, which in turn results in further BBB dysfunction[114].

BBB breakdown can lead to the occurrence of secondary injury processes including oedema, neuroinflammation, ionic disturbances and subsequent cell death, which may independently or synergistically contribute to TBI pathophysiology[118]. Post-traumatic changes in BBB function have been recently been identified as a major factor in determining injury progression[121], and their occurrence may have significant implications the clinical management of TBI patients. Understanding BBB dysfunction is important as it has implications for the release of biomarkers into peripheral circulation, which may have predictive utility following mTBI.

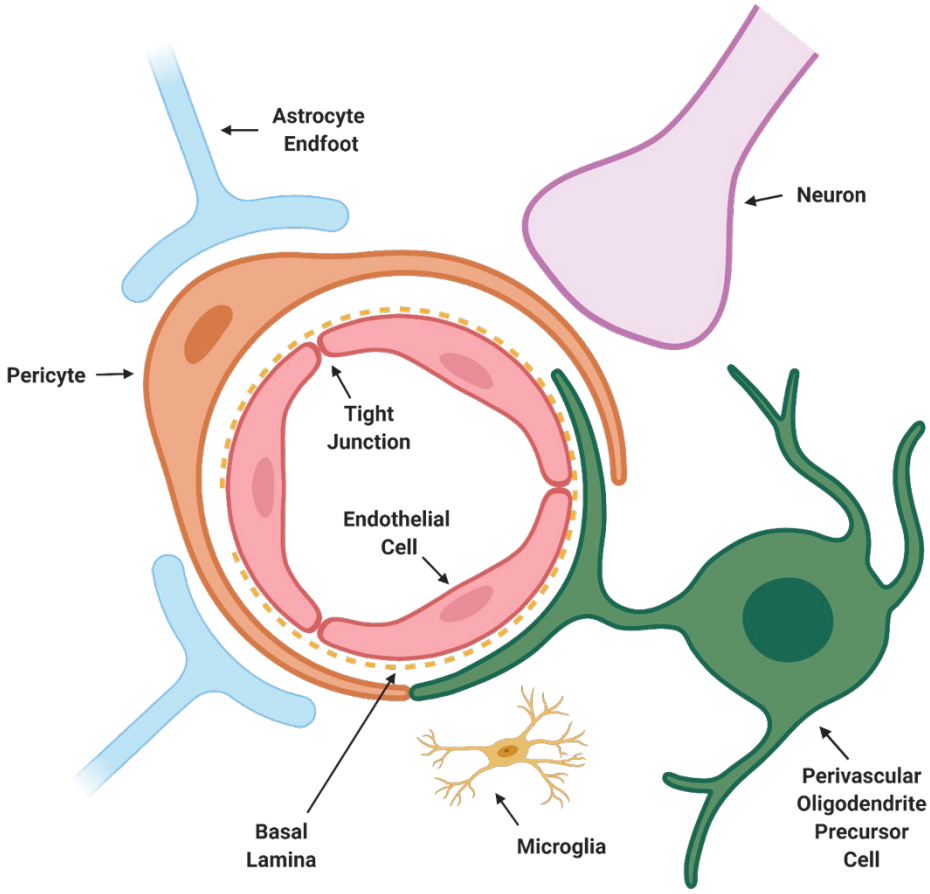

Figure 1. Components of the BBB. The BBB is a highly specialised vascular interface that comprises of astrocytic endfeet, microglia, neurons, pericytes and perivascular oligodendrocyte precursor cells. These cellular components collaboratively regulate the passage of molecules from circulating blood to the brain parenchyma. Figure adapted from Rustenhoven et al., 2017[122] and created using BioRender.com.

2.3.6 Neuroinflammatory Response

Neuroinflammation is a term used to denote inflammatory responses that occur within nervous tissue[123]. In TBI, damage occurring to the brain triggers a multifaceted inflammatory response involving numerous cellular components within the CNS and peripheral circulates. Depending on the time elapsed after injury, this inflammatory response can exhibit both neurodegenerative and/or neuroprotective effects[124,125], and is primarily mediated by two CNS cell types: microglia and astrocytes[126] (see Figure 2).

Microglia are the highly dynamic resident innate immune cells of the CNS[127], whose roles include surveying the CNS microenvironment for cellular debris[128,129] and facilitating it's removal *via* phagocytosis[130–132]. Microglia express an array of pathogen recognition receptors belonging to nucleotide-binding oligomerisation domain-like receptors and toll-like receptor families, which allow them to respond to pathogen-associated molecular patterns and danger-associated molecular

patterns that are secreted by neurons and other CNS cell types that are damaged by TBI[133]. Following trauma to the CNS, microglia become activated as part of the innate immune response, in which they transform from a resting 'ramified' state to a hypertrophic or 'bushy' morphology[134] and secrete numerous different factors, including anti- and pro-inflammatory cytokines, chemokines, growth factors, prostaglandins, and reactive nitrogen and oxygen species[119,135,136]. Collectively, these substances can exacerbate or ameliorate tissue damage resulting from TBI[136]. Post-TBI microglial activation has been studied in humans post-mortem using immunohistochemical techniques[137], as well as *in vivo* through the use of positron emission tomography[138]. Regardless of technique used, microglial activation has been observed to persist up to years after TBI has occurred[137,138].

Astrocytes are glial cells that play a critical role in sustaining physiological homeostasis within the CNS [126]. In addition, they help to maintain the integrity of the BBB through the formation of astrocytic end feet around endothelial cells[115,139,140]. Like microglia, astrocytes become reactive following CNS trauma[141]. This occurs through a complex and heterogenous process termed *reactive astrogliosis*[142], which involves changes in morphology, increased expression of glial fibrillary acidic protein (GFAP), intermediate filament proteins and vimentin, and heightened proliferation and secretion of immunomodulatory molecules (e.g. cytokines, chemokines and danger-associated molecular patterns) as well as growth factors[143–146]. Reactive astrocytes play a role in a variety of post-injury mechanisms, including the regulation of inflammation[141]. In fact, there are several ways in which reactive astrocytes can respond to inflammation, many of which exhibit both neurodegenerative and neuroprotective functionality[126,141,147].

One particularly noteworthy astrocytic-mediated response to TBI is the formation of astrocytic glial scars. Astrocytic glial scars are aggregates of several cell types, including astrocytes, microglia, endothelial cells, fibroblasts, and extracellular matrix[148], which are believed to serve as physical barriers that encapsulate damaged tissue. The neuroprotective functionality of astrocytic glial scars is two-fold, in that they prevent the leakage of toxic and inflammatory molecules and danger-associated molecular patterns from the injury site into surrounding healthy tissue, and limit access of invading cell types[126]. However, astrocytic glial scars have also been observed to have an inhibitory effect of axonal regrowth and regeneration[148,149].

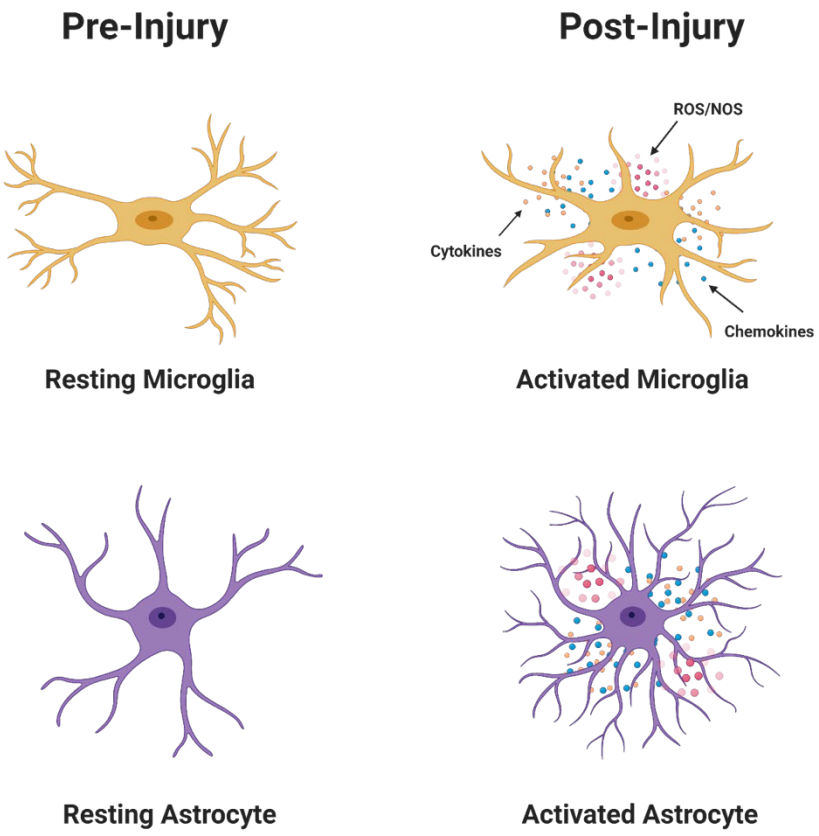

Figure 2. Neuroinflammatory glial response. Microglia and astrocytes play important homeostatic roles that are essential for maintaining neuronal function and survival. Together, these cells types also make up the primary immune component of the CNS. Under typical physiological conditions, microglia assume a highly ramified resting state and survey the CNS microenvironment for cellular debris and foreign bodies. Astrocytes, which play a central role in maintaining the integrity of the BBB, similarly exhibit a ramified appearance when in their resting state. Following TBI, microglia and astrocytes proliferate and undergo a range of molecular, structural and functional changes (including the secretion of different immunomodulatory factors, such as cytokines and chemokines) in response to the damage occurring within the brain parenchyma. These changes result in characteristic alterations in the morphological appearance of both types of cells, and help regulate brain inflammation and BBB permeability following TBI. *Note:* ROS/NOS: reactive oxygen species/ reactive nitrogen species. Figure created using BioRender.com.

2.3.7 Myelin Changes

Myelin is a lipid-rich substance[150] that forms a segmented, low-capacitance insulating coating around the axons of neurons in both the peripheral nervous system and the CNS called the *myelin sheath*[151,152]. Structurally, the myelin sheath is made up of repeating units of compacted double bilayers of myelin that are held together by myelin-specific structural proteins, such as myelin basic protein (MBP)[153] and proteolipid protein[154]. Mature myelin sheaths can comprise of up to 160 layers of compacted membranous lamellae that appear as interperiodic lines when visualised using electron microscopy[155]. The myelin sheath fulfils several important physiological functions. These include the facilitation of rapid transmission of action potentials *via* saltatory conduction[156,157], and

thereby refined neural signalling[157], as well as the protection and provision of trophic support to axons[155,158].

Within the CNS, myelin is produced by specialised macroglial cells called oligodendrocytes. Oligodendrocytes are characterised by their numerous cellular processes, which extend and wrap around neuronal axons in a spiral fashion to form discrete multilamellar nodes of the myelin sheath called *nodes of Ranvier*[159–161]. One oligodendrocyte can be responsible for producing up to 50 individual myelin segments that are located on different neurons[159,160], although this number can vary across different regions of the CNS[162,163]. Damage to, or the loss of, one oligodendrocyte can thus result in perturbed saltatory conduction in multiple pathways and render axons more vulnerable to further damage[164]. In addition to being responsible for myelination, oligodendrocytes are also known to influence axonal calibre and transport, the clustering of ion channels along axons and neuronal survival[165].

TBI can result in an array of abnormalities in the myelin sheath (see Figure 3). Falling under the umbrella term *dysmyelination*, specific examples of pathological myelin changes that have been found to occur as a consequence of TBI include myelin decompaction, fragmentation and complete degradation (i.e. demyelination/myelin loss)[166]. Damage to myelin can occur as a result of primary axonal damage and subsequent Wallerian degeneration[66] or from the secondary chemical cascades that occur in both acute and chronic stages of TBI[167]. Furthermore, myelin and oligodendrocyte cells have unique biological characteristics that make them both particularly susceptible to damage resulting from secondary degeneration processes. For example, the high lipid content of myelin inherently renders the myelin sheath vulnerable to oxidative stress and lipid peroxidation[157], which in turn can result in widespread dysmyelination following injury to the white matter (WM) of the CNS[168,169]. Similarly, oligodendrocytes and their progenitor cells known as *oligodendrocyte precursor cells*, are also susceptible to the effects of oxidative stress as they produce large amounts of ROS and have a low antioxidant reserves[170]. As such, oxidative stress can result in fewer oligodendrocytes and their precursor cells, thereby impairing the capacity for remyelination.

The effects of myelin damage and/or loss are thought to be clinically significant and are believed to contribute to impaired cognitive function that is seen following TBI[171,172], for which there is evidence in rodent studies of TBI[173].

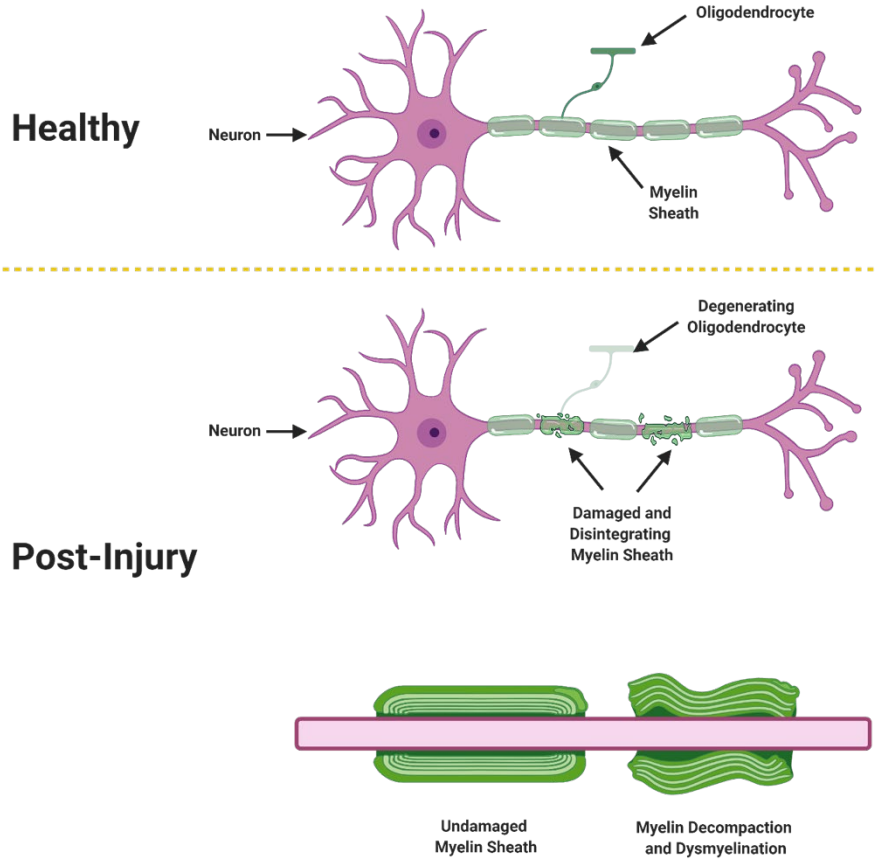

Figure 3. Myelin changes following mTBI. Oligodendrocytes are specialised glial cells that wrap around the axons of CNS neurons to form the myelin sheath. TBI results in a number of pathomechanisms that ultimately damage the myelin sheath. Damage to the myelin sheath may take the form of dysmyelination, myelin decompaction, myelin loosening, disruption to myelin sheath proteins, diffusion of myelin nodal components along the neuronal axon, increased number of atypical nodal complexes and well as incomplete remyelination. Figure created using BioRender.com.

2.3.8 Diffuse Axonal Injury

Diffuse axonal injury (DAI) is a progressive, delayed secondary form of CNS injury typically observed following high-impact traumatic insults involving sudden acceleration, deceleration or rotational movements of the head[155,174–177]. The most common cause of DAI are high-speed motor vehicle accidents[178], however, DAI is also thought to be present following blast injuries[179,180] and head injuries resulting from high-speed collision sports such as football, hockey and soccer[181]. The mechanical loadings associated with such injuries cause some regions of the brain to accelerate or decelerate faster than others[175], which generates shear and tensile forces that stretch and deform the brain tissue, and ultimately result in widespread, multifocal mechanical damage to axons[174,182]. Contrary to what the name implies, DAI is not diffuse throughout the whole brain[174,181]. Rather, when it occurs, DAI is predominantly found in the corpus callosum, brainstem, cerebellum and white-grey matter tissue interface[167,183,184]. The Adams DAI classification combines clinical

presentation and the locality of lesions to categorise the severity of DAI into to three distinct injury gradings, which range from microscopic white matter changes (Grade 1) to severe focal lesions (Grade 3)[155,183].

DAI is rarely seen on conventional neuroimaging, especially within the early stages of injury[181]. Only 10% of TBI patients with DAI have been found to demonstrate classic CT DAI findings, which are characterised by petechial haemorrhagic lesions present in the corpus callosum, white-grey matter junction of the cerebrum and the pontine-mesencephalic junction near the superior cerebellar peduncles[185]. In contrast, MRI is much more sensitive than CT in detecting DAI, which typically appears as hypointense foci at the white-grey matter interface on T2*-weighted gradient recalled echo (GRE) images[186,187]. Recent advances in susceptibility weighted imaging and diffusion weighted-MRI have further facilitated better detection and quantification of haemorrhagic and non-haemorrhagic DAI lesions, respectively[185].

DAI typically manifests in 40-50% of individuals requiring hospitalisation for their TBI, and is closely associated with LOC and poor outcome following head trauma[181]. Shear axonal injury can result in Wallerian degeneration, transection, and even cell death, which may compromise cortical and subcortical pathways and ultimately culminate in impaired functional ability[49,188]. DAI is hypothesised to contribute to many TBI symptoms, and may be the pathological substrate of enduring neurological impairments and cognitive dysfunction that have been observed to occur following TBI, including mTBI[189,190].

2.4 Symptoms of mTBI

The physiological disruption of brain function that occurs in mTBI can manifest as a number of different clinical *signs* that can be observed, and *symptoms*, which are subjective and only apparent to the affected individual. The signs and symptoms of mTBI can be subtle, are generally non-specific, and may not always present immediately following injury[191]. Furthermore, individual signs and symptoms are often accompanied by a unique onset, evolution and resolution trajectory[192], and it possible that clinical presentation may be influenced by the extent and location of injury[12].

Given that most signs and symptoms of mTBI are subjective, they are most commonly evaluated using self-report measures, of which there are many[193]. However, ongoing efforts to devise more objective methodologies for evaluating mTBI signs and symptoms have seen the development of assessments of exercise tolerance, functional gait/balance and vestibular-ocular motor function, as well as computerised neurocognitive testing batteries.

Individuals who have sustained a mTBI typically present with a constellation of symptoms that is unique to them[194], although some symptoms have been found to be endorsed more frequently

than others. Despite the considerable variation in clinical presentation, the signs and symptoms of mTBI can be broadly categorised into the following four distinct 'symptom clusters': *i) physical/somatic*, *ii) cognitive*, *iii) affective/emotional*, and *iv) sleep*[11,195,196] (see Figure 4).

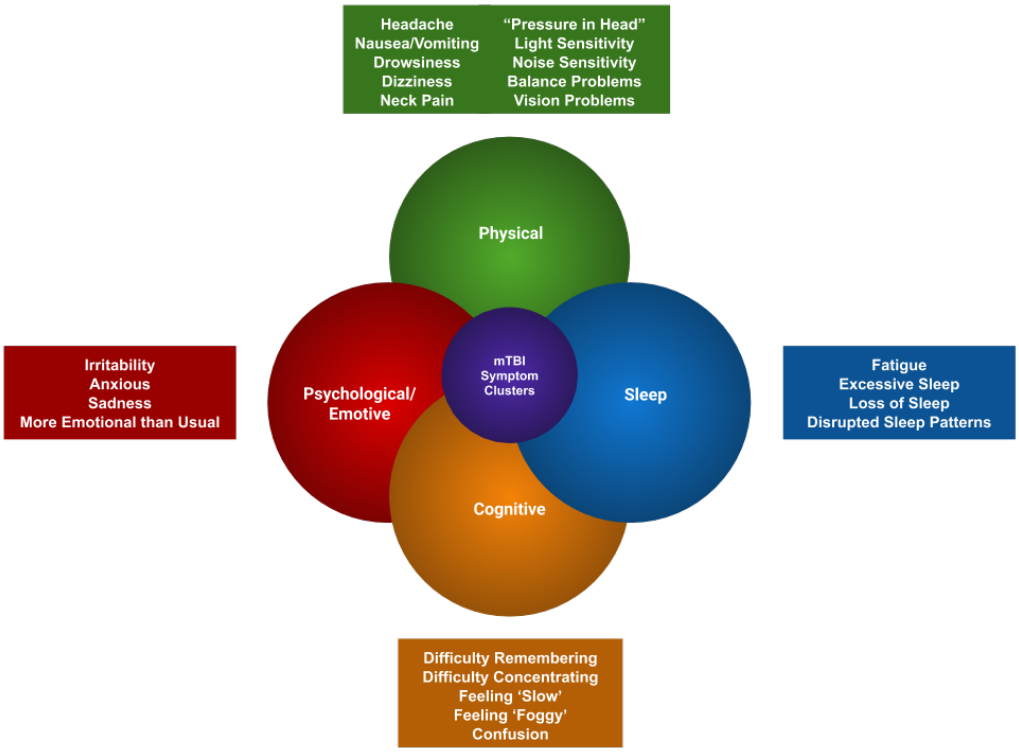

Figure 4. Categories of mTBI signs and symptoms. mTBI is a heterogeneous injury that is associated with a number of diverse signs and symptoms. These signs and symptoms can be broadly classified into physical, cognitive, emotional/affective, and sleep-related domains. Clinical presentation can differ considerably between individuals and individual instances of injury in cases where an individual has experienced several mTBIs over time. Original figure generated from information presented in Pardini et al., (2004)[11], Kontos et al., (2012)[195], and Merritt & Arnett (2014)[197].

The following section of this literature review serves to provide the reader with a brief overview of each of these symptom categories, providing explanations of the underpinning mechanisms where possible. Understanding mTBI symptoms and their theoretical underpinning is necessary to better understand the diversity in mTBI clinical presentation and the persisting symptoms that may ensue, as well as whether and how early symptom presentation may serve to predict poor outcome following injury.

2.4.1 Physical Symptoms

Physical signs and symptoms are amongst the most common complaints following mTBI, and include headache, dizziness, nausea and vomiting, balance disruption, sensitivity to light and

noise, vision and/or hearing disturbances, and lethargy[10,198,199]. Of these, the most commonly reported symptom is headache[191,192,195,200–206], although other frequently reported/observed physical symptoms include confusion, dizziness, balance problems, and fatigue[191,192,204–211]. The physical symptoms of mTBI are largely thought to arise from a host of physiological disturbances that result from injury and affect the brain and/or specific sensory systems (e.g. ocular, vestibular)[84], and may be maintained or exacerbated by interactions between individual underpinning and other extraneous mechanisms (e.g. behavioural changes).

2.4.2 Cognitive Symptoms

Relative to physical signs and symptoms, the cognitive symptoms of mTBI are less overt and can be more difficult to assess. As such, they may not always be immediately apparent to the affected individual or their treating clinician. Commonly endorsed cognitive symptoms include feelings of 'fogginess', difficulties concentrating and/or remembering, slower processing speed and confusion[198,199,212]. When present, cognitive symptoms typically develop within the first few hours to week following injury [213] and are generally believed to remit 1 to 3 months post-injury[206,214–222], however, the results of a recent meta-analysis suggest that chronic cognitive impairment may be present in as many as half of individuals who experience a single mTBI[223].

The effects of mTBI on cognition have been extensively investigated although the ability to draw firm conclusions from the reported results is often complicated by the divergent study methodologies. Factors complicating the interpretation of findings include the use of different cognitive tests, diagnostic criteria, post-injury assessment intervals, patient groups (e.g. collegiate athletes, consecutive hospital admissions, symptomatic referrals), mechanisms of injury (e.g. sports-related concussion, motor vehicle accidents, mixed causes) and levels of injury severity examined (i.e. only mild, mild and moderate, or mild to severe TBI)[224,225]. Nevertheless, it is generally thought that mTBI can affect the cognitive domains of *attention, memory*, and *executive functioning, visuospatial processing* as well as the generalised cognitive ability of *processing speed*.

Attention deficits are a common consequence of TBI irrespective of injury severity[226–228], and are thought to arise from damage to and disruption in the functioning of various components of the frontal, cingulate, parietal and temporal cortices, as well as the mid-brain region that are implicated in attention[229–231]. Amongst individuals who have sustained a mTBI, deficits in *selective*, *sustained*, *alternating* and *divided* types of attention are particularly prominent[217,220,224,232–237]. Attentional deficits may manifest as increased tendencies towards distractibility and impulsivity, and these too have been observed in individuals following mTBI[234,238,239].

Memory impairment is also evident across all TBI severity levels[240–242]. Trauma-induced memory deficits are primarily believed to result from dysfunction in the cerebral cholinergic[243] and

catecholaminergic[244] systems. Difficulty remembering recent events is a common complaint that has been observed amongst individuals with mTBI[204,245], although many other types of memory have been found to be impaired following mTBI, including *working*, *episodic*, and *immediate* and *delayed verbal* and *visual* memory[220,224,246–251]. Impaired memory ability can have significant ramifications for affected individuals, potentially impacting their ability to live independently and/or return to work[252].

Executive functioning is considered as a disparate, multifaceted neuropsychological construct, which consists of a broad set of higher-order neurocognitive processes that are necessary for the cognitive control of behaviour[253,254]. Numerous models of executive functioning have been proposed[255], and it is widely recognised that executive functioning is mutually interdependent with other cognitive domains, particularly attention and memory[256]. Impaired executive functioning following mTBI is generally believed to result from direct insult to the frontal lobes and/or from disruption to their connections with other brain regions[255]. mTBI has been found to affect a number of executive function processes, including *planning, sequencing, set-shifting, verbal and non-verbal fluency*, and *working memory* abilities[206,220,224,247,257–262], and it appears that executive functioning may also be particularly susceptible to the effects of multiple mTBI[214].

Visuospatial cognition comprises of a range of abilities that facilitate the processing of visual stimuli, comprehension of spatial relationships between objects and visualisation of different images and scenarios[263]. Primarily underpinning visuospatial functioning are the dorsal and ventral visual pathways[264], although it has recently been suggested that the parietal lobe may also play an important role in general visuospatial cognitive abilities while the right hemisphere may be dominant for certain visuospatial skills[263,265,266]. Additional brain areas implicated in visuospatial cognition include the anterior insular cortex, dorsolateral prefrontal cortex, temporal-occipital cortex, precentral gyrus, superior frontal gyrus/sulcus and the dorsal anterior cingulate cortex of the left hemisphere[263]. mTBI was previously not thought to have a profound effect on the visuospatial cognitive domain[249], however, there is mounting evidence of deficits in visual perception and visual-constructional reasoning amongst children and adults with mTBI[267–275].

Processing speed has been defined as the cognitive ability to "*identify, discriminate, integrate, make decisions about information, and to respond to visual and verbal information*"[276] and is known to play a role in attention, memory and executive functions. Typically measured using time-based tasks that involve motoric or oral response[276], processing speed has also been found to be compromised across all TBI severities[277,278]. Following mTBI, individuals have been observed to be slower and less accurate at processing information[235,279–281], and to have increased reaction times[281,282], particularly on forced choice reaction tasks[221,278,283,284]; although this may be contingent upon the working memory load associated with the task being performed[285].

2.4.3 Emotional Sequelae

The emotional sequelae of mTBI are numerous, and include anxiety or nervousness, depression, emotional lability and irritability[198]. Individuals who report experiencing depressive symptoms prior to mTBI have been found to be more likely to experience emotional symptoms following injury[286]. However, there is also evidence that suggests mood disturbance can also arise independently of premorbid mood conditions after sport-related concussion[287]. Affective symptomatology following mTBI may also occur as a consequence of other mTBI sequelae (e.g. physical symptoms, sleep disturbances), although further investigation is required to elucidate the nature of this relationship.

Depression is amongst the most commonly investigated emotional correlates of mTBI, particularly sports-related concussion[287]. Relative to the general population and orthopaedic injury controls, *major depressive disorder* has been observed to be more prevalent amongst individuals who have sustained a mTBI, with rates of around 20% being reported 3-6 months post-injury[288,289]. Each year, approximately 6% of individuals with a history of mTBI are diagnosed with clinical depression[212], and a dose-response relationship has been observed between the number of prior concussions and cases of depression reported in a large retrospective study of retired football players[290]. While it is common for individuals to report symptoms associated with depression following mTBI, it is important to note that not all meet the diagnostic criteria necessary to be diagnosed with a clinical disorder[291]. As such, it has become increasingly common for studies to examine the prevalence of *postconcussion depression symptoms*[292–294], as opposed to depression *per se*. When present, depressed mood subsequent to mTBI has been found to follow a similar time course to that which has been observed for impaired cognitive function. That is, depression-like symptoms typically emerge within the first few days to week following injury[286,295,296], may persist for up to two weeks[296], and generally resolve within a month[287,295].

The reasons as to why individuals may experience symptoms of depression following mTBI are poorly understood, though it is likely that they arise from complex interactions between interrelated neurobiopsychosocial factors that vary, not only between individuals, but also across the recovery trajectory itself[297]. Early accounts attributed post-mTBI depression and depression-like symptoms to psychosocial causes such as anger, experience of loss, frustration, and functional limitations due to injury[298,299]. However, it is increasingly being recognised that the neurochemical and metabolic disturbances, inflammatory responses, and changes in brain structure that accompany mTBI may also play a role[286,300–302].

Despite clinical reports of post-concussive irritability and *"nervousness"*[303], research investigating the extent to which anxiety disorders and anxiety-related symptoms emerge following mTBI is limited. Most studies conducted to date have involved small samples sizes and have

focussed on categorical classifications of anxiety, despite calls to adopt more dimensional conceptualisations[304]. Nonetheless, a study by Mooney & Speed (2001)[305] found that 24% of participants in a clinical sample developed an anxiety disorder following their mTBI, and virtually all types of types of anxiety disorder (e.g. *Post-Traumatic Stress Disorder, Obsessive Compulsive Disorder, Generalised Anxiety Disorder, Panic Disorder, Social Phobia*) have been documented following mTBI[304]. Furthermore, and as is consistent with the wider literature, anxiety and depression have been observed to co-occur following mTBI. For example, Jorge and colleagues[306] found 17% of individuals in a clinical sample of mTBI patients met the diagnostic criteria for both *Generalised Anxiety Disorder* and *Major Depressive Disorder*, which they termed *Anxious Depression*.

It remains unclear why some individuals develop anxiety disorders or experience anxiety-like symptomatology following mTBI while others do not, although several explanations relating to organic causes and psychogenic origins have been proposed. For example, it has been suggested that anxiety-related psychiatric sequelae may emerge as a general consequence of injury to the temporolimbic areas of the brain (e.g. amygdala, basal ganglia and frontal lobes) that are known to be affected in the regulation of anxiety[307,308]. Furthermore, there is also evidence that indicates that injury within other localised brain areas is associated more specific anxiety-related disorders. For example, a meta-analysis by Simmons & Matthews (2012)[309] concluded that the middle frontal gyrus, caudate nucleus, and anterior cingulate may be salient to the development of *post-traumatic stress disorder* following mTBI. From a psychogenic perspective, it is believed that the sudden and unexpected experience of acute (e.g. post-traumatic amnesia, loss of consciousness, hospitalisation) and/or long-term stressors (e.g. job loss, chronic symptomatology) has the potential to negatively influence an individual's mental health following mTBI by way of compromising their current psychological defences or coping strategies, and/or contributing to the exacerbation of pre-existing anxiety conditions[310].

2.4.4 Sleep-Related Changes

Disruptions to the sleep-wake cycle have been noted to occur following all severities of TBI[311–313]. Some of the most commonly reported sleep-related complaints include difficulty falling asleep and/or maintaining sleep, and excessive sleep[314]. The prevalence of sleep disturbances that occur after TBI vary widely across all severities, with reports ranging from 30-84%[315]. Numerous methodological factors contribute to these discrepancies, including variation in the criteria and measures used to assess sleep, as well as differences in sample characteristics[315]. Sleep-related disturbances, disorders and problems have only recently been recognised as a unique symptom cluster associated with mTBI, and while investigations are currently scarce[316–318], the field presents ample opportunity for study.

Sleep disturbances following mTBI are believed to result from a variety of causal mechanisms, which include trauma-induced biochemical and structural changes[287,319], co-morbid neuropsychiatric conditions such as depression[320] and/or neuromuscular pain[319], and are also likely to be impacted by the presence of pre-existing sleep-related problems[315]. Sleep problems may also arise from focal or diffuse lesions occurring within brain structures constituting the neuronal systems involved in sleep-wake cycle regulation[318,321], such as the brainstem[318,321,322], mid-brain[323], basal forebrain and hypothalamus[318]. Furthermore, specific sleep-related symptoms may also arise as part of the injury recovery process. For example, Kostyun et al., (2015)[324] have suggested that hypersomnia may arise in response to the brain's attempt to re-establish neurometabolic homeostasis to pre-injury levels. In doing so, hypersomnia may function as a protective mechanism used by the body to prevent the brain from engaging in exertional activities that may exacerbate symptoms and thereby potentially prolong recovery[324].

While sleep disturbances can be, and often are, easily overlooked by clinicians, it is important that they are recognised due to their potential to exacerbate both mTBI-related sequelae including anxiety, depression, cognitive deficits, fatigue, irritability, pain and functional impairments, and as pre-existing health conditions[314,315].

2.5 Clinical Profiles of mTBI

The recognition of heterogeneity among the clinical presentation of mTBI has led to the emergence of several clinical models featuring multiple mTBI clinical profiles, also referred to as *domains*, *phenotypes*, *subtypes*, or *trajectories*[201,325–327], which have the potential for more specific prognostic value and targeted treatment[328]. Although the concept is novel and does not reflect current clinical standards or norms, it is hoped that adopting such an approach may help improve patient outcomes through the facilitation of better clinical assessment and individualised patient management[328].

Kontos & Collins (2018)[329] recently described a clinical model that appears to be gaining traction within the mTBI community. In this particular model, the authors identify the following six distinct clinical profiles: *i) cognitive-fatigue, ii) post-traumatic migraine, iii) anxiety/mood; iv) vestibular, v) ocular* and *vi) cervical*. Each clinical profile is characterised by a specific set of symptoms, common findings observed at clinical examination, associated risk factors, and suggested strategies for targeted treatment. Acknowledging the important role of sleep, the authors identify sleep as a modifier capable of exacerbating symptoms and acting as a potential target for treatment. In this model, the authors point out that each clinical profile is not mutually exclusive, and that some symptoms and impairments may be common to several profiles. Furthermore, the authors also stress

that while some patients may present with a single clinical profile, it is possible for others to present with two or more clinical profiles simultaneously. In cases when multiple clinical profiles are present, the profiles ought to be distinguished into primary, secondary and tertiary profiles, with the most salient profile being prioritised and guiding the overall treatment approach to be undertaken. This model has since been revised and now features five clinical profiles, which are as follows: *i) cognitive-fatigue; ii) post-traumatic headache, iii) anxiety/mood; iv) vestibular* and *v) ocular*[330] (see Figure 5). With distinctions between cerebral and cervical-type concussions being increasingly recognised, the cervical clinical profile that was proposed in the original model is no longer considered a distinct profile, but rather is now considered to be a *profile modifier*, along with sleep. This revised model has recently undergone a preliminary empirical investigation in which the frequency of each of the five primary clinical profiles and the associations amongst the different profiles were examined[330]. Results of this seminal study, which involved 236 patients recruited from a specialty concussion clinic, found *post-traumatic migraine* (26%) and *anxiety/mood* (24%) to be the most common clinical profiles, while the *vestibular* and *ocular* profiles together accounted for over one-third (35%) of the clinical profiles observed. Furthermore, the study's findings supported the notion that individuals could present with multiple clinical profiles, with several relationships identified among individual clinical profiles.

2.6 Diagnosing mTBI

There are currently no universally accepted diagnostic criteria for mTBI. Consequently, the process of diagnosing a mTBI can be particularly challenging for health professionals. Numerous diagnostic criteria have been proposed, however, three of the most widely used definitions are those that have been developed by the *i) Mild Traumatic Brain Injury Committee of the Head Injury Interdisciplinary Special Interest Group of the American Congress of Rehabilitation Medicine (ACRM, 1993)*, *ii) US Center for Disease (CDC) Control working group (CDC, 2003)* and *iii) WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury (WHO, 2004)*[331,332]. Specifics of these definitions are presented in Figure 6 below.

The process of diagnosing mTBI can be further complicated by the fact that many of the signs and symptoms of mTBI, or assessment tools used as part of the diagnostic process (e.g. GCS) are difficult to assess and/or administer in paediatric patients, intoxicated individuals, and those with pre-existing neurological conditions[53]. Moreover, the clinical presentation of mTBI may also be influenced by extraneous factors, such as affected individual's ignoring, hiding or exaggerating their mTBI symptoms[333].

Revising definitions of mTBI remains an active area of research. Recently, a cross-sectional web-based survey was conducted with an international group of interdisciplinary clinician-scientists to update the ACRM 1993 definition of mTBI[334]. Results of this investigation identified several potential revisions to consider when updating the definition, which included placing a greater emphasis on observable signs, incorporating symptoms as acute test findings, and adding differential diagnosis considerations. It is anticipated that future conceptualisations and/or revisions of mTBI diagnostic criteria will emphasise such elements.

2.7 Assessing mTBI

mTBI can affect a variety of different brain functions, and as such, it is becoming increasingly recognised that it may be beneficial for clinicians to utilise a battery of tests to ensure adequate assessment and monitoring of injury. This multi-modal approach to clinical assessment is particularly important when considering that recovery rates are known to differ amongst the brain functions known to be affected by mTBI[213,335]. Recent recommendations suggest that a comprehensive clinical evaluation ought to include assessing patient's symptoms *via* self-report, as well as objective measures of their ocular function, vestibular function, gait, postural stability and neurocognitive functioning[328,336]. There is also scope to incorporate the use of fluid and genetic biomarkers, as well as advanced neuroimaging techniques into clinical assessment, however, further investigation into each of these is required to validate their clinical utility[337].

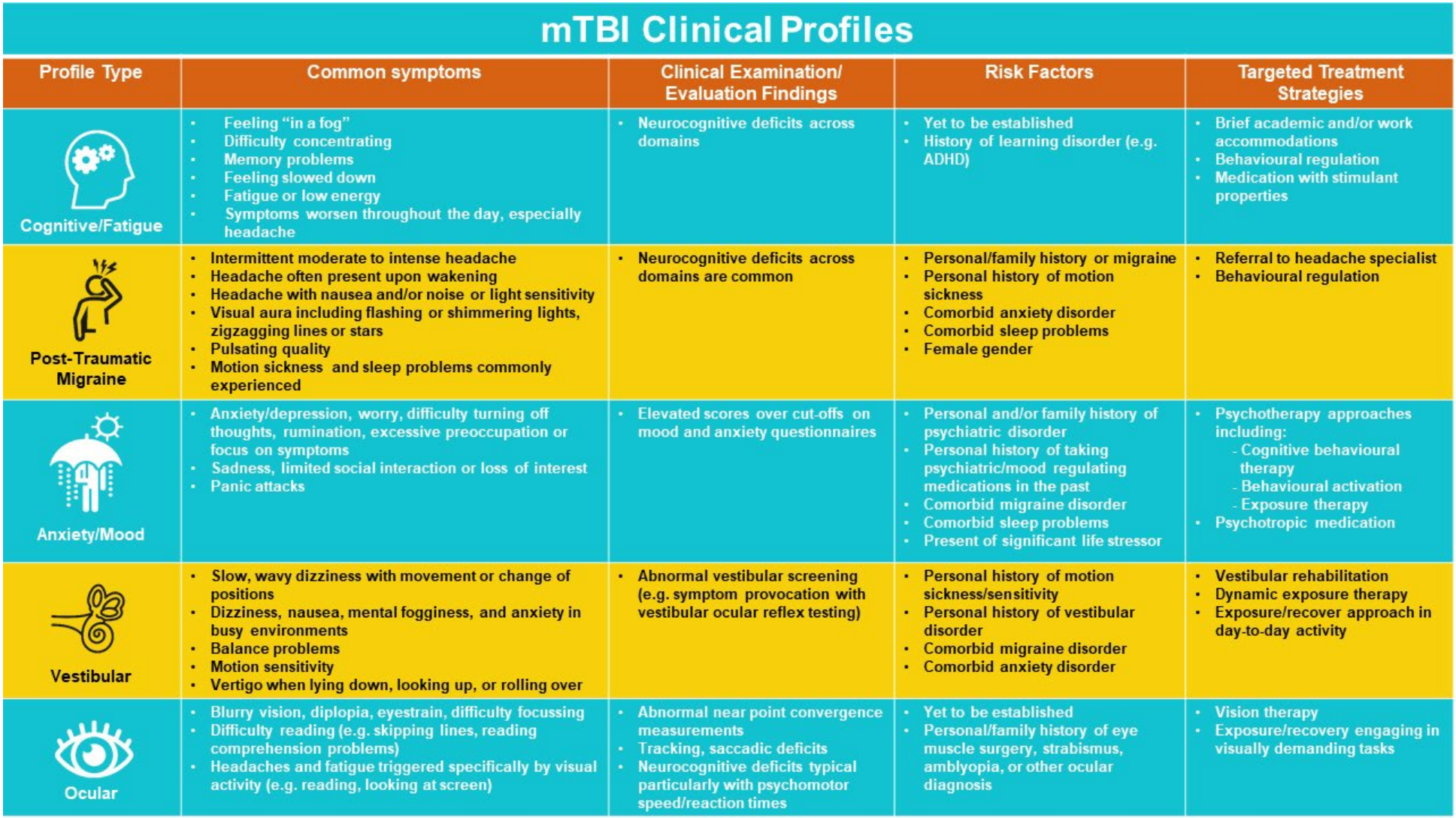

Figure 5. Emerging clinical profiles of mTBI. In an attempt to better capture the heterogeneous nature of mTBI, Kontos and colleagues have proposed a clinical model that comprises of five distinct mTBI clinical profiles. Each clinical profile is associated with its own set of commonly observed signs/symptoms, clinical examination/evaluation findings, risk factors and targeted treatment strategies. Original figure created based on information presented in *Table 1* in Kontos et al., (2019)[330].

mTBI Definitions

Organisation	Definition	Unique Features of Definition
American Congress of Rehabilitation Medicine (1993)	A traumatically induced physiological disruption of brain function, as manifested by *at least* one of the following: 1. Any loss of consciousness 2. Any loss of memory for events immediately before or after the accident 3. Any alteration in mental state at the time of the accident (e.g. feeling dazed, disoriented, or confused) and 4. Focal neurological deficit(s) that may or may not be transient But where the severity of the injury does not exceed the following • Loss of consciousness of approximately 30 minutes or less • After 30 minutes, an initial Glasgow Coma Scale (GCS) of 13-15 and • Postraumatic amnesia (PTA) not greater than 24 hours	• Broad definition • Includes persistent neurological deficits
Centers for Disease Control and Prevention (2003)	The conceptual definition of mTBI is an injury to the head as a result of blunt trauma or acceleration or deceleration forces that result in *one or more* of the conditions listed below Any period of observed or self-reported • Transient confusion, disorientation, or impaired consciousness • Dysfunction of memory around the time of injury • Loss of consciousness lasting less than 30 minutes • Observed signs of neurological or neuropsychological dysfunction, such as: - Seizures acutely following injury to head - Among infants and very young children: irritability, lethargy, or vomiting following head injury - Symptoms among older children and adults such as headaches, dizziness, irritability, fatigue or poor concentration, when identified soon after injury can be used to support the diagnosis of mild TBI, but cannot be used to make the diagnosis in the absence of loss of consciousness or altered consciousness. Research may proved additional guidance in this area. More severe brain injuries were excluded from the definition of mTBI and include one or more of the following conditions attributable to the injury • Loss of consciousness lasting longer than 30 minutes • Post-traumatic amnesia lasting longer than 24 hours • Penetrating craniocerebral injury	• Broad definition • Does not specify duration of PTA • Attempts to provide guidance for diagnosis of mTBI in infant/young children
World Health Organization Collaborating Centre for Neurotrauma Task Force on mTBI (2004)	mTBI is an acute brain injury resulting from mechanical energy to the head form external physical forces. Operational criteria for clinical identification include: (1) 1 or more of the following: confusion or disorientation, loss of consciousness for 30 minutes or less, post-traumatic amnesia for less than 24 hours, and/or other transient neurological abnormalities such as focal signs, seizure, and intracranial lesion not requiring surgery; and (2) Glasgow Coma Scale of 13-15 after 30 minutes post-injury or later upon presentation for healthcare. These manifestations of mTBI must not be due to drugs, alcohol, medications, caused by other injuries (e.g. symptom injuries, facial injuries or intubation), caused by other problems (e.g. psychological trauma, language barrier, or coexisting medical conditions) or cause by penetrating craniocerebral injury	• Broad definition • Describes neurological abnormalities as 'transient' only • Excludes cases where manifestation may be due to other causes

Figure 6. Commonly used definitions of mTBI. Figure generated from material sourced from Iverson & Lange (2011)[332] and Rosenbaum & Lipton (2012)[338].

2.8 Persisting Post-Concussion Symptoms

Persisting Post-Concussion Symptoms (PPCS) is a term used to describe a broad symptom complex in which individuals continue to experience the symptoms of mTBI beyond the time-frame that it typically takes for symptoms to resolve[339]. Generally speaking, mTBI symptoms are thought to resolve within 2 weeks of injury amongst adults[212], while paediatric and adolescent populations are typically expected to recover within 4 weeks of injury[340]. Like mTBI, the clinical presentation of PPCS is idiosyncratic and individuals experiencing this condition may report vastly different symptoms.

The concept of PPCS has undergone several iterations over time, which in turn can make it difficult to survey in the literature and draw conclusions. As is the case for mTBI, one of the primary reasons driving the apparent lack of consistency is the variation in the nomenclature used to refer to the condition and the definitions used to characterise it. For example, the terms *Post-Concussional Disorder* (*Diagnostic and Statistical Manual of Mental Disorders*- 4th Edition (*DSM-IV*)[341] and *Postconcussional Syndrome* (*International Classification of Diseases*-10th Edition (*ICD-10*)[342] have both been previously used to refer to PPCS-like conditions. While both definitions are associated with a unique set of diagnostic criteria, many studies have also used some sort of variation of these terms (e.g. *Post-Concussion Syndrome*), and do not always provide clear or consistent operational definitions. Furthermore, research centring upon the investigation of outcome following mTBI has recently expanded to incorporate additional facets of recovery that extend beyond mTBI-related symptomatology. This includes functional outcomes, such as return to work, return to study, return to physical activity, return to play (in sporting contexts), various activities of daily living (e.g. driving), and quality of life. While functional outcomes are not strictly the primary focus of this thesis, it is important that they are acknowledged.

PPCS has been subject to uncertainty and controversy over time. Much of this surrounds the reported prevalence rates of the condition[343,344], and the non-specific nature of many mTBI symptoms[59,345], which in turn can results in the condition mimicking several other diagnoses, such as anxiety, depression, chronic pain and somatisation disorders[196]. While this cannot be overlooked, it is also important to acknowledge that the effects of PPCS are debilitating to sufferers, as is illustrated in recent case studies[346,347] and patient perspective reports[348].

2.8.1 Defining PPCS

Historically, individuals who have experienced delayed recovery following mTBI have been diagnosed with *Postconcussional Disorder* or *Postconcussional Syndrome* using criteria listed in either the *DSM-IV*[341] or the *ICD-10*[342], respectively[343,344,349]. While both sets of criteria require a head injury to occur proximately to the onset of symptoms reported, there are subtle differences in the number of symptoms that are required to be endorsed by the individual, the duration of time that they are experienced, and the need for objective evidence of impairment and/or report of impaired functioning (see Figure 7).

Postconcussional Disorder	Postconcussional Syndrome
DSM-IV	**ICD-10**
1. History of head trauma with significant cerebral concussion 2. Difficulty with attention or memory based on objective testing 3. Three or more of the following symptoms occur shortly after trauma and last at least 3 months: a) Headache b) Dizziness or vertigo c) Becoming easily fatigued d) Irritability or aggression e) Disordered sleep f) Anxiety, depression or affective lability g) Changes in personality h) Apathy or lack of spontaneity 4. These symptoms start or substantially worsen after head trauma 5. Significant impairment in social, occupational or school functioning	1. History of head trauma with loss of consciousness 2. No clouding of consciousness or significant objective memory deficit 3. Three or more of the following symptoms: a) Headache, dizziness, malaise, fatigue or noise intolerance b) Irritability, emotional lability, depression or anxiety c) Subjective difficulty in concentration, mental tasks or memory impairment d) Insomnia e) Reduced intolerance to alcohol f) Preoccupation with the above symptoms or adoption of the sick role

Note: ICD-10 criteria does not specify a minimum length of symptom presence, and can begin any time within 1 month of the injury.

Figure 7. Diagnostic criteria for Postconcussional Disorder and Postconcussional Syndrome. Figure generated from material presented in Iverson & Lange (2011)[343].

In contrast to the above, there is currently no standardised diagnostic criteria for PPCS, nor is there an agreed upon duration of time that an individual must experience symptoms for in order to be considered as suffering from the condition. Many individuals experience mTBI-related symptoms following their injury. However, the vast majority recover spontaneously within 1-3 months[31,206] and thus, it is becoming increasingly common for researchers and clinicians to adopt 3 months as a cut-offs for PPCS. This particular choice of time point is further supported by research that indicates individuals with PPCS are generally seek help between three and six months following injury[350,351]. Furthermore, conventional wisdom states that early intervention is integral for optimising patient outcomes, and thus it may also be useful to examine PPCS as the 1 month. In doing so it may be possible to identify individuals' risk of delayed recovery following mTBI so that they may be triaged to personalised, evidence-based treatments that aim to prevent and reduce PPCS in a more timely fashion.

2.8.2 Prevalence

It is commonly cited that 10-20% of individuals who have sustained a mTBI will go on to develop PPCS[18,339]. However, upon closer examination of the existing literature it is clear that there is considerable variation in the reported prevalence rates of PPCS. Factors contributing to this include variation in the way in which PPCS has been ascertained amongst studies, which is partly driven by the lack of specificity in the symptom constellation[332], research design factors (e.g. study population, setting and timing of recruitment)[352], as well as the time points following injury that are examined by individual studies. Reported estimates of PPCS, or PPCS-like conditions (i.e. *Post Concussion Syndrome* and the like), range from 22-59%[353–355] at 1 month post-injury, 15-64% at 3 months post-injury[27,31,206,354,356–364], 21-49.8% at 6 months post-injury[27,354,363,365–367], and 21-47.9% at 12 months post-injury[363,366,368–370]. Investigation into the long-term effects and consequences of mTBI beyond 12 months after injury is currently limited though individuals who have sustained a mTBI have been found to report mTBI-symptoms for as long as 3[371], 5-7 years[372], and 11 years post-injury[371], in addition to reporting reduced quality of life[373] and life satisfaction[374]. Although the reported prevalence rates are likely to be subject to sample biases, it is possible that the true prevalence rate of PPCS is higher than is generally thought and may change as a function of time.

2.8.3 Aetiology

There has been a longstanding and controversial debate surrounding the aetiology of PPCS[339,375]. Seminal work by Lishman (1988)[376] proposed an aetiological model that distinguished between physiogenic and psychogenic factors underpinning the condition. Accordingly, this model suggested that neurobiological factors were most relevant in the acute stages of injury and accounted for the development of PPCS, while psychological factors became more salient in the chronic stages of injury and were significant for perpetuating the experience of ongoing symptoms. Silverberg and Iverson[375] recently performed a systematic review of the literature in which they re-visited and re-evaluated Lishman's hypothesis. Based on their findings, the authors concluded that both neurobiological and psychological factors play important roles in the development of PPCS but called for a revision of Lishman's model. Continued research has helped better understand the aetiology of PPCS, and Lishman's model has since been superseded by a biopsychosocial conceptualisation of the disorder (see Figure 8). This revised approach acknowledges that a myriad of individualised genetic, developmental, social, psychological and biological resilience and vulnerability factors individually contribute and interact with each other to both good and poor outcomes amongst individuals who have sustained a mTBI[377,378].

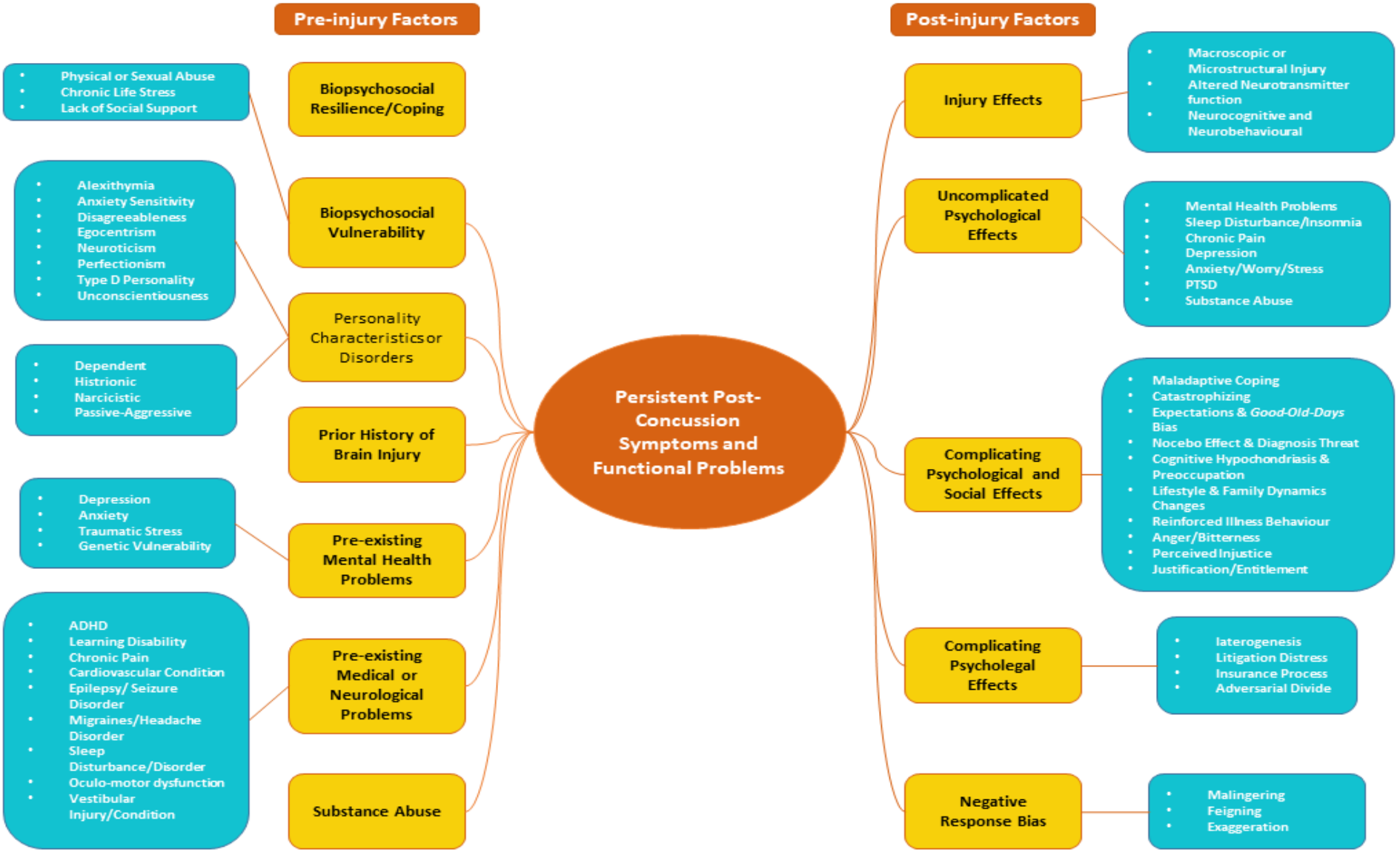

Figure 8. Biopsychosocial factors potentially influencing outcome following mTBI. A myriad of factors may singly, or in combination, contribute to an individual's injury experience and bear influence on their symptomatic and/or functional recovery following mTBI. Figure adapted from Silverberg & Iverson (2011)[375] and Young (2020)[379].

2.9 Predictors of Persistent Post-Concussion Symptoms

Prognostic factors are measurements or variables that can be used to estimate the chance of recovery from disease or disease relapse[380]. While it is widely acknowledged that some factors are likely to better predict outcome than others, research into identifying prognostic factors for PPCS has been largely heterogenous and subject to limitations. These include the lack of use of clear and consistent diagnostic criteria for both mTBI and PPCS, variation in outcome measures used, as well as attrition, missing and poor quality data[381]. Amongst the several factors have been found to be correlated with protracted recovery following mTBI[382] there is considerable variation in their ability to discriminate between recovery and PPCS[26]. It is becoming increasingly recognised that it is unlikely that any one variable will be sufficient to predict PPCS and it is anticipated that better success will be achieved by a prognostic model that combines multiple predictors[26,383–386].

The Importance of Predicting PPCS

The ability to determine which individuals may be at risk of developing PPCS is highly desirable and has implications for healthcare policy as well as clinical and research practice. PPCS has been broadly associated with disability and high utilisation of healthcare services[26,245,387–391], work absenteeism[392] and reduced health-related quality of life[392]. As such, having the ability to identify patients at risk of experiencing prolonged symptoms may assist with optimising patient outcomes (i.e. reduce number of missed days at work/school[393]) and thereby reduce the overall burden on the healthcare system attributable to PPCS. Specific to primary clinical practice, being able to predict who is at risk of developing PPCS may help healthcare practitioners to better tailor treatment plans to the needs of each individual patient[394,395], as well as assist with the provision of proper anticipatory guidance on necessary accommodations for academic, occupational, or leisure activities[395]. Furthermore, prognostic models could be used in research settings to identify and direct patients towards clinical trials of new therapies that may prevent or ameliorate the effects of PPCS, as well as the neurodegenerative diseases known to be associated with mTBI. Moreover, being able to identify which individuals are at risk of developing PPCS could help improve the statistical power of randomised controlled trials though risk stratification and covariate adjustment[26,396,397].

Prognostic Models to Date

A recent systematic review conducted by Silverberg and colleagues[26] found none of the multivariable prognostic models that have been generated to date to adequately predict individual patient outcomes following mTBI. The authors attributed the lack of reproducibility and clinical usefulness of these models to suboptimal research methodologies utilised across studies. The evaluation of individual factors for the prediction of outcome following mTBI and the development

of multivariate prognostic models remain active areas of research. Predictive factors that have been investigated can be broadly classified into pre-, peri-, and post-injury categories; that is, variables that are known to occur or can be ascertained before, at and after the time of injury, respectively (see Figure 9). The following section of this literature review provides an overview of a selection of pre-, peri-, and post-injury factors spanning demographic, injury-related characteristics, neuropsychological, blood-based biomarker and neuroimaging domains that are most relevant to this thesis.

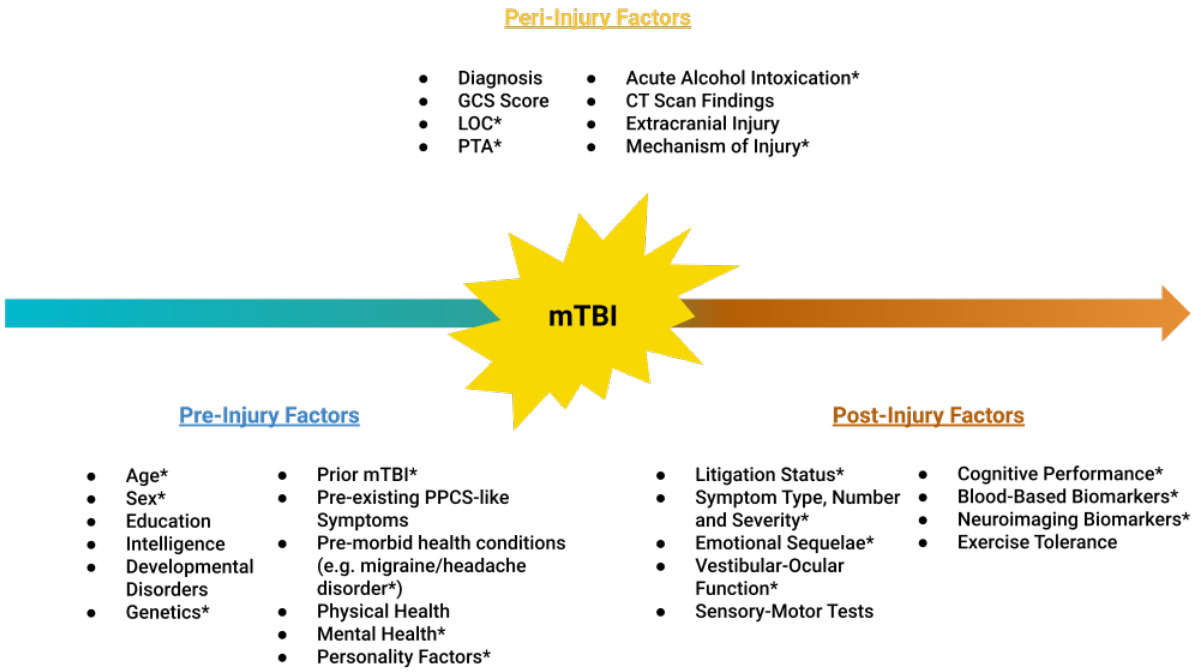

Figure 9. Prognostic variables for PPCS. A broad array of variables have been investigated to date as potential prognostic factors for PPCS. These variables can be categorised according to their temporal occurrence in relation to when mTBI is sustained (i.e. pre-, peri-, and post-injury). *Note:* * denotes variables that are covered in this literature review; CT: computerised tomography; GCS: Glasgow Coma Scale; LOC: loss of consciousness; PPCS: persisting post-concussion symptoms; PTA: post-traumatic amnesia. Original figure created using information presented in *Table 2* in Silverberg et al., (2015)[26].

2.9.1 Pre-Injury Factors

2.9.1.1 Age

The relationship between age and risk of unfavourable outcome following mTBI appears to be non-linear, with evidence suggesting adolescents and older adults are particularly vulnerable to poor outcome post-injury[26]. Amongst studies exclusively examining adult populations with mTBI, the risk of poor outcome following injury has been estimated to increase by 2-5% for each additional year of age[353,398]. Plausible explanations for this relationship include changes in brain plasticity, the

presence of extracranial comorbidities, as well as differences in clinical management associated with older individuals[399,400].

2.9.1.2 Sex

Relative to males, females have been found to be more likely to endorse more symptoms and/or be at a greater risk of poor outcome following mTBI in some studies[401–404] but not others[42,405,406]. Closer examination of studies in which females have been found to be at a higher risk of PPCS and/or poorer outcome at numerous timepoints following injury, including < 1 week[407,408], >14 days[409], 1 month[354,410], 6 weeks[411], 3 months[206,364,412,413] and 6 months[350] following injury.

Early accounts by King[10,414] suggested that females may be more vulnerable to poorer outcome following mTBI due to the higher prevalence of psychopathological presentations amongst women. However, recent investigations indicate that anatomical[415,416], hormonal[417,418], and biomechanical[415,416] differences that exist between males and females may also underpin observed sex differences. More specifically, axons in females are known to be smaller and contain fewer microtubules than in males. Axons in the brains of females have also been found to be more likely to be vulnerable to damage, and exhibit a greater degree of axonal pathology post-injury relative to male axons following equivalent force in simulated model of TBI[419]. Interhemispheric communication has been observed to be more pronounced in females than in males. As such, strain injuries resulting from mTBI that occur within the corpus callosum, the conduit for interhemispheric communication, may result in perceived symptom differences between sexes and affect females to a greater extent since their ability to process information may be relatively more disrupted[415]. In addition, metabolic differences have also been observed between males and females in several brain regions[420], and it has been suggested that this may help explain why males and females report different symptoms and why these differences may be exacerbated following injury[416]. Rodent models of TBI suggest that higher oestrogen levels may exacerbate brain injury effects in female rats[421], and differences in outcome following concussion have been observed amongst female collegiate athletes who do and do not use hormonal contraceptives[417]. Lastly, the weaker neck musculature of females may lead to them to experience greater angular acceleration of the head and/or neck[422–424], which in turn may mean females are more susceptible to sustaining a mTBI as well as subsequently experiencing post-concussion symptoms.

2.9.1.3 Education

Investigation into whether level of education attained may serve as a predictor of PPCS is limited. Initial studies appeared to indicate that education was not related to symptomatic outcome following mTBI[403,411], however, Cnossen et al., (2017)[425] recently found less years of education to be

a strong predictor of post-concussive symptoms 6 months post-injury. Furthermore, level of education appears to be significantly associated with and important for predicting functional outcome following injury[365,426–429]. Vocational rehabilitation literature suggests that individuals with more education tend to occupy positions in higher-level jobs, which are often associated with working conditions that are more conducive to resuming work, including greater autonomy, decision-making latitude and lower physical demands[365,429,430]. However, highly skilled and administration-type roles may also be difficult for some individuals to resume following mTBI if they require considerable simultaneous information processing, memory, and/or organisational skills[431]. Alternatively, it has been suggested that highly educated individuals may have better social support, adaptive coping skills, as well as cognitive and financial reserves that enable them to better deal with the consequences of mTBI and return to work sooner[425].

2.9.1.4 Previous History of mTBI

Despite the popular axiom that previous mTBI result in cumulative effects and/or delayed recovery following subsequent mTBI[432] investigation into whether previous history of mTBI may serve as a predictor of PPCS is limited, and the results yielded thus far are inconsistent. For example, while some studies have found a history of mTBI to be associated with prolonged experience of symptoms of mTBI at 3 months after injury[206] and able to predict PPCS[391,425], others have found neither to be the case[26,365,393,405,408,433]. The belief that prior mTBI may pose as a risk factor for PPCS may originate from early reports of prolonged recovery amongst athletes whom sustained multiple mTBI[218]. In fact, much of the research conducted investigating this factor has been within young, athlete populations with sport-related mTBI. The clinical significance of prior mTBI in other settings thus remains uncertain, and further investigation is required. The mechanisms underpinning the relationship between previous mTBI and recovery from subsequent mTBI have been poorly investigated, but are likely to be complex and may be modified by factors such as the overall number and recency of previous mTBI[432].

2.9.1.5 Previous History of Psychological Disorder

Previous history of psychological disorder (e.g. depression, anxiety, substance abuse disorder) is considered to be one of the strongest predictors of outcome following mTBI with numerous studies having found it to predict PPCS[206,298,305,407,408,412,434,435] and functional impairment following mTBI[436–439]. In both cases, it is speculated that individuals with a history of psychological disorder may be more vulnerable to experiencing poor outcomes following mTBI due to having greater anxiety sensitivity, less adaptive coping mechanisms or tolerance to stress, which in turn may culminate in a poorer ability to cope with the symptoms, cognitive slowing and stress that may

accompany their injury[206,408]. Alternatively, as has been suggested by Ponsford et al., (2011)[440], individuals with pre-existing psychiatric issues may be more inclined to respond to the experience of a mTBI with greater anxiety, which in turn may exacerbate PPCS.

2.9.1.6 Previous History of Headache/Migraine Disorder

Given the considerable overlap between mTBI and migraine pathophysiology[441] and the fact that headache is a symptom frequently reported by individuals who have experienced a concussion[442,443] as well as those suffering from PPCS[444,445], it seems plausible that individuals who have a history of headache/migraine disorder may be at a greater risk of developing PPCS. Investigations into this relationship are relatively recent, and while several studies conducted to date have not found an association between history of headache/migraine disorder and protracted symptom recovery[405,425,433,446], pre-existing headache/migraine has been identified as risk factor for worse functional and post-concussive outcomes at both 3 and 6 months post-injury[437]. Familial history of headache/migraine has been recently suggested as another factor that may influence an individual's recovery following mTBI. Research in this area is also lacking, although emergent findings from the paediatric literature appear to support this notion[447,448]. Further investigation is needed to determine the significance of family history of headache/migraine as a predictor of mTBI outcome amongst adult populations.

2.9.1.7 Coping Style

Coping is an umbrella term that is used to refer to the various thoughts and behaviours that people engage in to manage internal and external demands and situations that they perceive to be stressful[449,450]. Individual coping methods have been aggregated to form distinct *coping styles*, and it is common for people to use a number of different coping strategies when managing stressful situations[450], with choice of coping strategy selected often being dependent on the specific situation and phase of recovery[451]. Two coping styles that are thought to be particularly relevant to recovery within the context of brain injury are *avoidant (i.e. passive)* and *problem-focussed (i.e. active) coping*. Briefly, *problem-focussed coping* is characterised by active efforts to alter the situation and/or solve the problem that is being encountered. Examples of problem-focussed coping include seeking information and/or support from others to solve the problem and cognitive restructuring (i.e. thinking about the problem in different ways). Conversely, the *avoidant coping style* is characterised by behavioural and emotional efforts to avoid or escape the problem[449], such as mental and social disengagement, the use of alcohol or other substances, and wishful thinking[450].

It has recently come to the attention of researchers that certain coping styles could influence how people with mTBI cope with stressors that they may potentially incur after injury, which in

turn may moderate their recovery experience. There is currently a paucity of research investigating the association between coping and mTBI outcome, although the findings reported thus far have been insightful and, in some instances, rather surprising. Contrary to expectations, a study by Snell and colleagues[452] investigating the prognostic value of coping styles following mTBI found that individuals who utilised *active coping* strategies (which included problem-focussed coping) were at a greater risk of poor symptomatic and functional outcome 3 months following injury. In explaining this unexpected finding, the authors suggest that patient's representations and perceptions of illness (in their case, mTBI) may also influence the efficacy of different coping styles. With respect to functional outcome following mTBI, Maestas et al., (2014)[450] report that individuals with *avoidant coping styles* were more likely to experience worse emotional functioning and quality of life at 3 months post-injury. Observing that problem-focussed strategies were also frequently utilised by individuals within this cluster, the authors suggested that vacillation between the several coping styles may affect their individual efficacy, and proposed that the avoidant coping style is particularly maladaptive, and has the potential to 'override' the positive impacts that arise from using problem-focussed coping strategies. Furthermore, van der Naalt et al., (2017)[427] identified *passive coping style* to be a significant predictor of an incomplete functional recovery at 6 months post-mTBI, however, the study also found avoidant coping style to be offer some protective value. According to the authors of this study, the protective effect of the avoidant coping style, which has been observed to be engaged in more frequently in the chronic stages of TBI, may be attributable to its ability to help individuals avoid mentally challenging situations. More recently, coping style was not found to be associated with 6 month functional recovery amongst a cohort of elderly (>60 years) individuals with mTBI[453], which suggests that coping style may be pertinent to recovery in some age groups but not others.

2.9.1.8 Psychological Resilience

Psychological resilience is a construct that broadly refers to an individual's ability to recover following exposure to adverse events[454]. Defining resilience has been a difficult task due to its complex and multifaceted nature[455]. However, psychological resilience has been conceptualised as being a personality trait[456] and dynamic developmental process[457], but is most commonly thought of an innate characteristic that is possessed (and can be learned[458]) by all individuals, which can be amplified or diminished in response to encountered events and precipitating life circumstances[459].

Interest in examining the association between psychological resilience and outcome following mTBI, particularly amongst adult populations, has recently emerged. With only a handful of studies conducted to date and considerable variation in study design between them, the conclusions that can be drawn from the literature are tentative at best[460]. Nevertheless, studies

investigating psychological resilience as a pre-existing trait have found pre-injury resilience to be associated with higher symptom severity <24 hours of injury[42], and less fatigue and better quality of life at 1 month, less fatigue and depression and better quality of life at 6 months, and fewer post-concussion and post-traumatic stress disorder-related symptoms, less fatigue, insomnia and better quality of life at 12 months post-injury[461,462]. Investigating the prognostic capacity of pre-injury trait psychological resilience, McCauley et al., (2013)[42] found pre-injury resilience to predict symptom severity at 1 week and 1 month following injury, however they also noted that increased resilience was associated with increased symptom severity at both of these time points. According to the authors, this seemingly paradoxical finding may have been due to not enough time passing since injury during the study period for participants to 'bounce back'. Alternatively, they also suggest that this observation could be accounted for by another confounding variable (e.g. social supports) that was not investigated. Using a process-oriented definition of resilience and retrospective study design, Sullivan et al., (2015)[406] found *low perceived psychological resilience* to be a significant independent predictor of mTBI symptomatology. Further research is clearly warranted to better understand the relationship between psychological resilience and outcome following mTBI, and for excellent suggestions on future directions and discussion of their implications the reader is directed to a systematic review by Sullivan et al., (2016)[460].

2.9.1.9 ApoE Genotype

Apolipoprotein E (ApoE) is the major apolipoprotein within the central nervous system (CNS), and is synthesised by astrocytes and microglia[463]. ApoE is believed to play a critical role in cellular maintenance and repair *via* a number of intra- and extra-cellular processes within the CNS[464–466]. There are three common alleles (ε2, ε3, and ε4) that code for three protein isoforms (E2,E3,and E4). Of these, the APOE-ε4 allele is thought to influence cognitive performance[467,468] and decline[469], by conferring a survival disadvantage to injured neurons[470].

According to a meta-analyses by Zhou et al., (2008)[471] there appears to be an association between APOE-ε4 and elevated risk of poor outcome at 6 months after TBI. However, only a single study has been conducted investigating the prognostic value of APOE-ε4 to date. In comparing neuropsychological performance of APOE-ε4 carriers and non-carriers 6 months after mTBI, Müller and colleagues[472] found that patients negative for the APOE-ε4 genotype improved twice as much than those who were APOE-ε4-positive, and APOE-ε4 was identified as the only unique predictor of *less* improvement in neuropsychological performance 6 months post-injury. Although the findings are too limited to allow any firm conclusions to be made, it is possible that the APOE-ε4 genotype, along with other genes including those closely associated with cognition (e.g. Brain

Derived Neurotropic Factor, Catechol-O-Methyltransferase, DNA Methyltransferase 1), may be able to predict, moderate, and/or interact with other factors to influence outcome following mTBI.

2.9.2 Peri-Injury Factors

2.9.2.1 Loss of Consciousness

Loss of consciousness (LOC) is a term used to denote a state in which an individual loses awareness of their surroundings. Definitions of mTBI do not always require an individual to experience LOC in order to be diagnosed with mTBI, though if it is present, it is generally stated that LOC should not last more than 30 minutes. LOC is often used to indicate injury severity, and thus it is commonly believed that individuals who experience LOC at the time of their mTBI may have sustained a relatively more severe injury than those individuals that do not. This belief is likely to have stemmed from early animal studies examining the biomechanical forces of head injury in which it was found that a greater degree of rotational acceleration is required to produce LOC than other symptoms such as amnesia[473,474].

Determining whether LOC is a significant predictor of outcome following mTBI is difficult as relatively few studies have examined the prognostic capacity of LOC. While a systematic review by Silverberg et al., (2015)[26] concluded that LOC was an unlikely predictor of outcome, especially at longer follow-ups, more recent studies have found LOC to be predictive of symptom recovery at 1 month[405,475] and 7-months[434], and incomplete functional recovery 6 months post-injury[476]. LOC at time of injury was well documented in each of these studies, which highlights the importance of research design. Furthermore, LOC may be able to predict symptom duration[405] and when combined with amnesia may also be predictive of higher number of symptoms reported at follow-up[433], although both of these findings require further investigation. In addition, longer durations of LOC have been associated with increased incidence of *postconcussive syndrome* at 1, 3, and 6 months post injury[354], although LOC duration is yet to be investigated as an independent predictor of PPCS.

2.9.2.2 Post-Traumatic Amnesia

Post-Traumatic Amnesia (PTA) refers to an altered state of consciousness that occurs as a direct result of an individual sustaining a TBI. Despite the lack of a consistent definition, PTA is primarily characterised by an impaired ability to encode, store and retrieve memory, though it can also be accompanied with confusion, agitation and deficits in attention and executive function[477]. It is often not necessary for individuals to have experienced PTA to be diagnosed with a mTBI, however, if PTA is present, all three of the most commonly used mTBI diagnostic criteria stipulate that it should not last longer than 24 hours (refer to Figure 6). PTA can be further distinguished into

two forms according to the timeframe that is affected in relation to the injury. More specifically, these are *retrograde* PTA, which refers to defective memory of events occurring prior to the injury[477] and a*nterograde* PTA, which refers to reduced ability to recall or recognise information acquired subsequent to the injury[478]. Both anterograde and retrograde PTA are suspected to arise as a result of damage sustained within the Papez circuit (i.e. hippocampus → mammillary body → thalamus → limbic system → frontal lobe) although retrograde PTA may also occur as a consequence of injury occurring within neocortical structures of the temporal lobe[477].

PTA is generally considered to be an important marker of injury severity in the broader context of TBI, however, it is yet to be determined whether and how well it may serve to predict PPCS. Few studies conducted to date have examined PTA as a prognostic factor[26], and the reported results have been mixed[393,405,411,425,475,479]. Factors complicating the evaluation of PTA as a predictor of outcome following mTBI include co-occurring cognitive impairment and validity of measures used to assess PTA following mTBI. PTA is most frequently estimated by asking individuals about their first memory following injury. However, psuedoreminiscences and confabulations can occur[480] and the presence of 'islands of memory'[481] make it difficult not only for individuals to estimate the duration of PTA, but also for researchers to code PTA as a variable. Furthermore, it has also been pointed out that many of the standardised measures of PTA (e.g. Galveston Orientation and Amnesia Test[482], Westmead Post Traumatic Amnesia Scale[483]) that have been frequently used in studies were originally devised for use in moderate-to-severe TBI populations[477,484], and may thus not be sensitive enough for detecting PTA stemming from mTBI. Novel measures have recently been developed for use specifically among mTBI populations (e.g. Revised Westmead PTA Scale[485]), however, very few studies have implemented them and none have investigated its prognostic utility. Lastly, it is also important to note that only the prognostic capacity of anterograde PTA has been investigated thus far. This is likely to be due to its status as a clinical indicator of TBI severity as well as the fact that retrograde PTA has been observed to be generally less debilitating to individuals with TBI, particularly in the immediate stages post-injury[477]. As such, the prognostic capacity of retrograde PTA currently remains unknown.

2.9.2.3 Alcohol Intoxication

Albeit somewhat contrary to intuition, there is a growing body of evidence that suggests being under the influence of alcohol at the time of injury may be associated with better outcome following mTBI. In particular, acute alcohol intoxication has been associated with reduced neurocognitive deficits and neurological impairment in preclinical models of TBI[486–491], although there is also evidence that does not support this observation[492]. The underpinning mechanisms are yet to be fully understood, however, it is speculated that alcohol may exert neuroprotective

properties through an ability to inhibit NMDA receptor-mediated excitotoxicity[493–498] and/or delay the neuroinflammatory response that accompanies TBI[499]. Alternatively, it has been proposed that the association between acute alcohol intoxication and favourable outcome following injury amongst clinical populations may result from alcohol's ability to dampen the emotional impact of the trauma[500,501] and/or the depressant effects of alcohol on the central nervous system. Furthermore, it has also been pointed out that attending physicians could be misled to over-estimate the effects of head injury in intoxicated persons and/or diagnose mTBI when in fact none may be present[398] since the clinical presentation of acute alcohol intoxication can mimic that of mTBI. The latter is of particular significance for prognostic studies as it may lead to inaccurate conclusions being made about the role of alcohol in recovery following TBI, including mTBI.

Investigations into whether alcohol intoxication can influence assessments of TBI severity (e.g. GCS) have yielded inconsistent findings. While some studies have concluded that GCS scores are lowered by alcohol intoxication[502–508], and thus fail to provide an accurate evaluation of brain injury severity, other research has not found blood alcohol levels to affect GCS scores[509,510]. Fewer studies have investigated whether acute alcohol intoxication can affect initial TBI severity scores in a clinically significant manner, particularly amongst individuals with mTBI. Reported evidence from these studies is similarly mixed, with GCS scores having been observed to change over time amongst alcohol-intoxicated patients with TBI in some studies[504,511] but not in others[512–514]. Nevertheless, alcohol intoxication at time of injury appears to be an important factor that ought to be considered in prediction studies due to its potential to bias results.

Considering that between 30 and 50% of individuals are under the influence of alcohol when they sustain a mTBI[515], it is important to examine the prognostic utility of this factor. While only a handful of clinical studies have investigated the extent to which acute alcohol intoxication can predict outcome following mTBI, individuals who were intoxicated at time of injury have been found to be less likely to experience PPCS at 3[361] and 6[350] months post-injury, and were more likely to experience favourable functional outcome 6[398,427,500] and 12[436] months after mTBI.

2.9.2.4 Mechanism of Injury

Mechanism of injury is a term used to denote to the different ways in which an individual may sustain a mTBI. Possible reasons as to why some causes of injury may result in PPCS more so than others include differences in the amount of kinetic force involved in the injury and the context in which the injury occurred. For example, mTBI sustained during MVAs are likely to involve a greater amount of kinetic forces due to the velocity of impact, which may result in a greater amount of axonal injury or other physical trauma (e.g. neck or back pain) that in turn could contribute to or exacerbate an individual's experience of PPCS. Conversely, it is also plausible that recovery may be

more complicated for individuals who sustained a mTBI under what are typically considered to be highly traumatic events, such as an assault. In such instances, recovery from mTBI could be hampered by the emergence of psychological sequelae that are known to ensue from such events (e.g. post-traumatic stress disorder) and further exacerbated by other extenuating circumstances such as involvement in litigation proceedings, lack of social support and/or poor coping mechanisms.

Research investigating the prognostic utility of mechanism of injury has been fairly limited to date[26]. While some studies have not examined it as a variable or found it to be associated with outcome[365,411,516], other studies report findings that encourage further investigation. For example, Bazarian et al., (1999)[354] found patients with mild head injury due to sport had a significantly lower incidence of postconcussive syndrome compared to other mechanisms at 1 month post-injury, and a lower incidence at 3 and 6 months post-injury, although this did not reach statistical significance. Ponsford et al., (2000)[206] reported that individuals who continued to experience symptoms of mTBI 3 months post-injury were more likely to have been injured in a MVA, while McCauley et al., (2001)[410] found that postconcussional disorder resulted more frequently from MVAs and assaults although it must be noted that their study sample constituted of a mixture of both mild (n = 95) and moderate (n = 20) cases of TBI. "*Injury involved violence*" was found to be associated with an increased odds of PPCS at 6 months post-injury in an exploratory analyses conducted in a study by Cnossen et al., (2018)[350], while ordinal regression analyses by Booker et al., (2019)[436] found mTBI resulting from assault to be associated with worse functional outcome 12 months post-injury relative to the reference category of mTBI resulting from falls. Preliminary results also indicate that age and gender may interact with mechanism of injury to influence the risk of PPCS[517].

2.9.3 Post-Injury Factors

2.9.3.1 Litigation

The effect that involvement in litigation may have on outcome following mTBI is a highly contentious and complex issue. It is contentious because of the obvious financial gain that may be incurred from an individual malingering, exaggerating or fabricating their symptoms, and it is complex in that there are numerous reasons which may lead to an individual choosing to exaggerate of feign their level of impairment, just as there are many ways in which the litigation process may negatively influence an individuals' recovery. The litigation process in itself can be stressful for plaintiffs, often provoking feelings of anxiety[10] that have the potential to affect their mental state in negative ways through multiple pathways, including greater susceptibility to stressors, attitudinal and motivational changes, as well as biases in self-reported problems[344]. Furthermore, relative to

individuals who do not undertake litigation proceedings for their injury or emotional distress encountered following mTBI, individuals who elect to seek compensation often find themselves in a vastly different health care environment, which may bear influence on their recovery[344]. This may involve a number of additional personnel, some of which have a vested interest in the plaintiff's assessment and rehabilitation, while others have a vested interest in doubting the veracity of the plaintiff's health problems[344]. The effects of these vested interests can manifest in a number of different ways, including the plaintiff encountering more intense, frequent and possibly even hostile assessments of their claimed health problems, as well as incurring a level of scepticism associated with potential diagnoses of PPCS, post-traumatic stress disorder and major depressive disorder, all of which should be pointed out are generally accepted with less scepticism outside of the litigation context[344]. In some extreme cases, plaintiffs may also experience additional pressure to avoid effective treatments and/or to refrain from returning to work and other activities in order to illustrate the magnitude of the damages resulting from their injury[344]. Likewise, there are many potential underlying motivations for exaggeration and malingering, which include the plaintiff's own feelings of anger, resentment, entitlement and greed, desperate need for their problems to be recognised and/or concerns about not being taken seriously, depressive and/or negativistic thinking patterns, personality style or disorders and other secondary gains (e.g. avoidance of unpleasant activities)[344].

Investigations into whether litigation status may predict PPCS have yielded mixed results. An early meta-analysis by Binder & Rohling (1996)[518] concluded the overall effect of financial incentives on recovery following for mild head trauma are "particularly strong", and a subsequent review of 120 studies by the WHO Collaborating Centre Task force on mTBI identified compensation/litigation as the only risk factor that remained consistent across studies where symptoms persisted following mTBI[31]. At the level of the individual, litigation status has been found to predict number of post-concussion symptoms[433] and associated with protracted recovery[435,519], however, many studies have not found litigation status[27,408,412], compensation[206,405], or blaming and suing[354] to be associated with PPCS. While these discrepancies can be partly explained by factors pertaining to study design, such as research context and low proportions of study participants engaging in litigation, further investigation is needed to better establish the prognostic value of this factor.

2.9.3.2 Post-Injury mTBI Symptomatology

The presence of post-concussion symptoms in the acute stages of injury is an intuitive, albeit relatively poorly investigated, predictor of PPCS. mTBI symptomatology is typically quantified using self-report measures that require individuals to indicate the presence and/or rate the severity

of mTBI-related symptoms that they may be experiencing. Over the past 20 years, numerous post-concussion rating scales have been developed to accommodate different purposes and needs[193,520]. This has subsequently resulted in considerable variation in the nature and number of items that are featured in individual measures, test administration and scoring procedures, and the extent to which the psychometric properties of tools have been investigated and validation[520]. Despite this inter-measure variation, it is possible to examine the prognostic value of acute mTBI symptomatology according to the total number (i.e. overall symptom burden) or severity (i.e. total symptom severity score), as well as the specific type of symptoms that are endorsed by an individual.

Investigations into the prognostic utility of total symptom severity score have yielded mixed results, with some studies finding it able to predict outcome[365,403,411,435,521] and others not[472,479]. The prognostic capacity of total symptom burden does not appear have been investigated thus far, although Hiploylee et al., (2017)[522] report that for each additional post-concussion symptom reported, recovery rate was reduced by 20% in their sample. More recently, *average* symptom score has been suggested as another potential predictor of outcome following mTBI. A significant correlation between average symptom severity and longer recovery time has been reported in a study involving adolescents attending a specialty concussion clinic[523]; however, further investigation is required to follow-up on these preliminary findings.

Examination into whether the presence or severity of specific symptoms following mTBI, although limited, has also offered interesting insights. Acute headache has been found to be associated with mTBI symptom severity or functional outcome at 1[390,439] and 6[367] months following injury, and a statistically significant predictor of PPCS at 1[353], 3[359,361], and 6 months post-injury[350,367] in some studies but not others[365,411,516]. Somatic pain, including neck pain, has also been investigated as an independent predictor of PPCS. While some studies have not found the presence of somatic pain to be of prognostic value[408,524], others have found acute or sub-acute somatic symptom severity/burden to be associated with the duration/experience of mTBI symptoms within the first week of injury[407] as well as 45 days[405] and 3.5-months post injury[412], and predictive of PPCS at 3[521] and 6 months post-injury[350,521]. In addition, individuals who report experiencing low levels of pain in the acute stages of injury have also been observed to have more favourable odds of returning to 6 months after their injury[365]. Disturbances in balance and dizziness are frequently observed/endorsed following mTBI[208–211], and can arise from injury to the vestibular nuclei, labyrinthine complex of the inner ear and/or upper cervical spine[525]. Individual performance on measures of balance in the acute stages of injury have been associated with mTBI symptomatology at 1 month following injury[390], while acute postural instability has been found to predict PPCS at 3 months post-injury[359]. Dizziness is a well-established risk factor for prolonged clinical recovery in sports-related concussion[526]. Amongst clinical populations, individuals reporting acute dizziness

have been found to be 3 times more likely to experience mTBI symptoms 1 month post-injury[353], while the presence of dizziness in the acute stages of injury has been associated with greater severity of mTBI symptoms 6 months following injury[367]. Furthermore, dizziness to also be an independent predictor of failure to return to work 6 months following mild-to-moderate head injury[527] and functional disability ~15 months after mTBI[516].

2.9.3.3 Post-Injury Cognitive Performance

In a recent review, McInnes et al., (2017)[223] report that approximately half of individuals who experience a single mTBI demonstrate chronic cognitive impairment following their injury. As such, post-injury performance on objective neurocognitive tests, as measured in the acute to sub-acute stages of injury, presents as a logical factor that may be useful in predicting recovery after injury. Post-injury cognitive performance has indeed been identified as a predictor of symptomatic and neuropsychological outcome[26] and functional outcomes, such as return to work, at various time-points following mTBI[528]. However, not all studies support these findings[206,407,408,411,412,529], and the fidelity with which neurocognitive measure may prognosticate outcome has been questioned given that it is known to be influenced by factors such as age, levels of education, premorbid intelligence and current employment and socioeconomic status[521], as well as psychological disorders, such as anxiety and depression[524].

Of the cognitive domains examined, it appears that individual performance on traditional pen-and-paper tasks of memory, attention and executive functioning may be especially useful for predicting outcome following injury. Briefly, acute and sub-acute post-injury performance on tasks of attention, and immediate, delayed and working memory has been found to predict outcome at 1[354,359,361,390,521], 3[354,359,361,390,521] and 6 months post-injury[524]. The advent of computerised neuropsychological testing batteries has generated further opportunity to examine the prognostic utility of psychomotor ability. Whilst investigations into their ability to predict PPCS remain scarce, computer-based measures of reaction time, spatial processing and inhibition appear to be particularly promising[405,530].

2.9.3.4 Post-Injury Emotional Sequelae

Experiencing a mTBI can be a particularly traumatic event for some individuals. As such, some persons may go on to experience feelings of depression, anxiety and/or stress in the acute stages of injury, which may in turn result in or exacerbate PPCS and emotive symptom-cluster presentations. Depression, anxiety and stress symptomatology following mTBI have been commonly quantified using self-report measures completed by patients in the acute (e.g. presentation to the ED) to subacute (e.g. 1-2 weeks following injury) stages of injury. However,

investigations into the prognostic capacity of each have, overall, been severely limited and reported results have been mixed.

While acute post-injury depressive symptomatology has not been found to significantly predict the rate of change in post-concussive symptoms at 6 months post-injury in a cohort of adolescents with mTBI[531], a study by McCauley et al (2001)[410] found elevated self-reported depressive symptoms at 1 month post-injury to be a significant risk factor for PPCS 3 months post-injury. Post-injury anxiety has been identified as a significant predictor of PPCS at 3[27,408,479] and 6[27] months following injury, and is likely to interact with sex, with one study finding females who experience anxiety after mTBI to be at an elevated risk of developing PPCS at 3 months post-injury[364]. Acute post-injury stress has been found to be a significant predictor of post-concussion symptoms within the subacute stages (<2 weeks) of injury[407,412], and PPCS at 3.5[412] and 6 months post injury[350,365], in some studies but not others[205,407].

2.9.3.5 Vestibular-Ocular Function

mTBI can result in damage to cerebral networks responsible for vestibular and ocular function[532,533]. Dysfunction within these brain areas can in turn manifest in a host of symptoms and impairments. These include dizziness and balance problems[534] if the vestibular system is affected, and deficits in volitional saccades, oculomotor smooth pursuits, near point convergence, accommodation, and the vestibular ocular reflex if the oculomotor system is affected[521,533,535–537]. Recently, there has been much interest in devising screening tests of vestibular and ocular function (e.g. *Vestibular/Ocular Motor Screening Assessment*: *VOMS*[532]) that are sensitive to detecting damage within these sensory systems, which could be used to diagnose mTBI and predict recovery.

Research investigating the utility of vestibular-ocular function as a predictor of symptomatic and functional outcome following mTBI is in a relative state of infancy and has predominantly been conducted within paediatric/adolescent and athletic populations. However, reported findings appear to be promising. For example, an early study by Heitger et al., (2017)[521] found impaired eye movement, as assessed within the first week of mTBI, to be the most effective variable to distinguish between individuals who met PPCS criteria at 3 and 6 months post-injury, and significantly associated with functional outcome. More recently, Ellis et al., (2015)[538] found children and adolescents who exhibited vestibular-ocular dysfunction were over four times more likely to develop PPCS, whilst Anzalone et al., (2016)[539] found symptom provocation and/or clinical abnormality in all except for the near point convergence and accommodation domains of the VOMS to be associated with delayed recovery amongst a clinical sample of youth and adolescents attending a specialty concussion clinic. Similarly, Whitney et al., (2020)[540] observed abnormal scores on VOMS smooth pursuits, horizontal and vertical saccades, and convergence to be associated with increased

time-to-clearance for return to sport in a cohort of collegiate athletes who had sustained a mTBI. In addition to further research being required to evaluate the prognostic capacity of vestibular-ocular function in other populations (e.g. non-sport related paediatric samples, general adult, elderly *etc.*), it is important that future studies also account for the presence of pre-existing oculo-motor and balance disorders amongst participants, which could affect findings.

2.9.4. Blood-Based Biomarkers as Predictors of PPCS

Blood-based biomarkers are objective, physiological indicators that are detectable in blood samples, which can be used to determine the presence or severity of biological disease/injury states, prognosticate disease progression, predict recovery, and/or evaluate pharmacological responses to therapeutic interventions[333,541,542]. As a relatively non-invasive and cost-effective means of assessing and monitoring the physiological processes that underpin conditions of interest, blood-based biomarkers have received considerable attention over the past decade from the research community interested in identifying diagnostic and prognostic indicators of TBI, including mTBI. Much of the research conducted has focussed on biomarkers pertaining to pathophysiological processes of TBI, such as the structural and functional damage that occurs at the cellular level as well as the ensuing biochemical and molecular secondary injury cascades. To date, the most extensively studied blood-based biomarkers have been proteins associated with axonal, neuronal and glial injury, though research investigating the utility of genetic (e.g. APOE, Brain Derived Neurotrophic Factor) and inflammatory markers (e.g. cytokines, chemokines) as well as microRNA and metabolic products is increasingly emerging[543–547]. Figure 10 below provides a visual summary of candidate protein blood-based biomarkers that have been frequently examined to date within the context of TBI/mTBI.

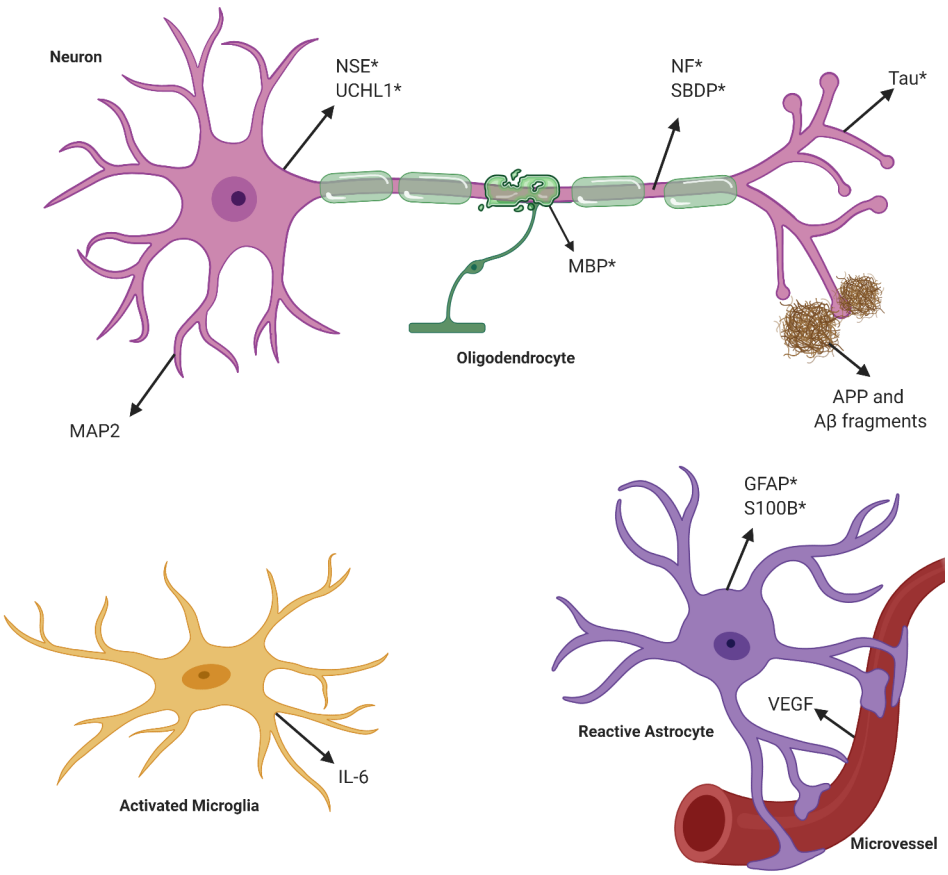

Figure 10. Commonly examined and emerging blood-based biomarkers in mTBI research. A number of protein blood-based biomarkers have been investigated to date for their ability to diagnose TBI/mTBI. In contrast, relatively few have been examined for their capacity to predict recovery following mTBI. Candidate protein blood-based biomarkers reflect the pathophysiological processes that underpin TBI/mTBI, and can be sourced from various CNS cell types (e.g. neuron, microglia, astrocytes), and may indicate damage to certain structural components (e.g. axonal or myelin sheath damage) or responses to injury (e.g. secretion of immunomodulatory factors). Newly emerging protein blood-based biomarkers include those pertaining to activated microglia (i.e. neuroinflammation) and the cerebrovascular system, in addition to genetic signatures, microRNAs, metabolomics and phenomics. *Note:* *: denotes protein blood-based biomarker reviewed in this literature review; Aβ: Amyloid Beta; APP: amyloid precursor protein; GFAP: glial fibrillary acidic protein; IL-6: interlukin-6; MAP2: microtubule associated protein 2A; MBP: myelin basic protein; NSE: neuron specific enolase; NF: neurofilament proteins; SBPDs: αII-spectin breakdown products; UCHL1: ubiquitin c-terminal hydrolase L1; VEFG: vascular endothelial growth factors. Original figure based on material presented in Zetterberg et al., (2016)[548] and Wang et al., (2018)[549], and created using BioRender.com.

Unfortunately, investigations into and the utility of CNS-derived blood-based biomarkers for TBI, and particularly mTBI, are complicated by several significant challenges[542,543,550]. In addition to low concentrations of proteins of interest in blood due to limited and/or transient BBB permeability, biomarker detectability can be influenced by proteolytic degradation, hepatic and renal clearance rates, and binding to other carrier proteins[542]. Furthermore, with numerous experimental and clinical studies indicating that levels of protein biomarkers in blood can change

substantially over time following mTBI[551–557], it is also important to consider the unique kinetic release profile of biomarkers (see Figure 11), especially when they are being evaluated for diagnostic and prognostic purposes. Often overlooked in study design and execution, technical considerations pertaining to blood sample collection, preparation, storage, stability and quality control, as well as choice of analytical platform are also increasingly being acknowledged as additional sources of variability that may affect blood-based biomarker detection and quantification, and warrant further investigation[558,559].

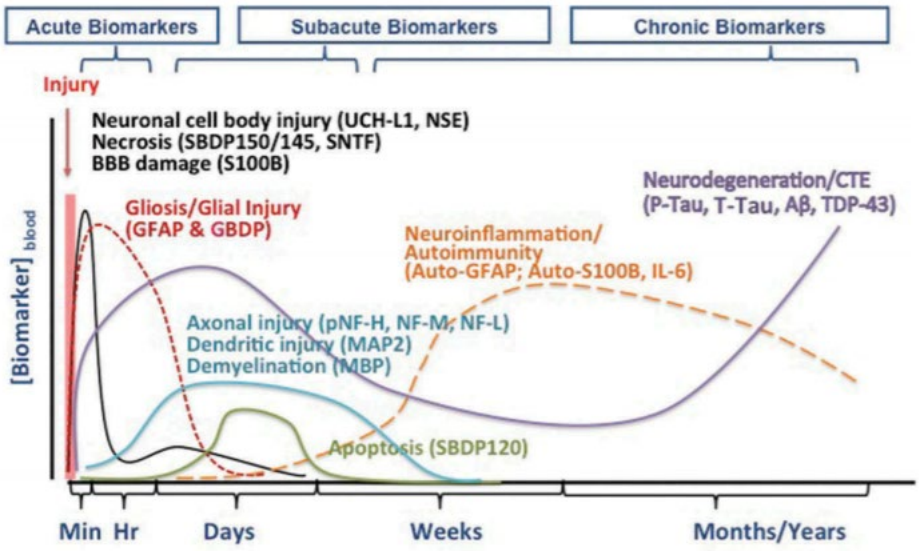

Figure 11. Temporal changes in blood-based marker release following TBI. Following TBI, protein blood-based biomarkers are released from their native cellular compartments and can be detected in peripheral blood circulation because of increased BBB permeability and/or glymphatic clearance. Different protein blood-based biomarkers indicate various pathomechanisms resulting from TBI, with their presence in the blood stream reflecting the temporal occurrence of said processes, and can vary as a function of time. Figure sourced from Wang et al., (2018)[549].

The following section provides a brief account of the blood-based biomarkers of astroglial, neuronal and axonal injury that have most frequently been investigated to date as prognostic biomarkers for the early identification of individuals at elevated risk of delayed recovery following mTBI.

Markers of Astroglial Injury

2.9.4.1 S100B

S100B is one of the most widely examined blood based biomarkers within the context of TBI research[333,560–563]. As a member of the S-100 family of calcium binding proteins, S100B plays an

important role in regulating intracellular levels of calcium[564]. S100B was initially thought to be exclusive to astrocytes but has since been found to also be expressed in oligodendrocytes[564] and other extracerebellar tissues, such as cartilage and skin[565,566]. S100B constitutes between 1-1.5µg/mg of all soluble protein in the brain[333], and is known to cross the blood-brain barrier in measurable quantities following head injuries, including mTBI, though the precise mechanism by which this occurs is yet to be elucidated[562,567,568]. Heightened levels of S100B have traditionally been considered as a marker for astrocytic activation and/or damage[569,570], and may also serve as a surrogate marker for diminished BBB integrity[571–573]. Amongst patients with mTBI, the mean half-life of S100B is reportedly 97 minutes[574] and its presence in blood samples appears to be relatively stable and not influenced by haemolysis, even if samples are not centrifuged and frozen immediately[575]; both qualities which have contributed to S100B's status as a highly attractive candidate in the early days of mTBI biomarker research.

Serum levels of S100B in healthy humans approximate 0.05 ng/mL[333,562,570], and have been found to be elevated amongst individuals with mTBI[353,576–581]. Studies investigating the clinical utility of S100B have found it to be a useful diagnostic marker of mTBI[582–584], and Scandinavian guidelines for the clinical management of mTBI patients currently recommend measuring S100B levels in order exclude the need to perform computed tomography (CT) scans[585], which itself can be resource-intensive process[586,587]. However, S100B's association with symptomatic and functional outcome following mTBI has been variable[560].

Individuals with elevated levels of S100B within the acute stages of injury have been found to be more likely to experience poor symptomatic outcome[411] and failure to return to work[588] 1 week after injury. Furthermore, acute levels of S100B have been found to predict S100B PPCS at 1 month following injury, including amongst paediatric populations[353,589,590] and poor functional outcome at 1[591] and ~15 months following mTBI[516]. In contrast, these findings are outweighed by the multitude of studies that have reported either only a trend towards a positive association[578,592] or no relationship between S100B and outcome following mTBI, including amongst child populations[582,593–600]. While it must be acknowledged that variability in study methodology (e.g. cut-offs used to define elevated levels of S100B, mTBI diagnostic criteria, post-mTBI outcome measures) may contribute to masking the relationship between S100B and outcome, it is generally thought that the predictive utility of S100B is limited[560]. Furthermore, the specificity of S100B as a marker of brain injury has been doubted as serum levels of S100B have also been found to be elevated amongst healthy individuals following physical activity[601–603] as well as in patients with extracranial injury (e.g. fractures)[604,605], thus restricting it's interpretability in individuals with mTBI and concomitant multitrauma.

2.9.4.2 Glial Fibrillary Acidic Protein

Glial fibrillary acidic protein (GFAP) is a monomeric intermediate filament protein that is predominantly expressed in glial cells that constitute the grey and white matter of the CNS[562,606,607], where it forms an integral component of the cytoskeleton of astrocytes[608]. Following TBI, GFAP is released from injured brain tissue into the CSF and peripheral blood circulation[609–612], and its presence is taken to indicate astrocytic injury, astrogliosis and possibly BBB disruption[562,563]. GFAP has been found to be a highly specific and robust marker in a variety of CNS pathologies[596,607,613], and has recently emerged as a promising biomarker for TBI[549], including mTBI. Blood levels of GFAP have been observed to be elevated within as little as 1 hour of mTBI[614], and appear to peak within 24-48 hours on injury[553,554,615–617], but may continue to remain elevated for several days in some individuals[552,553]. Furthermore, blood levels of GFAP have been found to effectively discern mTBI patients with a GCS score of 15 from uninjured controls[614]. Unlike other blood-based biomarkers, blood levels of GFAP do not appear to be elevated amongst polytrauma patients without brain injury[613] or orthopaedic controls[614,618]. Collectively, these findings suggest that GFAP may have good specificity for mTBI, particularly in the acute stages of injury.

Within the broader context of TBI research, GFAP has been found to be predict a host of pathophysiological events that are associated with TBI such as elevated intracranial pressure, reduced mean arterial pressure, low cerebral perfusion pressure, as well as poor functional outcome and increased mortality[333,613,619]. More specific to mTBI, serum GFAP level has been found to predict the need for neurosurgical intervention amongst patients with mild to moderate TBI[614,620]. Research investigating the capacity for GFAP to predict symptomatology and functional outcome following mTBI is currently scarce, especially amongst adult populations. Studies conducted thus far have reported findings that are consistent with initial observations of elevated serum/plasma levels of GFAP in the acute stage of mTBI[358,553,596,621]. However, multivariate models have not found GFAP to predict as a PPCS 3 months following injury[358] or functional outcome, including return to work, at 6[596] and 6-12 months post-injury[622], even though statistically significant associations with outcomes were initially observed in respective studies[596,621]. Given how few studies have been conducted, it is not currently possible nor appropriate to draw any conclusions about the utility of GFAP as a predictor of outcome following mTBI, and further research is certainly warranted.

2.9.4.3 Myelin Basic Protein

Myelin basic protein (MBP) is the second most abundant protein in the nervous system and is expressed by oligodendrocytes and Schwann cells in the central and peripheral nervous systems, respectively[623,624]. MBP is a major component of the myelin sheath and plays a key role in maintaining correct myelin structure *via* interactions with lipids of the myelin membrane[625,626].

Serum levels of MBP are typically very low (<0.3 ng/mL)[333] but are known to be elevated following TBI[627–631]. In TBI, MBP is speculated to be released into extracellular space as a consequence of damage to white matter resulting from DAI and ensuing demyelinating processes (e.g. myelin protease degradation[549,632,633]), whereby it facilitates its own entry into peripheral circulation, along with that of other CNS-derived biomarkers, by altering BBB permeability and tight junction expression[634].

At present, it is difficult to comment on the utility of MBP as a biomarker of TBI/mTBI and prognosis following injury due to the paucity of research in this space[635]. The few studies that have been conducted to date in which MBP has been examined have been limited to paediatric populations[630,631,636,637] and individuals with severe head injuries[638,639], although research has also emerged within the context of sports-related concussion[640]. Nevertheless, serum levels of MBP peak between 48-72 hours following head injury and have been observed to remain elevated for up two weeks[629], which suggests that MBP may be of greatest value in the post-acute stages of injury[384]. However, is it unknown whether this trajectory also applies to adult populations with mTBI as it has not yet been investigated. Furthermore, while no studies have been conducted to date in which the prognostic utility of MBP following mTBI has been evaluated, sub-acute CSF levels of MBP have been found to correlate with functional outcome at 7 days, 3 and 6 months post-injury amongst patients with severe TBI[638].

Markers of Neuronal Injury

2.9.4.4 Neuron Specific Enolase

Neuron specific enolase (NSE) is one of five isoenzymes of the glycolytic enzyme enolase[333]. NSE constitutes between 0.4% to 2.2% of the total soluble protein in the brain[641,642] and is abundant within the cytoplasm of neurons[643], but has also been found in lesser quantities within oligodendrocytes, erythrocytes, thrombocytes as well as cells of neuroendocrinal origin[384,561,644]. Being a cytoplasmic enzyme, NSE is typically constrained to the cellular environment under normal physiological conditions, although it is released into the extracellular space following insult to the cellular membrane[645], such as that which occurs in TBI, and is suspected to reach peripheral blood circulation *via* the glymphatic system[646]. Heightened levels of NSE are considered to be a marker of neuronal damage, and have been detected in CSF and peripheral blood samples obtained from individuals with TBI [643,647–650], including mTBI[411,516,651,652]. Normal levels of NSE are approximately 12.5 ng/mL[333] and appear to reach peak values within 6-12 hours of TBI[641].

Similar to S100B, NSE has received considerable attention as a putative biomarker for mTBI, although it is not generally considered to be a strong independent predictor of symptomatic, functional, or life satisfaction outcomes following mTBI[641]. Possible factors that may account for this

include the lack of specificity of NSE with respect to CNS injury, unknown optimal sampling time, and biased study designs[641]. Furthermore, haemolysis has also been observed to increase concentrations of NSE in both CSF and serum samples[653]. Although studies conducted to date appear to have seldom accounted for this factor, it is important to consider this confounder as it may result in false positives, thus affecting the utility of NSE as a prognostic marker for PPCS.

2.9.4.5 Ubiquitin C-terminal Hydrolase L1

Ubiquitin C-terminal hydrolase L1 (UCHL1) is a highly expressed deubiquitinase that is found in the cytoplasm of neurons[654–656]. Constituting around 1-2% of total soluble protein in the brain[333,657], this enzyme is also located in cells of the diffuse neuroendocrine system and neurons of the peripheral nervous system[657,658], and in particular, those that make up neuromuscular junctions[659]. Outside of the CNS, UCHL1 has been detected in cells of aortic endothelium, Leydig cells of the testis, renal tubule, smooth muscle tissue, ova, spermatogonia, and tumours[657,660–662]. UCHL1 plays a role in the addition and removal of ubiquitin from proteins that are destined for the ubiquitin proteosomal pathway, which is responsible for eliminating abnormal proteins and preventing the build-up of potentially toxic proteins within neurons under both typical and pathological conditions, including neurodegenerative disorders and TBI[384,663–665]. UCHL1 has historically been used as a histopathological maker for neurons [655] but has recently gained status as a candidate biomarker for mTBI[333,666].

UCHL1 is released into the extracellular space as a consequence of cell destruction under pathological conditions[664], and may enter peripheral circulation following TBI as a result of abnormal BBB function[667]. Serum levels of UCHL1 have been observed to be higher amongst individuals with TBI[668], including those with mTBI [669,670], with elevations having been detected within as little as 1 hour of mTBI[620,670] and peaking around 8 hours after injury[671]. However, evidence contrary to this has also been reported[554,672,673]. Acute serum levels of UCHL1 have also been found to be able to discriminate between patients with mild to moderate TBI and trauma controls without head injury, as well as mTBI/mild-to-moderate TBI individuals with and without intracranial lesions in some studies[670,674] but not others[672]. More recently, the United States Food and Drug Administration approved the combined use of acute blood measures of GFAP and UCHL1 with the aim of reducing radiation exposure by CT[675]. Elevated levels of UCHL1 and GFAP as detected within 12 hours of mTBI indicate the presence of intracranial lesions that require the patient to undergo a CT scan for further investigation[558].

With no studies conducted to date, the extent to which UCHL1 can predict symptomatic or functional outcome following mTBI remains to be elucidated. However, research conducted in a cohort of individuals of mixed TBI severities did not find an association between acute serum levels

of UCHL1 and functional outcome 3 months following injury[676]. Nevertheless, further research into the prognostic capacity of UCHL1 is required.

Markers of Axonal Injury

2.9.4.6 Neurofilament Proteins

Neurofilament proteins (NF) are intermediate (i.e. 10 nm diameter) filament proteins that are exclusively found within the axons and dendrites of neurons[677]. NF exist as bundles called as neurofibrils and comprise a major component of the neuronal cytoskeleton[549]. As such, they play an integral role in providing structural support to axons and dendrites and regulating axonal diameter[678–680]. Further to this, NF are also thought to be implicated in axonal and dendritic branching and growth[678,681–683] as well as the formation of synapses and neurotransmission[678–680]. The NF of the CNS constitute a triplet of polypeptide subunits of different molecular weights; *neurofilament-light protein* (NF-L; 68K), *neurofilament-medium protein* (NF-M; 150K) and *neurofilament-heavy protein* (NF-H; 200K)[384,549], and α-interexin[684]. In addition, NF-H is known to undergo process of phosphorylation following TBI, and hence also exists in a phosphorylated form (pNF-H)[682,685]. NF-L is the most abundant of the aforementioned subunits[686], and is predominantly expressed in the large-calibre myelinated white-matter axons that extend subcortically into the deeper layers of the brain and spinal cord[687]. Interestingly, this specific population of axons is known to be especially vulnerable to DAI that is caused by rotational forces present in mTBI[548]. Following insult to the CNS, NF are thought to be released into extracellular fluid by way of compromised cytoskeletal integrity that results from mechanical forces associated with injury[688]. As such, elevated levels of NF are taken to be a marker of axonal injury and NF release has been observed in both preclinical (*pNF-H*[689] ; *NF-L*[555]) and clinical studies of TBI (*pNF-H*[690]; *NF-M*[691]; *NF-L*[692–695]). In contrast to some other biomarkers, NF proteins are generally thought to be released into biofluids in a delayed fashion[549]. Furthermore, there is growing evidence that suggests that the concentration of NF-L in peripheral circulation may peak in a biphasic manner[553,696]. Most recently levels of serum NF-L have been observed to increase 6 and 13 days after sports-related concussion, relative to baseline, in a male but not female Australian Rules football players[554]. While the limited sample size of this study cannot be overlooked, this findings raise interesting questions regarding whether there may be an interaction between sex and the presence and timing of such biphasic peaks in biomarkers following mTBI.

Whilst studies have found serum levels of pNF-H to correlate with impact properties of mechanical injury in a rodent weight-drop model of closed head injury[697] and to predict mortality 6 months after TBI in both adult[698] and paediatric[699] populations, its prognostic utility within the context of mTBI does not appear to have been investigated to date. Doubts have also been cast about the utility of NF-M for TBI more generally[700]. In contrast, NF-L appears to be a particularly

promising biomarker for TBI may also have the capacity to predict outcome following mTBI. Acute levels of serum NF-L have been found to discriminate between survivors and non-survivors as well as predict clinical outcome 12 months in a cohort of patients with severe TBI[700]. Furthermore, acute levels of serum NF-L have also been shown to correlate with MR-DTI parameters measured at 12 months following TBI[701]. More specific to mTBI, serum levels of NF-L have been found to increase 7-10 days after bout in amateur boxers and correlate with the number of head impacts sustained during the match[695], while CSF levels of NF-L have been observed to be significantly increased amongst professional hockey players experiencing mTBI-related symptoms 1 year post-injury and found to correlate with lifetime concussion events (i.e. repeated mTBI)[702]. In regards to predicting outcome following mTBI, acute serum levels of NF-L have been associated with return to play time[696] following sports-related concussion, able to discern between ice-hockey players with rapidly resolving mTBI-related symptoms and those that continued to experience them for > 6 days[695], and predict functional outcome 6-12 months following injury in patients with mTBI[622].

2.9.4.7 Spectrin Breakdown Products

αII-spectrin is a highly expressed cytoskeletal protein that is found within the axons and pre-synaptic terminals of neurons[384] and plays an important role in stabilising the nodal and paranodal structures of myelinated axons[665,703]. Following cellular insult and cell death, aII-spectrin undergoes enzymatic cleavage that results in several spectrin breakdown products (SBDP) of distinct molecular weights[384,704–706]. Specifically, the enzyme calpain degrades α-II-spectrin at the C-terminal to produce two highly stable breakdown products weighing 150 and 145kDa (SBPD150 and SBPD145), and also at the N-terminal to produce a ~140kDa band called calpain-derived αII-spectrin *N*-terminal fragment (SNTF). Furthermore, αII-spectrin is also cleaved by the enzyme caspase-3 into a 120kDa breakdown product (SBDP120), although a 150kDa fragment is also known to be produced[705,707]. In addition to this, caspase-3 may further act upon the two calpain-derived SBDPs to also produce 120kDa fragments[706]. Broadly speaking, calpain-derived SBDPs are thought of as markers of neuronal injury and death due to necrotic and excitotoxic processes, whereas caspase-3 SBDPs appear to be indicative of apoptotic cell death[706].

αII-spectrin expression is not specific to the brain, and its presence has been detected in other organs and peripheral mononuclear blood cells[549]. Subsequently, the identification of heightened levels of spectrin breakdown products in peripheral blood cannot be treated as a conclusive indicator of the presence of TBI. Despite this confounding factor, the handful of studies that have been conducted to date suggest that SNTF/SBDPs are emergent biomarkers not only for mTBI, but for predicting outcome following injury as well. Elevated levels of blood and CSF SNTF, SBDP120, SBDP145, and/or SBDP150 have been observed following TBI[673,708,709], including mTBI/sports-

related concussion[710–712] where plasma levels of SNTF have been found to increase at 1 hour following sports-related concussion and continue to remain significantly elevated from 12 hours to 6 days after injury[711]. Pioneering research by Siman and colleagues conducted amongst professional ice hockey players found elevated plasma levels of SNTF to be associated with the development of mTBI-related symptoms following injury. Specifically, individuals whose symptoms lasted 6 days or longer were observed to have persistently higher serum SNTF levels at 1h to 6 days post-injury[711]. Amongst clinical adult populations with mTBI, the same group has also found acute elevations in plasma/serum SNTF levels to correlate with persistent impairments in cognition and sensory-motor integration 3 months following injury[710,712], and plasma levels of SNTF to correspond with significant differences in fractional anisotropy and the apparent diffusion coefficient in the corpus callosum and uncinate fasciculus measured by DTI[710].

2.9.4.8 Tau

Tau is microtubule-binding protein that is essential for maintaining the structural integrity of neuronal axons[713–715]. Tau is highly expressed within the nervous system and is most abundant within thin, unmyelinated axons[716,717], but has also been found within neuronal somatodendritic compartments[718] and oligodendrocytes[719]. Outside of the nervous system, tau has been detected in organs including the kidneys, testes and liver[720]. Tau undergoes a process of phosphorylation under both typical physiologic and pathologic conditions, and is hyper-phosphorylated (p-tau) to form the neurofibrillary tangles that characterise neurodegenerative diseases such as CTE[384,721]. In addition, tau can also be proteolytically cleaved by calpain and caspase enzymes to produce 17kDA fragments (c-Tau)[722].

Interest in tau as a potential biomarker for mTBI has been largely provoked by reports indicating that the development of tauopathies may be provoked by brain damage resulting from singular, episodic and repeated mTBI[666]. Serum and plasma levels of tau have been observed to be significantly elevated following mTBI in both paediatric[723] and adult populations[554,583,597,724]. The prognostic utility of tau has been relatively poorly studied and reported results have been inconsistent. A study by Shahim et al., (2014)[583] found acute plasma total tau levels to correlate with the number of days it took for mTBI-related symptoms to resolve in a cohort of ice hockey players who sustained sports-related concussion and return to play. Furthermore, a follow-up study by the same group also found serum Tau-A (a tau protein fragment resulting from enzymatic cleavage by ADAM metallopeptidase domain 10: ADAM10) levels to be significantly elevated amongst players whose mTBI-symptoms lasted >10 days and also correlate with the number of days it took for players to return to play[725]. Several studies have not found acute serum levels of c-Tau to predict PPCS at 3[358,597,724] or 6 months[726], or functional outcome 6-12 months[727] following injury. Given its

role in neurodegenerative disorders, it is possible that tau may be better suited to the prediction of long-term outcome following TBI and mTBI. Reports have emerged supporting this notion[728] although further study is needed.

2.9.5 Neuroimaging Techniques as Predictors of PPCS

Neuroimaging plays an important role in the diagnosis, clinical management, and prognosis of individuals with TBI[729]. As has been pointed out by others[729,730], neuroimaging can be utilised at each phase post-injury. For example, within the acute stages of injury, neuroimaging can help guide clinical decisions regarding the need for hospitalisation and early surgical intervention, while in the sub-acute period it can be used to monitor lesion or post-surgical changes, or to detect lesions that were not initially apparent. Furthermore, neuroimaging can be implemented in the chronic phase of recovery to characterise and monitor delayed brain tissue changes, and to inform subsequent rehabilitation practices.

Conventional CT and MRI are routinely utilised within the context of moderate and severe TBI though they are implemented relatively less frequently for cases of mTBI[731]. When applied within the context of mTBI, CT is typically used to identify the presence of additional types of injury (e.g. intracranial haemorrhage, skull fracture[732]), and thereby exclude more severe forms of TBI. mTBI has long been considered a noncritical injury[731] and both imaging modalities lack the sensitivity to detect mTBI-related abnormalities when used in their standard clinical capacity[733]. In contrast, *advanced* MRI techniques have the capacity to detect the relatively subtle changes that accompany mTBI, although their application is currently largely constrained to research settings due to the high costs and lengthy scan times often associated with them. As technology continues to advance, it is anticipated that the burden of these factors will lessen and the clinical uptake of these techniques will improve.

To date, an array of advanced MRI techniques has been used to investigate the various structural, functional and metabolic changes that accompany mTBI. For example, the structural integrity of WM has been studied using *diffusion MRI*[734–740] techniques, altered brain connectivity has been studied using *resting state*[741–744] and *task-based*[745–747] *functional MRI*, changes in CBF have been evaluated using *arterial spin labelling*[748–750], microhaemorrhages have been detected using *susceptibility weighted imaging*[751–753], while metabolites such as N-acetylaspartate have been quantified using *magnetic resonance spectroscopy*[754,755]. In addition, a number of non-MRI based neuroimaging techniques (e.g. *electroencephalography/quantitative electroencephalography (qEEG)*[756–758], *transcranial magnetic stimulation*[759,760], *functional near-infrared spectroscopy*[761], *transcranial Doppler ultrasonography*[762]) have been used to study electrophysiological and functional changes following

mTBI, although the number of investigations using these methods is comparatively less than those implementing advanced MRI techniques.

Similar to blood-based biomarkers, it is hypothesised that contemporary, advanced MRI techniques have the capacity to serve as objective biomarkers for mTBI[763] and potentially PPCS. Of the advanced MRI techniques listed above, those that are most pertinent to this thesis are diffusion MRI and a novel extension of susceptibility weighted imaging known as QSM. The following section of this literature review provides a very brief overview of diffusion MRI theory, its application within the context of mTBI, and summarises findings where it has been used as prognostic indicator for PPCS. A comprehensive overview of the theoretical grounding of QSM and emerging applications of this technique for detecting traumatic brain injury pathology is presented in the narrative review that comprises Chapter 5 of this thesis.

2.9.5.1 Diffusion MRI

Diffusion MRI, also referred to as *diffusion weighted imaging*, refers to a collection of advanced neuroimaging techniques that draw upon the diffusion properties of water molecules to non-invasively map out and discriminate between different types of brain tissue *in vivo*[764]. Capable of mapping complex tissue microarchitecture at the submillimetric level[734], this imaging modality is sensitive to the subtle structural changes resulting from DAI[765].

Diffusion tensor imaging (DTI) is a diffusion MRI method that uses complex mathematical algorithms to characterise the direction of diffusion of water molecules in 3D space[766], which can be used to quantitatively assess brain tissue integrity[767,768]. Given that the diffusion of water in biological tissues is highly influenced by tissue microstructure, DTI is particularly useful for measuring changes in highly ordered tissues, such as the WM tracts of the brain[732]. In WM, the myelin sheath, axonal membrane, neurofilaments, microtubules and other cellular components act as barriers that reduce the orthogonal diffusion of water, which in turn cause molecules to diffuse more readily along the orientation of axonal fibres relative to any other direction[769]. Perturbations in any of these microstructural elements, such as those seen in DAI, can alter the coherence of WM fibre tracts and therefore cause water molecules to move in random directions (i.e. isotropy)[769].

Two of the most commonly reported scalars in DTI studies are fractional anisotropy (FA) and mean diffusivity (MD)[770]. FA provides a measure of the extent to which the diffusion of water molecules is unidirectional[771]. Values range from 0 to 1, with higher values indicating a greater degree of unidirectional diffusion[772] while lower values indicate relatively isotropic diffusion[771]. Amongst healthy individuals, WM commissures, such as the corpus callosum, have been typically observed to have FA values between 0.6 and 0.8, although FA values are generally lower in other white matter bundles[732,766,773]. MD is a measure of the average diffusion of water molecules across

three principle directions, and is often inversely related to FA[774]. Following TBI (as well as other conditions in which WM is damaged), FA and MD values are expected to decrease and increase, respectively, at the site of damage due to fewer microstructural elements hindering diffusivity due to pathological factors, such as myelin sheath and axonal membrane damage, reduced axonal packing density and/or reduced axonal coherence[772,775].

Studies in which DTI has been used to investigate mTBI date back to 2002[776]. A number of studies have been conducted since then, though most have been limited to cross-sectional investigations focussing on either the acute or the chronic stages of injury[732,772]. With few longitudinal studies conducted to date, comparatively little is known about the longitudinal course of DAI [735], though research in this space is increasing[737,777,778]. Altered FA and/or MD values have been found in various regions of the brain, most of which are also consistent with those in which DAI has been observed to typically occur in individuals with mTBI[779,780]. Given that the pathomechanisms of mTBI evolve over time, the time elapsed between injury and MRI scanning is a crucial factor that needs to be taken into consideration when interpreting DTI findings[734,765]. At the acute stages of injury, significant differences have been observed in the mean FA for the corpus callosum, external capsule and right temporal subcortical WM, although not all studies have not found this to be the case[734,781,782]. At the subacute or chronic stages of mTBI, FA values have been found to be altered in several brain areas, including the corpus callosum, centrum semiovale or internal capsule, right superior corona radiata and cingulum[783–789]. Furthermore, it also appears that the posterior part of the corpus callosum may be more vulnerable to damage resulting from mTBI relative to the anterior part[782]. MD has been observed to be increased in the external capsule, left cingulum and right uncinate fasciculus, and the genu and splenium of the corpus callosum in the chronic stage of mTBI in some[783,789–791], but not all studies[792].

A considerable number of studies have been conducted investigating associations between DTI parameters and PPCS, although there is significant variation in research design (e.g. sample characteristics, technical aspects of imaging and analysis methods) and quality, with many studies failing to provide information on how mTBI and PPCS was diagnosed[735,765,793]. Nevertheless, relative to healthy controls or typically recovering mTBI patients, individuals with PPCS have been found to have decreased FA and increased MD values in several brain regions, including the corpus callosum, anterior corona radiata, internal capsule, right anterior thalamic radiations and superior longitudinal fasciculus, the inferior longitudinal fasciculus, and the fronto-occipital fasciculus bilaterally[736,781,783,790,793,794]. Abnormal DTI parameters have also been correlated with increased number and/or severity of mTBI-related symptoms[736,793,795], which suggests that PPCS may be underpinned by greater axonal damage[736,793], however, there is also evidence contrary to this[355]. Furthermore, WM abnormalities following mTBI detected using DTI have been associated with

worse cognitive performance[737,771,783,796–798] as well as functional outcome[789], including return-to-play[778].

With respect to predicting PPCS, relatively few investigations have been conducted into whether FA or MD may serve as potential prognostic biomarkers. However, a study by Messé and colleagues found MD values of six WM tracts (namely the corpus callosum, the right anterior thalamic radiations and the superior longitudinal fasciculus, the inferior longitudinal fasciculus and the fronto-occipital fasciculus bilaterally), which were obtained from DTI-MRI scans performed on average 15 days post-injury, could discriminate between individuals with and without PPCS at 3 months post-injury with 69% sensitivity and 77% specificity[794].

2.10 Concluding Remarks

The literature review presented in this chapter has identified and described a comprehensive range of established and novel pre-, peri- and post-injury factors that may have utility in predicting PPCS following mTBI. Given that no one indicator is, or is likely to be, sufficiently sensitive and specific enough to predict outcome at the level of the individual, a multivariate suite of outcome measures is required. This thesis aims to enrich the existing literature and work towards the development of a multivariate prognostic model for PPCS by investigating a range of demographic, injury-related characteristics, blood-based biomarker, and neuropsychological outcome measures, as well as emerging advanced MRI data analysis metrics using data collected in a unique, Western Australian context.

Introduction to Series One: Pilot Study and *CREST* Protocol

Series One of this thesis consists of two papers that are centred on the notion of developing multivariate prognostic models for predicting poor outcome following mTBI. The first has been published in *Brain Sciences* and reports the findings of a pilot study that was conceived with the goal of developing a suite of measures that could be readily collected within an Emergency Department (ED) setting in order to identify individuals at risk of PPCS.

Recruitment for the pilot study took place between September 2015 and January 2018, and participants were patients presenting with mTBI to the ED at Royal Perth Hospital (RPH), Western Australia. Implementing a prospective research design, potentially predictive data elements were collected within 48 hours of injury and participant follow-up was conducted approximately 28 days later. The primary aim of the pilot study was to assess the utility of blood-based biomarkers, particularly those measured using the highly sensitive Quanterix single molecule enzyme-linked immunoassay (Simoa™), as well as neuropsychological and MRI outcomes for predicting PPCS. Presence of PPCS was ascertained using the *Rivermead Post-Concussion Symptoms Questionnaire* (King, Crawford, Wenden, Moss, & Wade, 1995; abbreviated to *RMPCQ* in the published journal article), and individuals were considered to be experiencing PPCS if they scored in the moderate (25-32 points) to severe (33+ points) on the *RMPCQ* at follow-up. A cohort of healthy, age and sex-matched uninjured community-dwelling participants was also recruited to serve as a comparison group for neuropsychological and blood-based biomarker outcomes, while a separate cohort of healthy, age and sex-matched controls was recruited as MRI control subjects.

This pilot study represents our research team's first foray into prospective, observational cohort clinical study design. I joined the team as a doctoral candidate mid-way though the study's recruitment and managed all elements of subsequent data collection from study participants and controls, and co-ordinated blood-based biomarker assessments. At the study's conclusion, I performed all data analyses in consultation with the study's primary investigators and was chiefly responsible for writing and editing the manuscript, preparing figures and tables, integrating feedback from co-authors, and handling submission and correspondence. Finally, I also led the preparation of responses to journal reviewer comments and manuscript revisions.

Unfortunately, a number of logistical challenges were encountered over the course of the study, and particularly within the early stages with regard to participant follow-up. Before I joined the study team, follow-up and health control data collection was predominantly conducted by volunteer research staff (e.g. Honours and international exchange students) working on the project.

This resulted in a high staff turnover rate and a subsequent lack of continuity in follow-ups. While follow-up rates improved as the study progressed, the final number of participants for which a complete set of neuropsychological data (that was required to determine whether an individual was experiencing PPCS at follow-up) was available at the pilot study's conclusion was a little over thirty participants. Given that PPCS occurs at a prevalence rate of ~10%, this limited sample size resulted in an insufficient number of PPCS participants to perform the intended between-group analyses across the various types of data collected (e.g. neuropsychological, blood-based biomarker, MRI etc.).

Overall, the pilot study presented the research team with numerous insights with respect to both study findings and research design. Whilst the low number of individuals with PPCS must be taken into consideration when drawing inferences about the findings of the pilot study, a few things became apparent in terms of the selected study outcomes and implications for future studies. Firstly, the results of the pilot study seemingly suggested that neuropsychological tests had prognostic capacity, particularly those that assessed the cognitive domains of attention, executive function and memory. This finding warranted further investigation in larger longitudinal prediction studies of PPCS. Secondly, blood-based biomarkers measured using the Quanterix Simoa™ system, which was experiencing an exponential increase in popularity at the time that the pilot study was being conducted, were not found to predict PPCS. While the pilot study may have been underpowered to detect significant differences, future studies ought to consider broadening the scope and novelty of biomarkers being investigated. Thirdly, MRI analysis revealed that individuals with mTBI had higher levels of FA within the left inferior frontal occipital fasciculus relative to healthy age and sex-matched controls. While no assessment could be made of the predictive power of MRI measures for PPCS due to the study's limited sample size and variable timeframe in which participants underwent MRI scans, the reported high sensitivity of diffusion tensor imaging in the literature suggests the MRI findings should not be discounted.

In terms of research design, our experience in conducting this pilot study highlighted the impost of conducting follow-ups that required participants to attend in person. As such, the need to make follow-ups as easy as possible for participants was recognised, particularly if future study endeavours were to include multiple follow-up time points, which would also help circumvent participant attrition. We also appreciated the need to broaden participant recruitment beyond a single site through the use of a multi-modal recruitment pathway. Lastly, from an operational standpoint, the value of regular communication between the study team was recognised to help maintain awareness of the study to help with participant recruitment.

The second study comprises a protocol paper for the prospective, longitudinal observational *CREST Concussion Recovery Study*, which has recently been published in *BMJ Open*. Drawing heavily

upon the key learnings and insights gained from the pilot study, *CREST* is a new study that incorporates a much broader range of prognostic markers, which is facilitated through its two-part research design and multiple follow-up time points. More specifically, *CREST* consists of a telephone interview *(Phase I)* and a single comprehensive testing session that is conducted at a centralised research hub *(Phase II)*. Follow-ups are conducted by telephone interview at 1, 3, 6 and 12 months following injury. *CREST* has two overarching goals: *i)* establish a research dataset of mTBI that occur in the state of Western Australia, which has not been previously conducted to date, and *ii)* identify outcome measures that are particularly promising for use in multivariate prognostic models of PPCS.

Relative to the pilot study, *CREST* collects more detailed information about the participant demographics, injury-related characteristics and relevant aspects of their medical history in *Phase I*. *Phase II* features the assessment of numerous outcome measures including quantitative electroencephalography (qEEG), blood-based biomarkers (e.g. protein biomarkers of neuronal and glial structure and function, microRNAs, genetic signatures, phenomics and metabolomics), neuropsychological testing (including mood and personality), exercise tolerance, vestibular-ocular motor screening, and MRI. Notably, the MRI arm of the study has also been considerably expanded to include sequences for brain morphometry, arterial spin labelling, functional MRI, susceptibility weighted imaging (that will facilitate the novel analysis technique known as QSM, which is the focus of *Series Two* of this thesis), in addition to diffusion MRI that was reported on in the pilot study.

Recruitment strategies were also refined for *CREST*. While the pilot study recruited from only one metropolitan hospital ED, *CREST* recruitment has been expanded and encompasses both medical and community-based recruitment pathways. That is, in addition to hospital EDs, *CREST* also recruits participants from General Practitioners (GPs), sports physicians and allied health professionals, community and semi-professional sporting groups as well as directly from the general community *via* self-referral. Furthermore, the hospital ED recruitment pathway has been thoroughly expanded for *CREST,* and the study is currently actively recruiting from 7 of the 8 EDs that serve the Greater Perth Area, as well as two major regional EDs, with scope to recruit from additional regional/rural and remote areas of Western Australia in future.

It was intended that data collected for *CREST* would be analysed for the purposes of this thesis, however, issues pertaining to the ongoing *COVID-19* pandemic precluded this. Most significantly, recruitment efforts were stifled by decreased presentations to Eds, the shortening or complete cancellation of many community and professional sporting seasons, and local and national guidelines that prevented face-to-face interaction with participants. In addition, recruitment from GPs was minimal, although it is not clear whether this is related to the *COVID-19* pandemic. *Phase I* of the study was able to continue in a reduced capacity over the duration of the lockdown, however,

guidelines that were in place in Western Australia for 6 months in 2020 prohibited the ability to conduct *Phase II* of the study during this time. Nevertheless, numbers of people presenting to EDs and other sources of recruitment remained low throughout the remainder of 2020 and only began to return to more anticipated numbers in April 2021. It is also worth noting that given the anticipated incidence of mTBI in Australia has been estimated as high as 170,000 per annum, it can be expected that approximately 17,000 mTBIs occur in Western Australia. Considering that the *CREST* study recruits from a pool of 8 EDs in the metro area and several regional areas, it is therefore surprising that the current number of individuals being referred to the study is ~5 per week, which suggests that previous numbers reported are overestimated, or people are not seeking medical care for their injury. At the time of submitting this thesis, participant enrolment into *CREST* was n = 153 for *Phase I* and n = 21 for *Phase II*. Even considering that *i)* recruitment is limited to adult participants under 65 years of age, *ii)* research staff are not available 24/7 and *iii)* not all eligible individuals are interested in participating in the study, we appear to be notified of only 1-2% of our anticipated pool of participants.

This thesis originally intended to develop a multivariate prognostic model for predicting PPCS using data collected from the *CREST* research project, the design of which was informed by the findings and experience gained from conducting the pilot study. Due to a combination of issues relating to recruitment and *COVID-19*, it became apparent that the target number of participants for *CREST* were unlikely to be reached within the PhD candidacy. Although over 150 participants were recruited into *Phase I* of *CREST* at the time of that this thesis was being submitted, this data was not examined within thesis as doing so would constitute an unplanned interim analysis, which could potentially bias study results. Furthermore, the Australian New Zealand Clinical Trials Registry record for *CREST* (ACTRN12619001226190) does not specify that an interim analysis will be performed as part of the study's research design.

Subsequently, this thesis pivoted to investigating the utility of advanced MRI metrics as potential indicators of poor outcome following mTBI. However, to comprehensively evaluate the range of MRI sequences that were being implemented in *CREST*, approximately n = 120 participants would need to be recruited into *Phase II* of the study. Even if the number of MRI outcomes assessed was reduced for the purposes of this thesis, data would still need to have been collected from 30-50 participants in order to facilitate a meaningful analysis. As this number of participants had also not been reached at the close of this thesis, it was not possible to execute the intended analyses, and it was in turn decided that this thesis would focus solely on QSM due to its emerging status within the MRI field. Note that recruitment for *CREST* is ongoing, and other staff and students will be analysing data upon its conclusion, including that which is being collected as part of the study's

MRI arm. Given all this, a protocol paper was deemed to be a suitable alternative for this thesis and is presented in Chapter 4.

3 Pilot Study

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Towards the Development of an Integrative, Evidence-Based Suite of Indicators for the Prediction of Outcome Following Mild Traumatic Brain Injury: Results from a Pilot Study

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Abstract: Background: Persisting post-concussion symptoms (PPCS) is a complex, multifaceted condition in which individuals continue to experience the symptoms of mild traumatic brain injury (mTBI; concussion) beyond the timeframe that it typically takes to recover. Currently, there is no way of knowing which individuals may develop this condition. Method: Patients presenting to a hospital emergency department (ED) within 48 h of sustaining a mTBI underwent neuropsychological assessment and demographic, injury-related information and blood samples were collected. Concentrations of blood-based biomarkers neuron specific enolase, neurofilament protein-light, and glial fibrillary acidic protein were assessed, and a subset of patients also underwent diffusion tensor–magnetic resonance imaging; both relative to healthy controls. Individuals were classified as having PPCS if they reported a score of 25 or higher on the Rivermead Postconcussion Symptoms Questionnaire at ~28 days post-injury. Univariate exact logistic regression was performed to identify measures that may be predictive of PPCS. Neuroimaging data were examined for differences in fractional anisotropy (FA) and mean diffusivity in regions of interest. Results: Of $n = 36$ individuals, three (8.33%) were classified as having PPCS. Increased performance on the Repeatable Battery for the Assessment of Neuropsychological Status Update Total Score (OR = 0.81, 95% CI: 0.61–0.95, $p = 0.004$), Immediate Memory (OR = 0.79, 95% CI: 0.56–0.94, $p = 0.001$), and Attention (OR = 0.86,

95% CI: 0.71–0.97, $p = 0.007$) indices, as well as faster completion of the Trails Making Test B (OR = 1.06, 95% CI: 1.00–1.12, $p = 0.032$) at ED presentation were associated with a statistically significant decreased odds of an individual being classified as having PPCS. There was no significant association between blood-based biomarkers and PPCS in this small sample, although glial fibrillary acidic protein (GFAP) was significantly increased in individuals with mTBI relative to healthy controls. Furthermore, relative to healthy age and sex-matched controls ($n = 8$), individuals with mTBI ($n = 14$) had higher levels of FA within the left inferior frontal occipital fasciculus (t (18.06) = −3.01, $p = 0.008$). Conclusion: Performance on neuropsychological measures may be useful for predicting PPCS, but further investigation is required to elucidate the utility of this and other potential predictors.

Keywords: persistent post-concussion symptoms; blood-based biomarkers; neuropsychological assessment; MRI; prediction

1. Introduction

Mild traumatic brain injury (mTBI), also known as concussion, accounts for approximately 70–80% of all traumatic brain injuries worldwide [1]. mTBIs are caused by the head hitting an object or by forceful mechanical impacts external to the body that result in an abrupt acceleration/deceleration of the craniocervical complex [2]. mTBIs are characterised by a rapid onset of transient changes in neurological function which manifest as physical, cognitive, psychological/emotional, and sleep-related signs and symptoms [3]. Whilst most patients who sustain a mTBI present with their own unique constellation of symptoms [4], the most commonly reported symptoms include headache, difficulties in concentrating and maintaining attention, and alterations in mood and sleep [5]. Symptoms typically resolve within two weeks following injury [6–8], however, 10–20% of individuals who have sustained a mTBI continue to experience mTBI-related symptoms for months to years [9,10]. These individuals are said to be suffering from a complex condition known as persisting post-concussion symptoms (PPCS) [10–12]. PPCS is associated with significant disability [13–17] and a heightened use of health services [18,19], making it an emergent public health issue.

Previous studies have identified factors that can predict PPCS, however, these have not been found to be sufficiently precise to use on an individual patient basis. Having the ability to identify individuals at-risk of developing PPCS is necessary for both clinical and research practice. For clinicians, prognostic models would assist in tailoring treatment plans to better suit the needs of the individual and, more importantly, facilitate the early provision of targeted treatment strategies to circumvent ongoing problems [20]. Moreover, researchers could use prediction models to help enrich clinical trials in order to accelerate the development of evidence-based therapies [21] that aim to prevent or ameliorate the effects of PPCS, as well as other neurodegenerative diseases that have been found to be associated with mTBI, such as Alzheimer's disease [22–24] and chronic traumatic encephalopathy [25–30].

A range of demographic and injury-related factors, as well as neuropsychological, physiological and structural measures have been investigated for their ability to predict outcome following mTBI. However, variations in study methodologies have often resulted in conflicting results being reported [31], and many of the studies conducted thus far have been limited to investigating only one or a small subset of prognostic factors [32]. Demographic and injury-related factors have received considerable attention, partly because of the convenience with which they can be extracted from medical records. Of those examined, factors frequently associated with increased incidence of PPCS include being female [33–36], previous history of mTBI [16,37], and affective and anxiety-related psychological disorders [16,38]. A variety of neuropsychological measures have also been examined as possible predictors of PPCS because cognitive deficits have been observed in both individuals who have sustained a mTBI [39,40] and those suffering from PPCS [41]. In particular, individuals who perform poorly on tasks of executive function [42], memory [33,43–45], and psychomotor function [21] have been found to be at a

heightened risk of developing PPCS. However, the fidelity with which neuropsychological measures can prognosticate PPCS has been questioned, as individual performance can be confounded by extraneous factors such as age, socio-economic status, and prior education [46–49]. Hence, there is a need to identify and examine the prognostic capabilities of additional objective physiological and morphological variables.

Blood-based biomarkers are surrogate markers of disease that can be quantified from blood samples, and present as an option for use as diagnostic and prognostic indicators as they are a relatively cost-effective means of assessing the physiological mechanisms underpinning the condition of interest [50]. Of particular relevance to mTBI are blood-based biomarkers pertaining to neuronal injury, such as neuron-specific enolase (NSE) and neurofibrillary protein-light (NFL), as well as glial structure and function, such as glial fibrillary acidic protein (GFAP). These biomarkers are constrained to their respective locations in the cellular environment under normal physiological conditions, however following events where cellular membrane integrity is compromised, these biomarkers are released into the extracellular space [51–60]. In mTBI, damage to the cellular membrane primarily occurs as a result of mechanical forces that are present at time of injury [61]. Although there is a lack of normative values associated with any of these biomarkers, a relatively elevated presence of NSE, NFL, and GFAP in blood samples is considered to be indicative of neuronal injury, axonal injury, and astrocytic damage and possible blood–brain barrier disruption [62,63].

Diffuse axonal injury (DAI) is also thought to play a role in maintaining mTBI symptoms [64,65], through disruptions to axolemma and neurofilament organisation that result in compromised structural integrity of white brain matter [66]. DAI can be identified and quantified through the use of diffusion tensor imaging (DTI), an advanced magnetic resonance imaging technique that estimates the displacement of water molecules in biological tissue [67,68]. A growing body of literature suggests that certain DTI parameters may serve as biomarkers for the microstructural damage to white brain matter seen in mTBI [66], including fractional anisotropy (FA) and mean diffusivity (MD) [65,69]. FA is a normalised measure that describes the degree to which diffusion of water is unidirectional in each voxel [70], and is an indicator of complex biophysiological processes such as axonal density [64,69,71–73]. MD corresponds to the average apparent diffusion coefficient measured along the three principal diffusion directions in a voxel [65]. Disease processes that affect the integrity of the cellular membrane are known to affect MD [74,75], which is considered to be a non-specific, albeit sensitive, measure of alterations to brain tissue [76]. Although several studies have reported differences in DTI scalars amongst mTBI patients, research into whether they could be used to predict PPCS is limited.

Given that PPCS is a complex multifactorial condition that affects several aspects of functioning, it is unlikely that any single factor will be sufficiently capable of predicting the disorder at the individual level. Moreover, the predictive power of models is found to increase when several measures of clinical assessment (e.g., symptom, neurocognitive, balance) are considered together [21]. This suggests that model accuracy is likely to be optimised when a multidimensional approach that acknowledges the neurobiopsychosocial aspects of PPCS is undertaken. Thus, the aims of the present pilot study were twofold. Firstly, to evaluate the potential for a suite of demographic, neuropsychological, blood-based biomarker and MRI-DTI outcomes to be incorporated into future multivariate analyses aiming to predict PPCS. Secondly, to contribute data for the blood-based biomarker and MRI outcomes for which there is currently relatively little literature regarding the differences between mTBI and controls. These two aims were addressed in this small pilot study with the intention that the outcomes be validated in a larger scale study designed to generate a suite of outcomes that could be used to predict PPCS.

2. Methods

2.1. Participant Recruitment and Inclusion Criteria

Recruitment for this prospective, observational pilot study took place between September 2015 and January 2018. Participants were patients presenting to the Emergency Department at Royal Perth

Hospital (RPH), Western Australia, with mTBI. mTBI was defined in accordance to criteria specified by the American Association of Neurological Surgeons (https://www.aans.org/Patients/Neurosurgical-Conditions-and-Treatments/Concussion). That is, patients verbally verified that their closed head injury resulted from mechanical force or trauma and involved an immediate and transient alteration in brain function, including alteration of mental status and level of consciousness. Participants were enrolled into the study if they were aged between 18 and 50 years of age, presented to the ED within 48 h of head injury with symptoms that were attributable to that injury, and cranial CT scan revealed no presence of intracranial injury or CT was not performed. Participants were excluded if at time of presentation to the ED they scored 13 or less on the Glasgow Coma Scale (GCS), were a ward of state, prisoner, or under a mental health treatment order, were unsuitable for undertaking MRI procedures according to standard practice (e.g., metal implants), were homeless, were substance dependent, their head injury was deemed to be entirely due to primary seizure, were non-English speaking; or reported to suffer from pre-existing cognitive impairment. All MRI scans performed were reviewed by a consultant neuroradiologist, and any participants identified with abnormality not associated with mTBI were excluded from the study. A cohort of age and sex-matched, uninjured controls were recruited from the community to serve as a comparison group for neuropsychological and blood-based biomarker outcomes, while a separate cohort of healthy, age and sex-matched uninjured community-dwelling participants was recruited as MRI control subjects. Ethics approval for the study was obtained through the RPH Human Ethics Committee (RPH Ethics Approval Number REG 15-062/ANZCTR:123615000543583).

2.2. *General Data Collection Protocol*

Study participants were recruited by on-duty research nursing staff during daylight hours. Written consent was obtained directly from participants, or from their accompanying next-of-kin and then reconfirmed by the participants. Following consent, blood samples were procured by nursing staff and trained research assistants administered the neuropsychological testing battery, both of which occurred within 48 h of mTBI injury. A convenience subset of participants was later referred to the Radiology Department at RPH for MRI.

At ED presentation, participants were invited to attend a follow-up assessment that was scheduled approximately 28 days later. This involved the procurement of another non-fasting blood sample and re-administration of the neuropsychological test battery. Alternative forms of the neuropsychological tests where used where possible (i.e., RBANS® Update Test form B).

2.3. *Measures*

2.3.1. Neuropsychological Test Battery

A custom neuropsychological test battery was compiled to assess the physical, cognitive, and psychological symptoms associated with mTBI. More specifically, the physical symptoms associated with mTBI were measured using the Rivermead Post-Concussion Symptoms Questionnaire (RMPCQ) [77]. This self-report measure, which has been used to evaluate PPCS in previous prognostic studies [37,42–45,78–82], comprises of 16 symptoms that are commonly experienced following head injury. Respondents indicate the severity of their symptoms over the past 24 h (h) on a five-point Likert scale (0 = 'none', 4 = 'severe'), relative to their experience before sustaining their mTBI. Total scores range from 0 to 64, with higher scores indicating increased severity of symptoms being experienced by participants.

Cognitive functioning, namely the domains of attention, language and memory was measured by the Repeatable Battery for the Assessment of Neuropsychological Status Update (RBANS® Update) [83]. The RBANS® Update comprises of five subscales: Immediate Memory, Visuospatial/Constructional Ability, Language, Attention and Delayed Memory, which like the original RBANS® are combined to produce a composite Total Score [84]. Executive function was also measured using the Trails Making Test B (TMT B) [85]. Briefly, this measure comprises an array of numbers and letters that are

individually presented within circles. To complete the task, examinees are instructed to connect all items in the correct numerical and alphabetical orders, whilst switching between the two sets as quickly and accurately as possible. Examinees are timed and are allowed a maximum of 5 min to complete the task. Longer completion times indicate slower, or impaired, executive functioning capabilities.

Mood was measured using the Depression, Anxiety and Stress Scales-21 item version (DASS-21) [86]. The DASS-21 is a self-report questionnaire consisting of 21 items which has been designed to provide a quantitative measure of an individual's subjective experience of three related emotional states (subscales): depression, anxiety and stress. Individuals complete the DASS-21 by indicating the frequency with which they have experienced the listed symptoms on a four-point Likert Scale (i.e., 0 = items did not apply to me at all: 'never' to 3 = Item applied to me very much, or most of the time: 'almost always') over the week prior to mTBI. Individual's scores are summed and interpreted according to provided guidelines for each subscale, with higher scores indicating greater severity of symptoms experienced by the individual.

Lastly, effort was evaluated using the Rey 15-item Memory Test [87], a brief visual memory test that is frequently used as a screening measure to assess symptom validity and/or feigned memory impairment [88]. The test involves presenting individuals with a 3×5 matrix of meaningful symbols for a duration of 10 s before asking them to freely recall or draw the items that they can remember in the correct sequence. Individuals unable to recall at least 9 of the 15 items (that is, at least 3 of the 5 character sets) can be suspected of malingering [85] (p. 778). Overall, the total time taken to complete the neuropsychological tests spanned approximately 40 min on both testing occasions.

Consistent with the study's ethics approval, each individual participants' neuropsychological test results were reviewed by research assistants. Cases of concern (e.g., elevated scores on the RMPCQ, DASS-21 scores and/or impaired performance on tests of cognitive and executive function) were referred to the chief neuropsychologist (C.P.), and in cases where physical symptomatology was prevalent; to the emergency physician (D.F.), for further clinical review. Moreover, psychological counselling was offered free-of-charge to all study participants for any distress or symptomatologies that they may have experienced relating to their mTBI incident. This was coordinated by C.P. at the Robin Winkler Clinic at the University of Western Australia.

2.3.2. Blood Collection and Blood-based Biomarker Quantification

Non-fasting blood samples were collected from patients by on-duty research nursing staff at presentation to the ED and stored at −80 °C until use. Biomarker concentrations in plasma (GFAP) and serum samples (NFL, NSE) were measured with a digital array technology (Quanterix Corporation; Lexington, Massachusetts: USA) that uses a highly sensitive single molecule enzyme-linked immunoarray (Simoa™) method previously described [89]. More specifically, levels of GFAP were determined using Simoa™ GFAP Discovery Kit (Product number 102336, Lot 501277), while NFL and NSE were determined using the Simoa™ NF-Light Advantage Kit (Product Number 103186, Lot 501213) and NSE Discovery Kit (Product Number 102475, Lot 501345), respectively. Blood-based biomarkers were quantified on two separate occasions in two batches to minimise duration of storage prior to analysis (June 2017 and August 2018). Concentrations of blood-based biomarkers were multiplied by the dilution factor to generate corrected concentrations.

2.3.3. MRI Data Collection

Imaging was conducted at the Department of Radiology at Royal Perth Hospital using a 3T Siemens Skyra scanner (Siemens Healthcare, Erlangen, Germany) and a 32-channel head coil. Sequences included an axial 3D T1-weighted gradient echo sequence (TE/TI/TR = 2.48/900/2200 ms, flip angle = 8°, field of view (FOV) = 230×230 mm^2, matrix size = 256×256, slice thickness = 1 mm, voxel size = $1 \times 1 \times 1$ mm^3) and DTI using a spin echo-planar sequence (TE/TR = 110/8800 ms, b = 3000 s/mm^2, 64 directions, one average, FOV = 240×240 mm^2, matrix size = 96×96, 60 slices, slice thickness = 2.5 mm, voxel size = $2.5 \times 2.5 \times 2.5$ mm^3). Total scan time took approximately 40 min.

2.4. Diagnosis of PPCS

Participants were classified as experiencing PPCS if at the 28-day follow-up they scored in the moderate (25–32 points) to severe (33+ points) range on the *RMPCQ* [90]. Individuals who did not meet this criterion were considered to have experienced typical mTBI recovery.

2.5. Statistical Analyses

Data are summarised using means and standard deviations (SD) or counts and proportions, as appropriate. Data were screened for extreme outliers defined according to Tukey's outlier detection method; that is, equaling to or exceeding 3 times the inter-quartile range (3xIQR) below the first quartile or above the third quartile. Outliers meeting this criterion were investigated and determined to be implausible values and removed prior to analyses: only 2 GFAP values and 1 NSE value met these criteria. All statistical analyses were conducted using IBM SPSS software v25 (Armonk, NY, USA), except for univariate exact logistical regressions which were performed using SAS software v9.4 (Cary, NC, USA), and $p < 0.05$ was used to indicate statistical significance, unless otherwise specified. No adjustment for multiple testing was made due to the exploratory nature of the study.

Predictors of PPCS

Univariate exact logistic regression was conducted in order to determine the odds of an individual developing PPCS based on the demographic, injury characteristic, neuropsychological, and blood-based biomarker data collected. Results are reported as odds ratios (OR) with 95% confidence intervals (95% CI).

Conditional logistic regression was conducted to determine whether blood-based biomarkers examined could discern between individuals who had sustained a mTBI, regardless of recovery status, and healthy controls. In this instance, individuals with mTBI that were identified as an outlier, or for whom blood samples were not available, were excluded from the analyses, along with their matched healthy controls.

2.6. MRI Data Analyses

Due to the small number of individuals who sustained a mTBI, underwent MRI and were classified as having PPCS, all neuroimaging data collected was examined for between-group differences amongst individuals who sustained a mTBI, regardless of their recovery status, and healthy controls.

2.6.1. Tract-Based Spatial Statistics

DTI processing and voxelwise statistical analysis were carried out using FMRIB Software Library (FSL; http://www.fmrib.ox.ac.uk/fsl, [91], using tract-based spatial statistics [92]). First, FA images were created by fitting a tensor model to the raw diffusion data using FMRIB's Diffusion Toolbox (FDT), and then brain-extracted using the Brain Extraction Tool [93]. All participants' FA data were then aligned into a common space using the FSL nonlinear registration tool (FNIRT) [94,95], which uses b-spline representation of the registration warp field [96]. Next, the mean FA image was created and thinned to create a mean FA skeleton which represents the centres of all tracts common to the group. Each subject's aligned FA data was then projected onto this skeleton and the resulting data fed into voxelwise cross-subject statistics. Clusters were tested for significance at $p \leq 0.05$, corrected for multiple comparisons across space using the Threshold-Free Cluster Enhancement (TFCE) approach.

2.6.2. Region of Interest Analyses

FA and MD values were computed for a selection of regions of interest (ROIs), which were chosen based on previous findings reported in the literature indicating that they were either affected following mTBI or implicated in the neuropsychological functions that were assessed by the measures used in the present study. More specifically, the ROIs examined were the anterior corona radiata [97], the anterior, retrolenticular, and posterior components of the internal capsule [64,97,98], cingulum [64,99], corpus

callosum [67,97,100], the superior and inferior fronto-occipital fasciculi [67] as well as the superior and inferior longitudinal fasciculi [64,67,101]. All ROIs were evaluated in both left and right hemispheres apart from the corpus callosum, which was examined as a whole as well as genu, body, and splenium segments.

Maps of the ROIs were created first, using the atlas panel featured in FSLeyes (v2.1). All ROIs were located on either the JHU ICBM-DTI-81 White-Matter Labels or the JHU White-Matter Tractography Atlases. All ROI masks were then binarized using FSLmaths, with a threshold of 30 being applied to masks for ROIs that were identified using a probabilistic map (i.e., JHU White-Matter Tractography Atlas). FA and MD values were then extracted for each participant for all ROIs using 'fslmeants'.

Extracted FA and MD values were then exported to IBM SPSS software to evaluate between-group differences. Data corresponding to each ROI was screened for outliers as per Tukey's outlier detection method described above. *t*-tests were conducted in order to examine whether there were between group differences in FA and MD of the ROIs extracted. Partial correlations controlling for the effects of age, sex and time elapsed between presentation at the ED and MRI scanning procedures, were subsequently conducted to examine whether there was a correlation between extracted ROI FA and MD values and participants' performance on outcome measures pertaining to neuropsychological functions previously reported in the literature.

3. Results

3.1. Study Sample

A total of $n = 63$ participants who had sustained a mTBI were enrolled into the study. Of these, $n = 60$ had a complete data set for measures obtained at presentation to the ED, $n = 39$ presented at follow-up ~28 days later and of these, $n = 36$ completed all elements of neuropsychological testing. Details of eligibility and missing data are provided in Figure 1. On average, patients in the $n = 36$ study sample presented to the ED 10.86 h following injury (SD = 10.49, Range = 1.25–45 h, $n = 35$) and attended follow up 34.61 days later (SD = 7.19, Range = 25–55 days); MRI scanning procedures were conducted on average 30.07 days following injury (SD = 18.78; Range = 3–60 days). No participants required intervention by the neuropsychologist, emergency physician or neuroradiologist, or requested the use of these services.

3.2. Characteristics of Participants Included in the Study and Participants Lost to Follow-Up

Descriptive statistics for individuals who did and did not return for follow-up in this study are presented in Table 1. Unfortunately, a number of logistical difficulties were experienced during the establishment of processes early in the study and these partially account for the relatively low retention rate of participants in the present study. Statistical tests have been performed to assess differences between groups, however, results need to be interpreted in light of the present study's small sample size and resulting low power and thus, non-statistically significant results do not necessarily indicate no difference between the groups. As such, the data appear to suggest that a history of headache/migraine and neurological disorder was more prominent amongst individuals who presented for follow-up, relative to those individuals who did not present for follow-up. Furthermore, a lower proportion of individuals who presented for follow-up reported a history of mTBI and psychological disorder relative to those individuals who did not present for follow-up. In addition to this, participants who presented for follow-up also tended to score higher on the RBANS® Update Total, Immediate Memory, and Attention Index scores, and were faster at completing the TMT B at presentation to the ED, relative to those who did not.

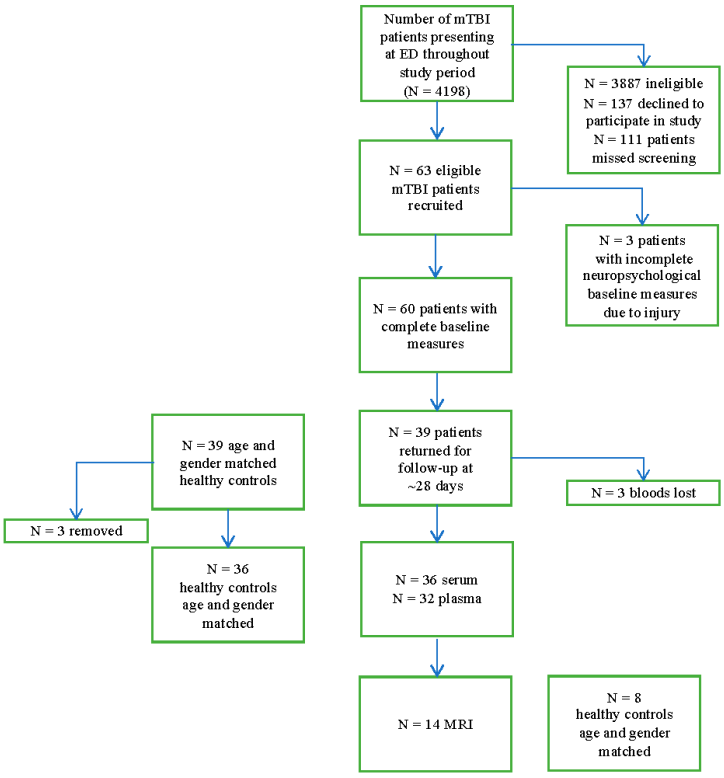

Figure 1. Consort diagram of study sample.

Table 1. Characteristics of participants included in the study and those lost to follow-up.

	Participants Lost to Follow-Up (n = 21)		Participants Presenting at Follow-Up (n = 39)			
Demographic and Pre-Injury Characteristics	n	Missing (n)	n	Missing (n)	p	Test
Age: M ± SD	30.62 (7.95)	-	28 (8.89)	-	0.264	t-test
Sex: Female (%)	9 (42.86)	-	16 (41.03)	-	0.883	χ^2
Education (years; M, (SD))	12.68 (2.12)	5	13.74 (1.83)	5	0.089	t-test
History of previous mTBI: Yes (%)	12 (57.14)	-	18 (46.15)	-	0.417	χ^2
History of any psychological disorder: Yes (%)	9 (42.86)	-	11 (28.95)	1	0.274	χ^2
History of neurological disorder: Yes (%)	1 (4.76)	-	6 (15.39)	-	0.222	χ^2
History of headaches/migraines: Yes (%)	0 (0)	-	3 (7.69)	-	0.192	χ^2
General co-morbidities: Yes (%)	7 (33.33)	-	12 (30.77)	-	0.839	χ^2
Currently on medication: Yes (%)	6 (28.57)	-	11 (28.21)	-	0.976	χ^2
Injury Characteristics	n	Missing (n)	n	Missing (n)		
Loss of Consciousness: Yes (%)	9 (60)	6	22 (66.67)	6	0.433	χ^2
Δ time between injury and ED assessment (hours; M (SD))	10.48 (6.57)	3	8.75 (7.20)	2	0.409	t-test

Table 1. *Cont.*

Performance on Neuropsychological Measures at Presentation to ED	Mean (SD)	Missing (*n*)	Mean (SD)	Missing (*n*)		
RMPCQ	22.57 (14.68)	-	18.38 (10.82)	-	0.213	*t*-test
RBANS® Update Total Score	80.78 (13.46)	3	92.16 (13.13)	2	0.004	*t*-test
RBANS® Update Immediate Memory	73 (14.57)	3	88.28 (15.37)	0	0.001	*t*-test
RBANS® Update Visual Constructional	94.35 (17.66)	1	98.45 (17.40)	1	0.400	*t*-test
RBANS® Update Attention	80.06 (18.55)	1	89.46 (16.65)	2	0.069	*t*-test
RBANS® Update Language	94.60 (10.56)	1	99.59 (15.07)	0	0.192	*t*-test
RBANS® Update Delayed Memory	86.47 (13.29)	1	90.19 (10.89)	2	0.282	*t*-test
TMT B Completion time (sec)	85.91 (47.37)	4	54.87 (14.77)	1	0.017	*t*-test
DASS-21 Total Score	17.05 (16.01)	-	11.58 (8.73)	-	0.159	*t*-test
DASS-21 Depression Subscale	5.62 (5.97)	-	3.31 (3.89)	-	0.120	*t*-test
DASS-21 Anxiety Subscale	5.05 (5.34)	-	3.50 (3.00)	-	0.231	*t*-test
DASS-21 Stress Subscale	6.24 (5.70)	-	4.73 (3.31)	-	0.276	*t*-test
RMT	13.38 (2.16)	5	14.13 (1.48)	7	0.163	*t*-test

Note: M: mean; SD: standard deviation; RMPCQ: Rivermead Post-Concussion Symptoms Questionnaire; RBANS® Update: Repeatable Battery for the Assessment of Neuropsychological Status, Update; TMT B: Trails Making Test version B; DASS-21: Depression, Anxiety and Stress Scales- 21 item version; RMT: Rey Malingering Test.

3.3. Characteristics of Patients with mTBI and PPCS

The cause of mTBI varied amongst study participants, with the most frequent cause being falls (47%) (Figure 2).

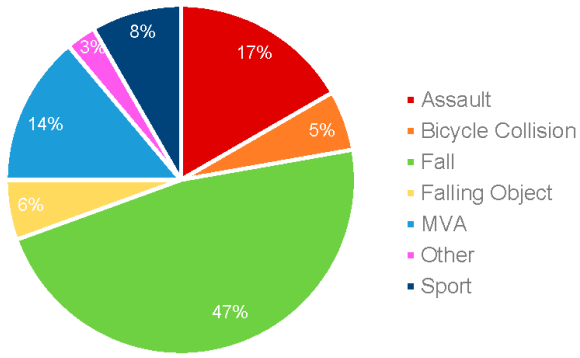

Figure 2. Breakdown of mTBI by type of causal mechanism (n = 36).

Of the individuals that returned for follow-up, n = 3 (8.33%) were classified as having PPCS. For patients classified as having PPCS, the causal mechanisms of mTBI were falls (n = 2) and falling objects (n = 1). Descriptive statistics suggest that individuals in the study sample who were diagnosed with PPCS may have been younger relative to those that recovered typically from mTBI, and all reported a history of prior mTBI. There were no statistically significant differences across the other demographic and injury-related characteristics surveyed (Table 2).

3.4. Predictors of PPCS

None of the demographic variables or injury-related characteristics surveyed were found to be statistically significant predictors of PPCS in this pilot study (Table 2). Amongst individuals who had sustained a mTBI, increased performance on the RBANS® Update Total Score (OR = 0.81, 95% CI: 0.61–0.95, p = 0.004), and the RBANS® Update Immediate Memory (OR = 0.79, 95% CI: 0.55–0.94, p = 0.001) and Attention (OR = 0.86, 95% CI: 0.71–0.97, p = 0.007) indices at presentation to the ED

suggested a decreased odds of developing PPCS. Faster completion of the TMT B at ED presentation also suggested decreased odds of developing PPCS (OR = 1.06, 95% CI: 1.00–1.12, $p = 0.032$). No significant associations were detected between PPCS and performance on the other neuropsychological measures examined (Table 2). Scatterplots illustrating individual mTBI and PPCS patient performance on each of the neuropsychological outcomes as measured at ED presentation are depicted in Figure 3.

Table 2. Demographic variables, injury-related characteristics, and performance on neuropsychological outcome measures of participants who recovered typically and those who developed PPCS, with exact logistic regression odds ratios for PPCS.

	mTBI Typical Recovery ($n = 33$)		PPCS ($n = 3$)				
Demographic Variable	***n***	**Missing (*n*)**	***n***	**Missing (*n*)**	**OR**	**95% CI**	***p***
Age (years): M (SD)	28.64 (9.09)	-	21 (2.65)	-	0.80	0.52–1.03	0.122
Range	18–49		18–23				
Sex: Female (%)	14 (42.40)	-	1 (33.33)	-	0.69	0.01–14.42	1.000
Years of education: M (SD)	13.89 (1.99)	5	12.33 (1.53)	-	0.85	0.14–7.85	1.000
Range	10–17		11–14				
<12 years education (%)	9 (27.30)	5	2 (66.67)	-	0.25	0–5.37	0.563
History of previous mTBI: Yes (%)	16 (48.50)	-	3 (100)	-	3.76 *	0.38–†	0.271
Number of previous mTBI	1 previous mTBI: $n = 10$		1 previous mTBI: $n = 2$		1.33	0.58–2.69	0.444
	≥2 previous mTBI: $n = 6$		≥2 previous mTBI: $n = 1$				
History of any psychological disorder: Yes (%)	9 (27.30)	1	2 (66.67)	-	4.84	0.23–314.29	0.454
History of neurological disorder: Yes (%)	5 (15.20)	-	0 (0)	-	1.58	0–16.86	1.000
History of headaches/migraines: Yes (%)	3 (9.10)	-	0 (0)	-	2.90	0–34.44	1.000
General co-morbidities: Yes (%)	9 (27.30)	-	2 (66.67)	-	5.05	0.24–327.39	0.431
Currently on medication: Yes (%)	9 (27.30)	-	1 (33.33)	-	1.32	0.02–28.44	1.000
Smoker: Yes (%)	7 (21.20)	1	1 (33.33)	-	1.75	0.03–38.57	1.000
>10 cigarettes/day	5 (15.20)	1	0 (0)	-	1.53	0–16.29	1.000
Exercise each week: Yes (%)	31 (93.90)	-	2 (66.67)	-	0.14	0.01–11.39	0.472
Number of hours exercised/week: M (SD)	14.67 (14.73)	-	22.67 (20.53)	-	1.03	0.96–1.11	0.398
Alcohol consumer: Yes (%)	23 (69.70)	3	2 (66.67)	-	0.62	0.03–40.99	1.000
Number of standard drinks consumed per week: M (SD)	4.38 (4.95)	4	4.67 (5.03)	-	1.01	0.76–1.27	0.870
Injury-related Characteristics		**Missing (*n*)**		**Missing (*n*)**	**OR**	**95% CI**	***p***
Loss of consciousness: Yes (%)	20 (60.60)	3	1 (50)	1	0.51	0.01–43.14	1.000
Δ time between injury and ED assessment (hours; M (SD))	10.84 (10.66)	1	11.08 (10.47)	-	1.00	0.88–1.11	0.810
Performance on Neuropsychological Outcomes at ED Presentation	**Mean (SD)**	**Missing (*n*)**	**Mean (SD)**	**Missing (*n*)**	**OR**	**95% CI**	***p***
RBANS® Update Total Score	94.12 (12.38)	-	73.00 (9.84)	-	0.81	0.61–0.095	0.004
RBANS® Update Immediate Memory	91.76 (13.57)	-	63.67 (12.22)	-	0.79	0.55–0.94	0.001
RBANS® Update Visual Constructional	100.61 (16.85)	-	93.67 (8.51)	-	0.97	0.90–1.05	0.498
RBANS® Update Language	102.67 (11.82)	-	92.67 (8.15)	-	0.92	0.81–1.03	0.174
RBANS® Update Attention	91.52 (15.58)	-	65.33 (12.86)	-	0.86	0.71–0.97	0.007
RBANS® Update Delayed Memory	92.45 (12.24)	-	79.00 (3.46)	-	0.90	0.79–1.01	0.071
TMT B Completion Time (sec)	56.83 (19.34)	-	87.70 (20.54)	-	1.06	1.00–1.12	0.032
DASS-21 Total Score	11.94 (10.36)	-	12.67 (6.35)	-	1.01	0.88–1.12	0.838
DASS-21 Depression	3.36 (4.05)	-	3.00 (3.61)	-	0.98	0.65–1.31	1.000
DASS-21 Anxiety	3.48 (3.81)	-	4.67 (1.16)	-	1.08	0.77–1.42	0.562
DASS-21 Stress	5.09 (3.96)	-	5.00 (2.00)	-	0.99	0.69–1.35	1.000
RMT	14.39 (1.37)	5	12.50 (0.71)	1	0.52	0.17–1.37	0.202

Note: M: mean; SD: standard deviation; * median unbounded estimate; † 95% CI upper limit not definable; RMPCQ: Rivermead Post-Concussion Symptoms Questionnaire; RBANS® Update: Repeatable Battery for the Assessment of Neuropsychological Status, Update; TMT B: Trails Making Test version B; DASS-21: Depression, Anxiety and Stress Scales- 21 item version; RMT: Rey Malingering Test; ORs have been calculated per one unit increase for all continuous variables.

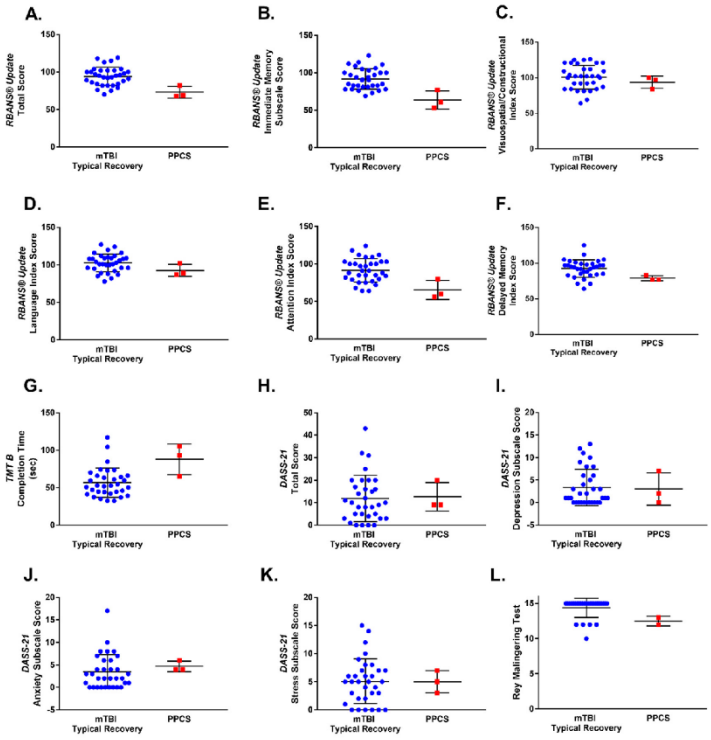

Figure 3. Scatterplots depicting patient performance on neuropsychological outcomes, as measured at presentation to the ED. Mean and standard deviation are shown for mTBI typical recovery and PPCS groups for all neuropsychological outcomes assessed, which were as follows: (**A**) the RBANS® Update Total Score, (**B**) RBANS® Update Immediate Memory subscale, (**C**) RBANS® Update Visual/Constructional subscale, (**D**) RBANS® Update Language subscale, (**E**) RBANS® Update Attention subscale, (**F**) RBANS® Update Delayed Memory subscale, (**G**) TMT B, (**H**) DASS-21 Total Score, (**I**) DASS-21 Depression subscale, (**J**) DASS-21 Anxiety subscale, (**K**) DASS-21 Stress subscale, (**L**) Rey Malingering Test.

None of the blood-based biomarkers examined were found to be statistically significant predictors of PPCS in this pilot study (Table 3). As an example, for GFAP the OR of 0.998 for a 1 pg/mL change equates to an OR of 0.905 for a 50 pg/mL change. The corrected concentrations of blood-based biomarkers measured in blood samples from individual mTBI and PPCS patients, collected at presentation to the ED, are presented as scatterplots in Figure 4.

Table 3. Exact logistic regression odds ratios for PPCS for blood-based biomarkers.

	mTBI (n = 33)		PPCS (n = 3)				
Blood-Based Biomarker	n	**Mean (SD)**	n	**Mean (SD)**	**OR**	**95% CI**	p
GFAP (pg/mL)	27	482.12 (553.95)	3	231.00 (139.31)	0.998	0.992–1.002	0.540
GFAP (50 pg/mL)	27	9.64 (11.08)	3	4.62 (2.79)	0.905	0.669–1.105	0.540
NFL (pg/mL)	32	5.89 (2.28)	3	6.33 (4.38)	1.075	0.650–1.688	0.706
NFL (50 pg/mL)	32	0.12 (0.04)	3	0.13 (0.09)	37.19	4.42×10^{-10}–2.34×10^{11}	0.706
NSE (pg/mL)	32	5950.88 (4476.00)	3	7939.33 (4921.24)	1.00008	0.9998–1.0003	0.417
NSE (50 pg/mL)	32	119.02 (89.52)	3	158.79 (98.43)	1.004	0.9900–1.015	0.417

Note: Odds ratios have been calculated per one unit increase for all blood-based biomarkers examined. Means and standard deviations are provided for descriptive purposes only.

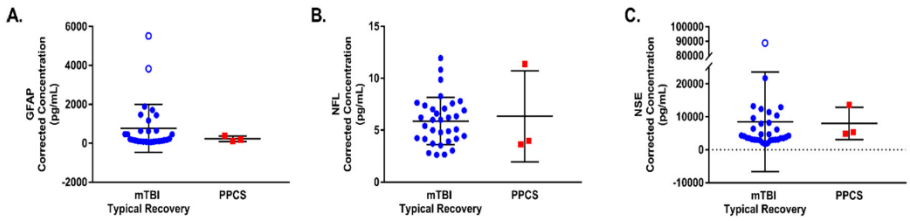

Figure 4. Scatterplots depicting the corrected concentrations of blood-based biomarkers (**A**) GFAP, (**B**) NFL and (**C**) NSE measured from blood samples obtained upon patient presentation to the ED. Mean and standard deviation are shown for mTBI typical recovery and PPCS groups for all outcomes assessed. Outliers are denoted by empty circles (mTBI Typical Recovery) and squares (PPCS), which were excluded from OR analyses presented in Table 3.

3.5. Differences in Biomarkers between mTBI and Healthy Controls

A significant association was identified between GFAP and mTBI where a one unit increase in GFAP generated a 2.8% increase in the odds of mTBI compared to healthy controls (OR = 1.028, 95% CI: 1.001–1.056, $p = 0.042$). Note that the OR of 1.028 for a 1 pg/mL change equates to an OR of 3.978 for a 50 pg/mL change. No significant association was detected between the odds of mTBI and levels of NSE or NFL (Table 4). However, the estimated effect size for NFL warrants further investigation. Blood-based biomarker concentrations from mTBI patients obtained at ED presentation (regardless of recovery status) and from healthy control participants are presented as scatterplots in Figure 5.

Table 4. Conditional logistic regression odds ratios for mTBI for blood-based biomarkers.

	mTBI (Total $n = 36$)		Healthy Controls (Total $n = 36$)				
Blood-Based Biomarker	***n***	**Mean (SD)**	***n***	**Mean (SD)**	**OR**	**95% CI**	***p***
GFAP (pg/mL)	30	457.01 (531.35)	30	96.68 (35.43)	1.028	1.001–1.056	0.042
GFAP (50 pg/mL)	30	9.14 (10.63)	30	1.94 (0.71)	3.978	1.051–15.247	0.042
NFL (pg/mL)	36	5.92 (2.42)	36	5.41 (1.93)	1.125	0.90–1.41	0.310
NFL (50 pg/mL)	36	0.12 (0.05)	36	0.11 (0.04)	361.099	$0.005–2.89 \times 10^7$	0.310
NSE (pg/mL)	35	6121.31 (4473.30)	35	4675.26 (2179.96)	1.0001	1.0000–1.0002	0.144
NSE (50 pg/mL)	35	122.43 (89.47)	35	93.51 (43.60)	1.005	1–1.01	0.144

Note: Odds ratios have been calculated per one unit increase for all blood-based biomarkers examined. Means and standard deviations are provided for descriptive purposes only.

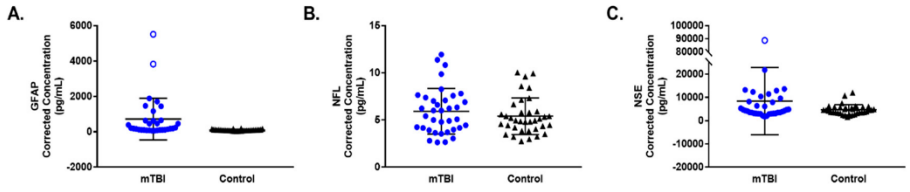

Figure 5. Scatterplots depicting the corrected concentrations of blood-based biomarkers (**A**) GFAP, (**B**) NFL and (**C**) NSE measured from blood samples obtained upon patient presentation to the ED. Mean and standard deviation are shown for individuals who sustained a mTBI (mTBI) and healthy controls (control) for all biomarkers assessed. Outliers are denoted by empty circles (mTBI), which were excluded from OR analyses presented in Table 4.

3.6. Neuroimaging Outcomes

Results of the TBSS analyses revealed no statistically significant differences between groups on both the FA (*t*-statistic corrected $p = 0.683$) or MD skeleton (*t*-statistic corrected $p = 0.601$). Similarly,

ROI analyses revealed no statistically significant differences between mTBI and healthy control groups for all regions examined in terms of MD values extracted, however, a significant between group difference was found in the FA of the left inferior fronto-occipital fasciculus (IFOF; Figure 6A) (t (18.06) = −3.01, p = 0.008; Figure 6B). Given that previous reports have indicated that this region is implicated in visuo-spatial constructional ability [102–105], a partial correlation was conducted between RBANS® Update Visuospatial/Constructional Index Scores, as measured at patient presentation to the ED, and FA values extracted from this region for the mTBI group. Insufficient neuropsychological data was available for healthy controls therefore precluding investigation for this group. Results of the partial correlation indicated a statistically significant correlation between RBANS® Update Visuospatial/Constructional Index Score and extracted FA values from the left IFOF (r = 0.63, p = 0.038) (Figure 6C) amongst individuals who sustained mTBI.

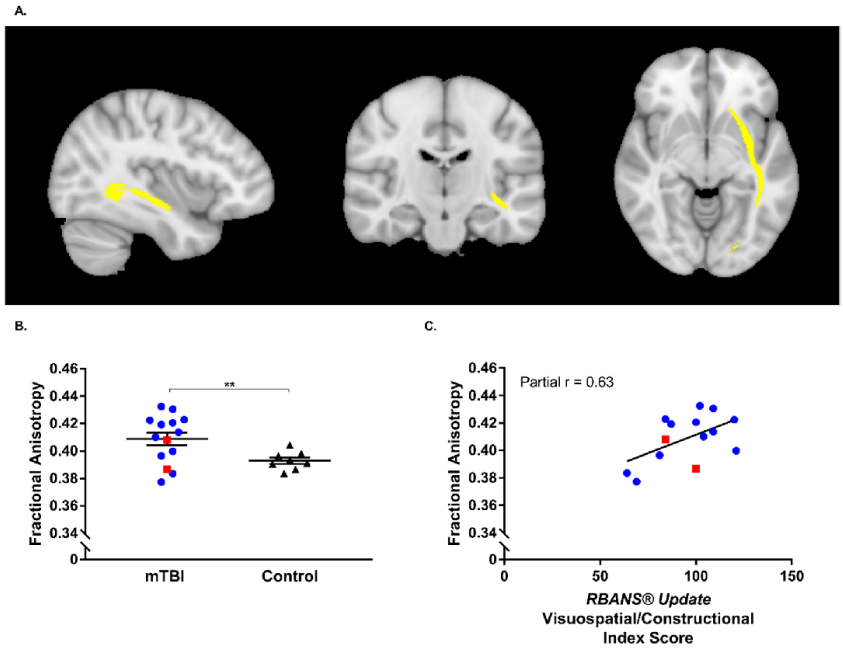

Figure 6. (**A**) Left inferior fronto-occipital fasciculus (IFOF) threshold of 30 presented in radiological view (voxel coordinates 128,108,65); (**B**) Scatterplot depicting fractional anisotropy values extracted from the left IFOF threshold of 30 from individuals who had sustained a mTBI and healthy control participants. Data from individuals who sustained a mTBI and underwent MRI and were classified as having PPCS at follow-up are identified as red squares; ** $p < 0.01$; (**C**) Scatterplot depicting the partial correlation between the RBANS® Update Visuospatial/Constructional Index Scores at ED Presentation and fractional anisotropy values in the left IFOF threshold of 30 for individuals who had sustained a mTBI and underwent MRI procedures. Data from individuals who underwent MRI procedures and were classified as having PPCS at follow-up are identified as red squares.

4. Discussion

The first aim of this pilot study was to univariately examine demographic, neuropsychological, blood-based biomarker and MRI-DTI outcomes for their potential to predict PPCS. In this context, neuropsychological measures and demographic variables appear to show promise. The blood-based biomarkers assessed did not indicate predictive potential and the number of participants assessed for MRI precluded statistical analyses for the intended purpose. Given the small sample size and limited power to detect associations in this pilot study, statistical significance was not the only indicator of potential utility

of a variable considered. Failure to detect a significant association is not proof of no association, and this is particularly relevant when power is low. Therefore, variables with substantial estimated effect sizes have been highlighted as warranting further investigation without intention to imply a confirmed association.

The overall incidence of PPCS in this study was consistent with the literature. Of the demographic variables examined, none were identified as statistically significant predictors of PPCS. However, prior history of psychological disorder was estimated to have a positive and substantial OR, which is consistent with findings that have previously been reported with the literature [16,20,37,38]. The magnitude of the estimated ORs for previous history of mTBI and general comorbidities are also thought to be clinically meaningful and should not be dismissed as potential predictors on the basis of *p*-values derived from this pilot. Acknowledging that there was a tendency for relatively less cognitively impaired individuals to return for follow-up in the present study cohort, examination of neuropsychological data identified three components of the RBANS® Update test battery to be predictive of PPCS. These results suggest that this brief neuropsychological screening tool, which can be easily administered bedside in an ED setting by a suitably trained individual, may add significant value to a multi-modal suite of measures that aim to predict PPCS amongst patients who present to hospitals for mTBI. To the authors' knowledge, the present study was the first of its kind to use the RBANS® Update for predicting PPCS following mTBI. As such, further investigation is warranted in order to validate the use of the RBANS® Update Total Score, Immediate Memory and Attention indices, and potentially the Delayed Memory index, as robust predictors of PPCS. Similarly, the results of the present study suggest that an individual's performance on the TMT B may also be relevant for the prediction of PPCS and warrants further investigation, particularly in conjunction with assessments of oculomotor function. This is consistent with a previous study by Heitger and colleagues (2007) [106] which identified TMT B performance as a potentially clinically meaningful predictor of PPCS.

The second aim of the present study was to contribute data for blood-based biomarker and MRI-DTI outcomes. None of the three blood-based biomarkers examined were found to be statistically significant predictors of PPCS. However, mean concentration of GFAP at ED presentation differed between mTBI and healthy controls. This finding is consistent with recent reports that suggest that GFAP may be useful for detecting the occurrence of traumatic brain injuries, including mTBI [107–111]. Given that each biomarker is associated with its' own release kinetic profile, it may be that variation in the time between injury and acquisition of blood samples resulted in the lack of statistically significant differences for the NFL and NSE biomarkers. GFAP is believed to have a relatively stable release kinetic profile, with peak levels occurring approximately 20 h post injury in cases of mild to moderate traumatic brain injury [108] and 1–2 days following incidents of severe traumatic brain injury [111–114]. In contrast to this, peak levels of NSE appear to be reached 6–12 h post traumatic brain injury [115], while levels of NFL have been observed to peak 144 h following sports-related mTBI [58]. The temporal stability of blood-based biomarkers is increasingly being recognised as a potential limitation to their routine clinical use. Thus, non-protein based biomarkers such as microRNAs may be better suited for the prediction of outcome following mTBI [116–119].

Given the exploratory nature of the present study and the fact that PPCS affects a relatively small, albeit significant proportion of individuals who sustain a mTBI, an insufficient number of individuals underwent MRI procedures to determine the prognostic utility of the MRI analyses conducted. However, the results obtained are in line with those previously reported, which suggest that the inferior fronto-occipital fasciculus may be particularly vulnerable to the diffuse axonal injury that is present in mTBI [120]. Contrary to previous studies which have observed FA to be decreased in this region [121], our results indicate that relative to healthy controls, FA was, on average, higher amongst participants with mTBI. This increase in FA may be accounted for by the time elapsed between injury and the time at which study participants were imaged. Furthermore, it is also worth noting that in the present study sample, the FA values of the two participants that were classified as having PPCS did not appear to deviate from the other mTBI participants that experienced typical recovery. Additional larger scale studies are required to better establish whether differences in FA can be observed

amongst individuals that do and do not recover typically following mTBI, and whether FA may also function as a prognostic biomarker.

Follow-up analyses of neuroimaging data also revealed a statistically significant positive correlation between performance on the RBANS® Update Visuospatial/Constructional index at ED presentation and FA value amongst individuals who had sustained a mTBI. This finding is consistent with previous reports that suggest that the IFOF is implicated in visuospatial functioning. Taking into consideration that mTBI can result in compromised oculomotor function [122], which can also persist in cases of PPCS [123], future studies investigating the association between performance on neuropsychological tests and/or tests of oculomotor function with aberrant MRI-DTI findings, as well as whether microstructural damage within corresponding brain areas may be predictive of PPCS, are warranted. Given the continuous advancement in neuroimaging sequences and analysis techniques, the potential remains for MRI to elucidate biological changes occurring within the brain that result in PPCS.

Having the ability to identify individuals at risk of PPCS has important implications for both clinical and research practice. At the primary healthcare level, it would help clinicians customise treatment plans so that they best meet the unique needs of each individual patient [124] and, most importantly, facilitate the triage of patients to treatment interventions in a more timely manner. Considering that early intervention is anticipated to be integral to optimising patient outcome, and given that PPCS is associated with high utilisation of healthcare services [19,31,43,125–128], being able to identify at-risk patients and directing them to treatment sooner may assist in reducing the overall burden on the healthcare system that is attributed to PPCS. Furthermore, knowing which individuals may go on to develop PPCS following mTBI would also help clinicians to better manage patient expectations, as it would allow them to provide more accurate advice about the anticipated trajectory of recovery and the academic, occupational, and/or leisure activity accommodations that may be necessary to assist in the process [129]. Prognostic models can also be used to bridge the gap between clinical and research settings. For example, clinicians could use them to direct at-risk patients towards clinical trials of novel therapies that may ameliorate, or even prevent, the development of PPCS and chronic neurodegenerative diseases, such as Alzheimer's disease [22–24] and Chronic Traumatic Encephalopathy [25,27–29], that are variably associated with mTBI. Within the context of randomised controlled trials, prognostic models can also be used to increase statistical power through risk stratification and covariate adjustment [31,130,131].

The present study found that recovery status at one-month following mTBI could be predicted by patients' performance on selected neuropsychological outcome measures when assessed at presentation to the ED. As such, neuropsychological performance may add value to multi-modal prognostic models of PPCS developed in the future. However, PPCS is a multifaceted condition and as performance on neuropsychological tests can also be influenced by extraneous factors, further investigation is needed to examine the utility of additional blood-based and neuroimaging biomarkers in the prediction of PPCS at the individual level.

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3.1 Supplementary Analyses

The following section of this thesis presents the results of additional supplementary analyses that were performed on data collected as part of the pilot study reported in Chapter 3. These analyses were performed to provide further insight into differences in neuropsychological test performance within the acute stages of injury between mTBI patients and control participants recruited to the study. In addition, differences on neuropsychological test performance within the acute stages of injury were examined amongst mTBI patients according to select injury-related characteristics, and correlations between blood-based biomarkers and neuropsychological test performance were also explored.

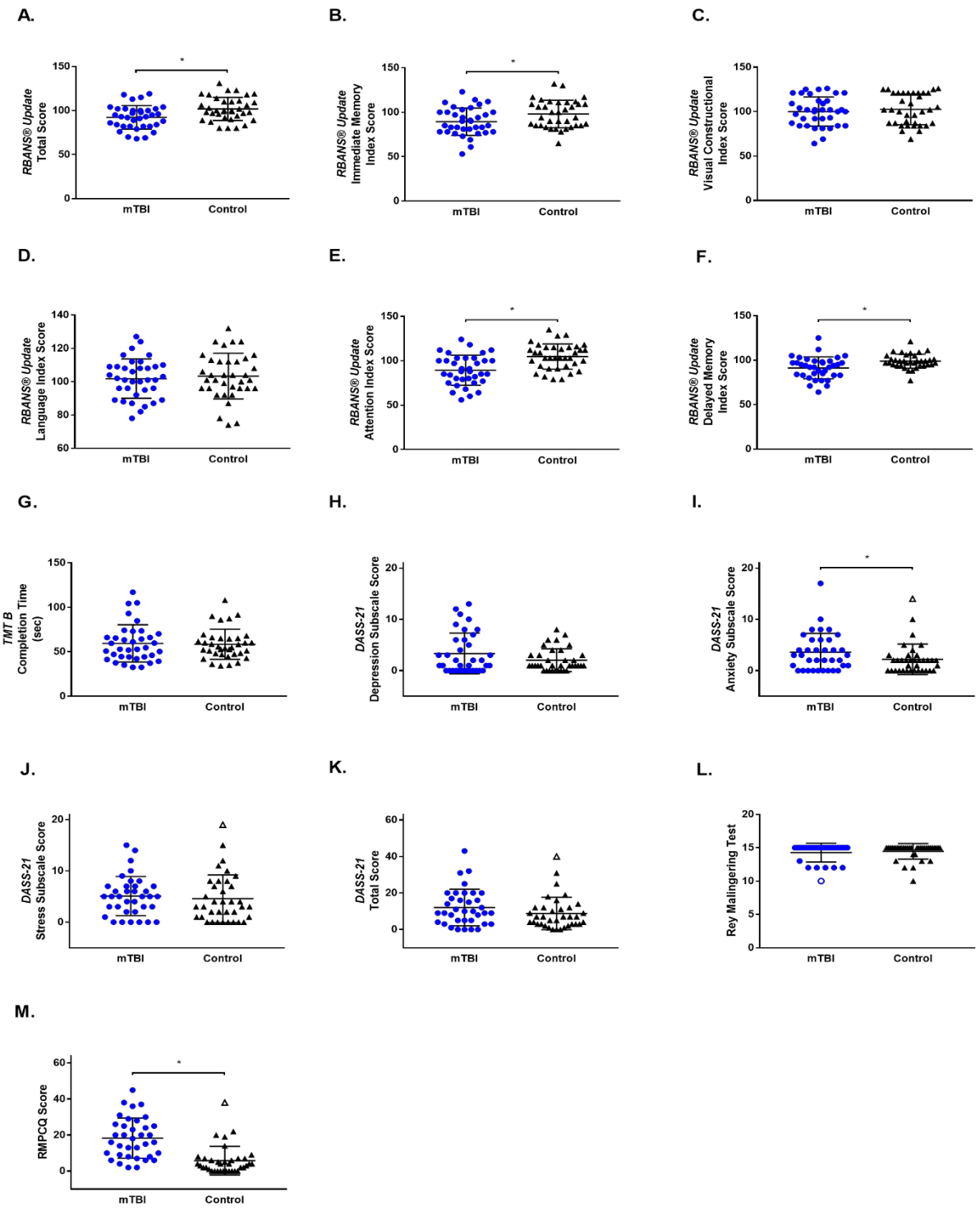
Specifically, the following was investigated:

- Differences in acute performance on neuropsychological measures of cognition and affect between mTBI patients and controls.
- Differences in acute performance on neuropsychological measures of cognition and affect between mTBI patients with and without a history of mTBI.
- Differences in acute performance on neuropsychological measures of affect between mTBI patients with and without a history of psychological disorder.
- Differences in acute performance on neuropsychological measures of cognition and affect between mTBI patients who did and did not experience LOC at time of injury.
- Correlations between blood-based biomarkers (i.e. GFAP, NFL, and NSE) and acute performance on neuropsychological measures of cognition and affect amongst mTBI patients.

Supplementary Table 1. Performance on neuropsychological outcome measures for mTBI patients assessed in the acute stage of injury relative to control participants.

	mTBI *n* = 36		**Control** *n* = 36		***p***	**Test**
Neuropsychological Measure	**Mean (SD)**	**Missing (*n*)**	**Mean (SD)**	**Missing (*n*)**		
RMPCQ	18.33 (11.13)	-	4.86 (5.80)	-	**<.0000***	*t*-test
RMT	14.27 (1.41)	6	14.46 (1.17)	1	.782	*Mann-Whitney U-Test*
RBANS® Update Immediate Memory	89.42 (15.46)	-	98 (15.51)	-	**.022***	*t*-test
RBANS® Update Visual Constructional	100.03 (16.35)	-	102.56 (17.27)	-	.526	*t*-test
RBANS® Update Language	101.83 (11.81)	-	103.33 (13.68)	-	.620	*t*-test
RBANS® Update Attention	89.33 (16.87)	-	104.72 (14.19)	-	**<.001***	*t*-test
RBANS® Update Delayed Memory	91.33 (12.32)	-	98.89 (7.90)	-	**.003***	*t*-test
RBANS® Update Total Score	92.36 (13.25)	-	101.94 (13.18)	-	**.003***	*t*-test
Trail Making Test Form B *(seconds)*	59.40 (21.02)	-	58.53 (16.96)	-	.848	*t*-test
DASS-21 Depression	3.33 (3.96)	-	2.03 (2.21)	-	.090	*t*-test
DASS-21 Anxiety	3.58 (3.67)	-	1.86 (2.23)	-	**.019***	*t*-test
DASS-21 Stress	5.08 (3.82)	-	4.56 (4.64)	-	.600	*t*-test
DASS-21 Total Score	12.03 (10.01)	-	7.89 (7.19)	-	.050	*t*-test

Abbreviations: RMPCQ: Rivermead Post-Concussion Symptoms Questionnaire; *RMT:* Rey Malingering Test; *RBANS®:* Repeatable Battery for the Assessment of Neuropsychological Status: *DASS-21:* Depression Anxiety Stress Scales-21 item version. *Note:* *: $p < 0.05$.



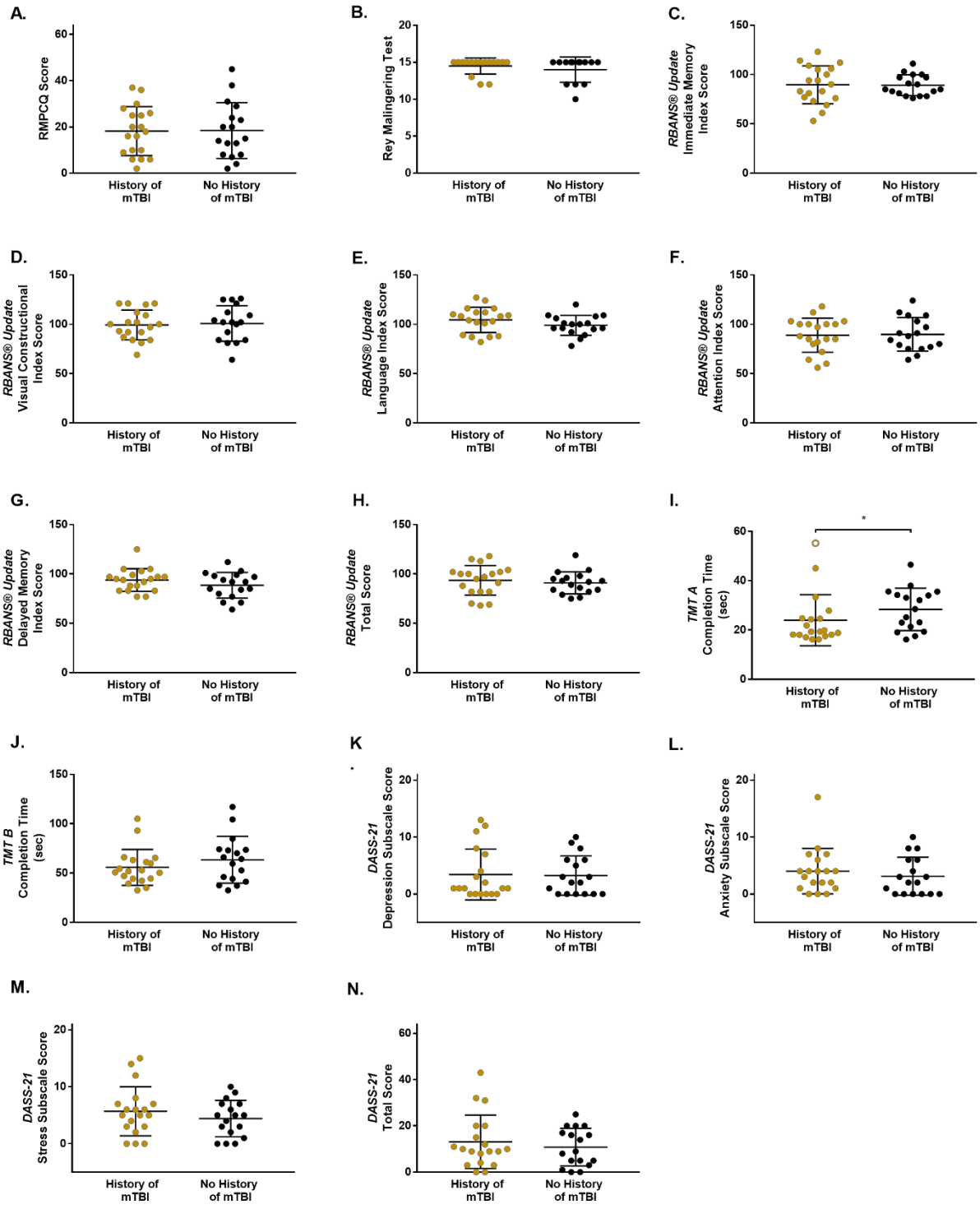
Supplementary Figure 1. Scatterplots depicting mTBI patient and control performance on neuropsychological outcome measures of cognition and affect. Mean and standard deviation shown. Outliers, represented by hollow circles, were identified using *Tukey's Outlier Detection Method* and removed prior to undertaking analyses. Neuropsychological outcomes assessed were as follows: (**A**) Repeatable Battery for the Assessment of Neuropsychological Status *(RBANS®)* Update Total Score; (**B**) *RBANS*® Immediate Memory Index score; (**C**) *RBANS*® Update Visual Constructional Index score; (**D**) *RBANS*® Language Index score; (**E**) *RBANS*® Attention Index score; (**F**) *RBANS*® Delayed Memory Index score; (**G**) Trail Making Test Form B *(TMT-B)*; (**H**) Depression Anxiety Stress Scales- 21 item *(DASS-21)* Depression subscale score; (**I**) *DASS-21*Anxiety subscale score; (**J**) *DASS-21* Stress subscale score; (**K**) *DASS-21* Total score; (**L**) Rey Malingering Test; (**M**) Rivermead Post-Concussion Symptoms Questionnaire *(RMPCQ)*. *Note:* *: $p < 0.05$.

Supplementary Table 2. Demographic characteristics mTBI patients with and without a history of mTBI, and performance on neuropsychological outcome measures of cognition and affect assessed in the acute stage of injury.

	History of mTBI *n* = 19	No History of mTBI *n* = 17	*p*	Test
Demographic characteristics				
Age *(years)*				
Mean (SD)	28.95 (8.78)	26.94 (9.34)	.511	*t*-test
Range	18 – 49	18 - 48	-	-
Gender: Female (%)	9 (47.37)	6 (35.29)	.538	*Fisher's Exact Test*

Neuropsychological Measures	Mean (SD)	Missing (*n*)	Mean (SD)	Missing (*n*)	*p*	Test
RMPCQ	18.21 (10.55)	-	18.47 (12.07)	-	.945	*t*-test
RMT	14.50 (1.01)	3	14 (1.71)	3	.431	*Mann-Whitney U*-test
RBANS® Update Immediate Memory	89.63 (19.16)	-	89.18 (10.47)	-	.929	*t*-test
RBANS® Update Visual Constructional	99.32 (15.16)	-	100.82 (18.03)	-	.787	*t*-test
RBANS® Update Language	104.47 (12.89)	-	98.88 (10.03)	-	.159	*t*-test
RBANS® Update Attention	88.95 (17.21)	-	89.76 (17.00)	-	.887	*t*-test
RBANS® Update Delayed Memory	93.84 (11.44)	-	88.53 (13.01)	-	.201	*t*-test
RBANS® Update Total Score	93.58 (15.05)	-	91.00 (11.11)	-	.567	*t*-test
Trail Making Test Form A *(seconds)*	22.27 (7.28)	-	28.37 (8.59)	-	**.031***	*t*-test
Trail Making Test Form B *(seconds)*	55.76 (18.19)	-	63.46 (23.67)	-	.278	*t*-test
DASS-21 Depression	3.42 (4.46)	-	3.24 (3.46)	-	.891	*t*-test
DASS-21 Anxiety	4.00 (3.99)	-	3.12 (3.33)	-	.479	*t*-test
DASS-21 Stress	5.68 (4.31)	-	4.41 (3.18)	-	.325	*t*-test
DASS-21 Total Score	13.11 (11.55)	-	10.82 (8.14)	-	.503	*t*-test

Abbreviations: SD: Standard deviation; *RMPCQ:* Rivermead Post-Concussion Symptoms Questionnaire; *RMT:* Rey Malingering Test; *RBANS®:* Repeatable Battery for the Assessment of Neuropsychological Status: *DASS-21:* Depression Anxiety Stress Scales-21 item version. *Note:* *: $p < 0.05$.

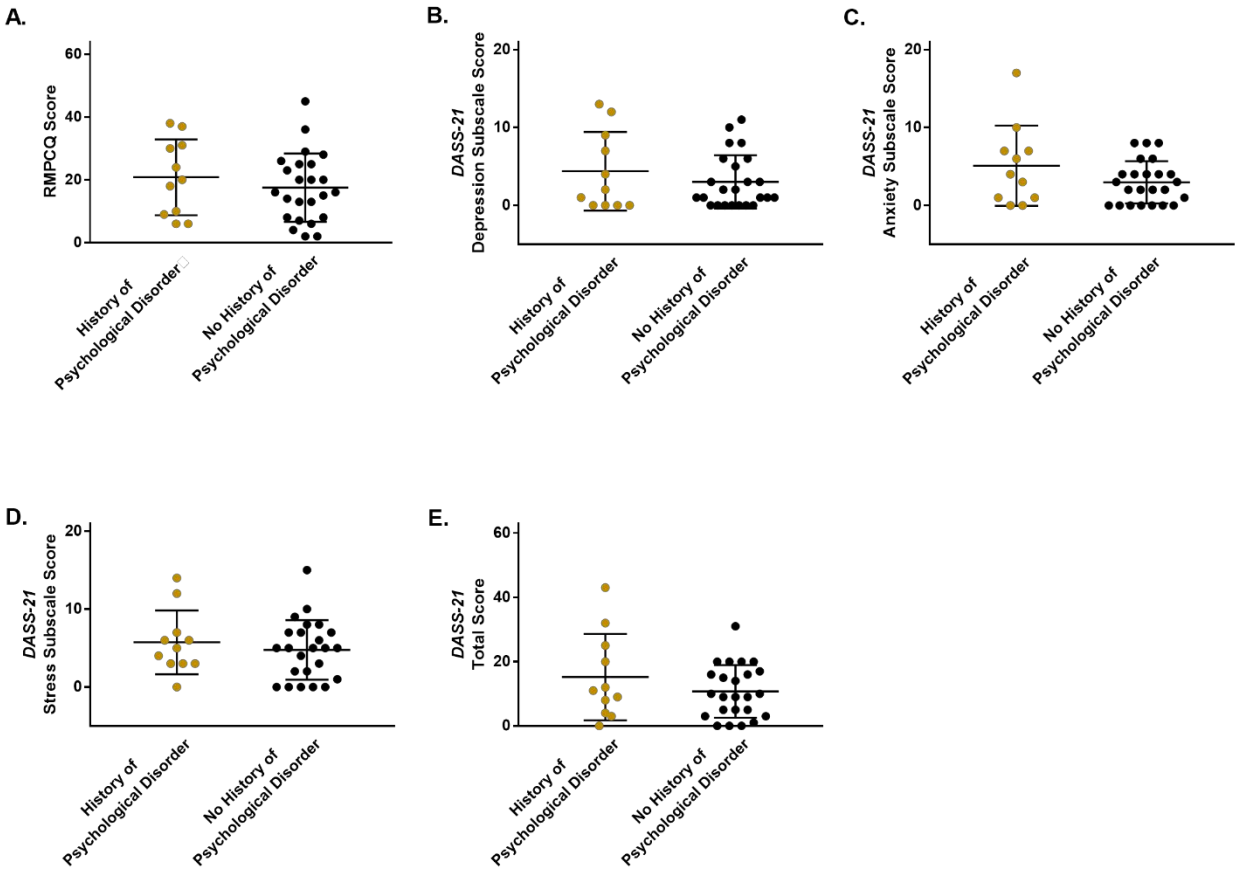


Supplementary Figure 2. Scatterplots depicting acute performance on neuropsychological measures of cognition and affect between mTBI patients with and without a history of previous mTBI. Mean and standard deviation shown. Outliers, represented by hollow circles, were identified using *Tukey's Outlier Detection Method* and removed prior to undertaking analyses. Neuropsychological outcomes assessed were as follows: (**A**) Rivermead Post-Concussion Symptoms Questionnaire *(RMPCQ)*; (**B**) Rey Malingering Test; (**C**) Repeatable Battery for the Assessment of Neuropsychological Status *(RBANS®)* Update Immediate Memory Index score; (**D**) *RBANS®* Update Visual Constructional Index score; (**E**) *RBANS®* Language Index score; (**F**) *RBANS®* Attention Index score; (**G**) *RBANS®* Delayed Memory Index score; (**H**) *RBANS®* Total Index score; (**I**) Trail Making Test Form A *(TMT-A)*; (**J**) Trail Making Test Form B *(TMT-B)*; (**K**) Depression Anxiety Stress Scales- 21 item *(DASS-21)* Depression subscale score; (**L**) *DASS-21* Anxiety subscale score; (**M**) *DASS-21* Stress subscale score; (**N**) *DASS-21* Total score. *Note:* *: $p < 0.05$.

Supplementary Table 3. Demographic characteristics and performance of mTBI patients with and without a history of psychological disorder on neuropsychological outcome measures of affect and the RMPCQ, as assessed in the acute stage of injury.

	History of Psychological Disorder *n* = 11	**No History of Psychological Disorder *n* = 24**	***p***	**Test**
Demographic Characteristics				
Age *(years)*				
Mean (SD)	25.27 (7.96)	28.58 (8.94)	.301	*t*-test
Range	18 - 42	18 - 49	-	-
Gender: Female (%)	9 (81.81)	6 (25)	**.003***	*Fisher's Exact Test*
Neuropsychological Measure	**Mean (SD)**	**Mean (SD)**	***p***	**Test**
RMPCQ	20.82 (12.08)	17.54 (10.88)	.430	*t*-test
DASS-21 Depression	4.36 (5.05)	3.00 (4.43)	.354	*t*-test
DASS-21 Anxiety	5.09 (5.15)	2.96 (2.71)	.219	*t*-test
DASS-21 Stress	5.73 (4.10)	4.75 (3.81)	.496	*t*-test
DASS-21 Total Score	15.18 (13.41)	10.75 (8.20)	.235	*t*-test

Abbreviations: RMPCQ: Rivermead Post-Concussion Symptoms Questionnaire; *DASS-21:* Depression Anxiety Stress Scales- 21 item version. *Note:* *: $p < 0.05$.

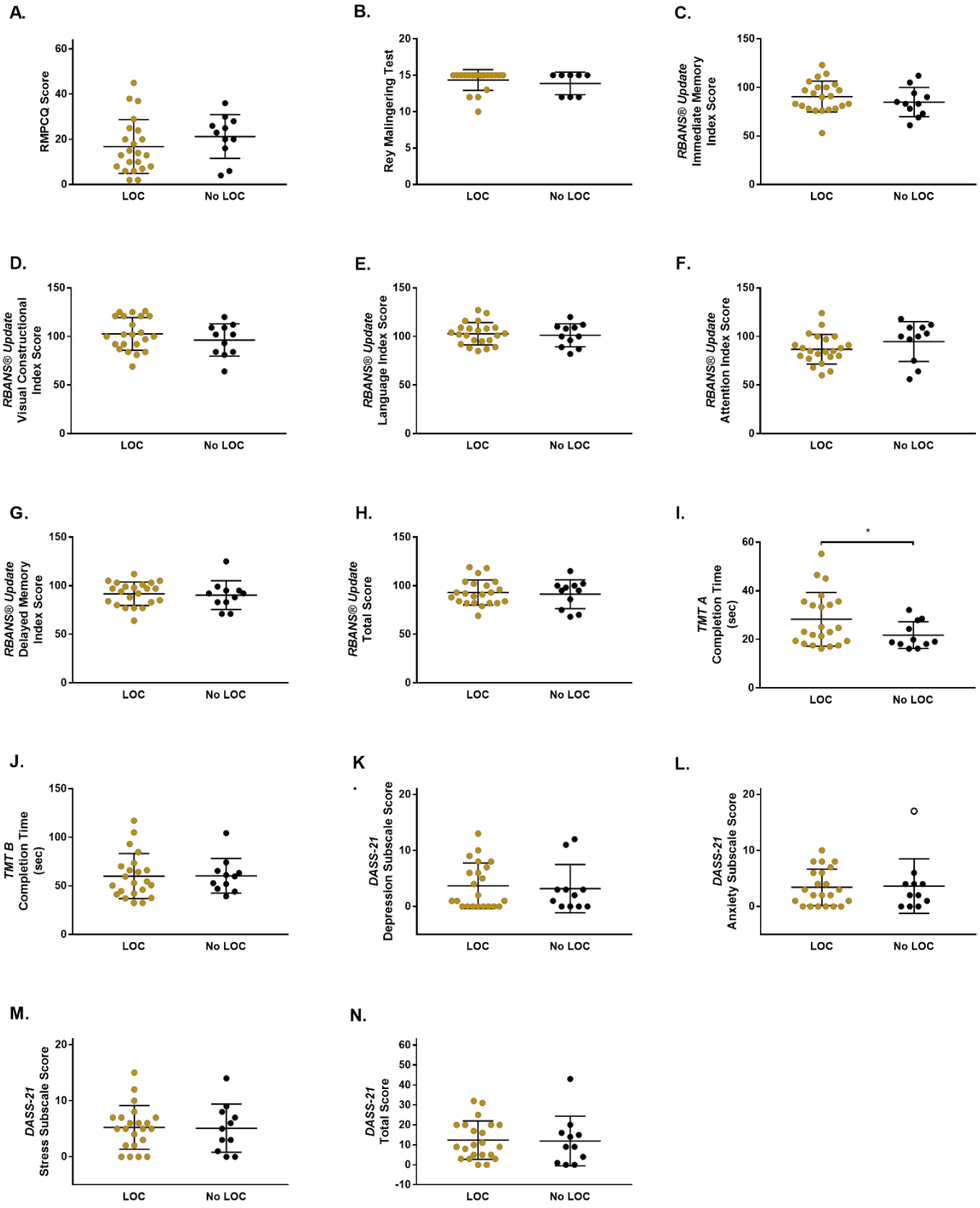


Supplementary Figure 3. Scatterplots depicting acute performance on neuropsychological measures of affect between mTBI patients with and without a history of psychological disorder. Mean and standard deviation shown. Data were screened for outliers using *Tukey's Outlier Detection Method* prior to undertaking analyses. No outliers were identified. Neuropsychological outcomes assessed were as follows: (**A**) Rivermead Post-Concussion Symptoms Questionnaire *(RMPCQ)*; (**B**) Depression Anxiety Stress Scales- 21 item *(DASS-21)* Depression subscale score; (**C**) *DASS-21* Anxiety subscale score; (**D**) *DASS-21* Stress subscale score; (**E**) *DASS-21* Total score.

Supplementary Table 4. Performance of mTBI patients with and without reported LOC at time of injury on neuropsychological outcome measures of cognition and affect, as assessed in the acute stage of injury.

	LOC *n* = 22		No LOC *n* = 11		*p*	Test
Demographic characteristics						
Age *(years)*						
Mean (SD)	25.77 (8.86)		31.36 (9.21)		.102	*t*-test
Range	18 - 48		18 - 49		-	-
Gender: Female (%)	7 (31.82)		6 (54.55)		.270	*Fisher's Exact test*
Neuropsychological Measures	**Mean (SD)**	**Missing (*n*)**	**Mean (SD)**	**Missing (*n*)**	***p***	**Test**
RMPCQ	16.82 (11.95)	-	21.27 (9.70)	-	.293	*t*-test
RMT	14.35 (1.42)	2	13.88 (1.53)	3	.347	*Mann-Whitney U*-test
RBANS® Update Immediate Memory	90.55 (15.91)	-	84.82 (15.06)	-	.329	*t*-test
RBANS® Update Visual Constructional	102.64 (16.76)	-	96.36 (16.69)	-	.318	*t*-test
RBANS® Update Language	102.82 (11.50)	-	101.18 (11.85)	-	.705	*t*-test
RBANS® Update Attention	86.82 (15.23)	-	94.82 (20.52)	-	.215	*t*-test
RBANS® Update Delayed Memory	91.73 (12.03)	-	90.36 (14.79)	-	.778	*t*-test
RBANS® Update Total Score	93.00 (13.00)	-	91.27 (14.80)	-	.733	*t*-test
Trail Making Test Form A *(seconds)*	28.28 (11.05)	-	21.74 (5.52)	-	**.031***	*t*-test
Trail Making Test Form B *(seconds)*	60.10 (23.16)	-	60.33 (17.81)	-	.977	*t*-test
DASS-21 Depression	3.68 (4.08)	-	3.18 (4.31)	-	.747	*t*-test
DASS-21 Anxiety	3.41 (3.25)	-	3.64 (4.86)	-	.259	*t*-test
DASS-21 Stress	5.23 (3.90)	-	5.09 (4.30)	-	.928	*t*-test
DASS-21 Total Score	12.36 (9.61)	-	11.91 (12.43)	-	.908	*t*-test

Abbreviations: RMPCQ: Rivermead Post-Concussion Symptoms Questionnaire; *RMT:* Rey Malingering Test; *RBANS®:* Repeatable Battery for the Assessment of Neuropsychological Status: *DASS-21:* Depression Anxiety Stress Scales-21 item version. *Note:* *: $p < 0.05$.

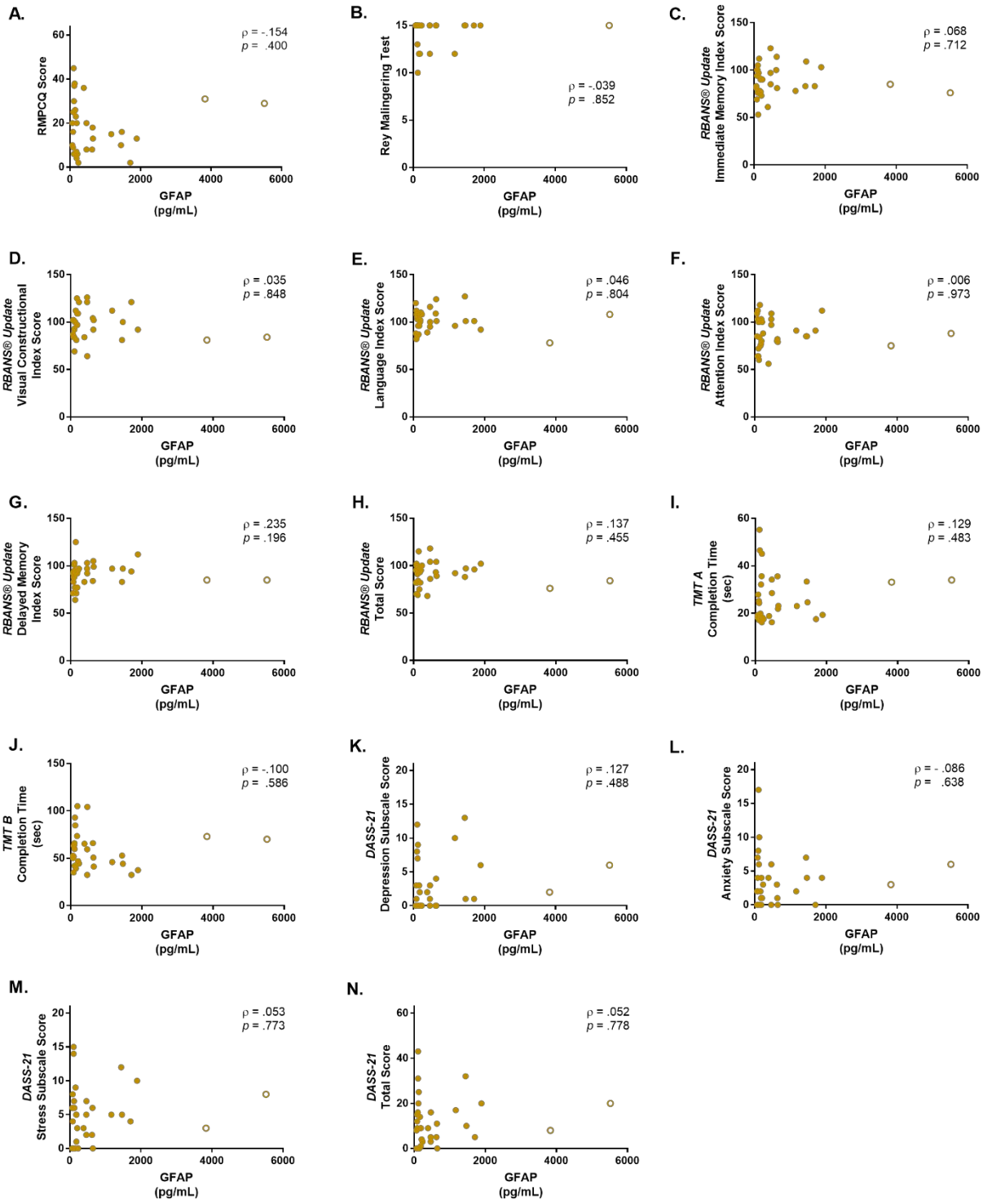


Supplementary Figure 4. Scatterplots depicting acute performance on neuropsychological measures of cognition and affect between mTBI patients with and without LOC at time of injury. Mean and standard deviation shown. Outliers, represented by hollow circles, were identified using *Tukey's Outlier Detection Method* and removed prior to undertaking analyses. Neuropsychological outcomes assessed were as follows: (**A**) Rivermead Post-Concussion Symptoms Questionnaire *(RMPCQ)*; (**B**) Rey Malingering Test; (**C**) Repeatable Battery for the Assessment of Neuropsychological Status *(RBANS®)* Update Immediate Memory Index score; (**D**) *RBANS®* Update Visual Constructional Index score; (**E**) *RBANS®* Language Index score; (**F**) *RBANS®* Attention Index score; (**G**) *RBANS®* Delayed Memory Index score; (**H**) *RBANS®* Total Index score; (**I**) Trail Making Test Form A *(TMT-A)*; (**J**) Trail Making Test Form B *(TMT-B)*; (**K**) Depression Anxiety Stress Scales- 21 item *(DASS-21)* Depression subscale score; (**L**) *DASS-21* Anxiety subscale score; (**M**) *DASS-21* Stress subscale score; (**N**) *DASS-21* Total score. *Note:* *: $p < 0.05$.

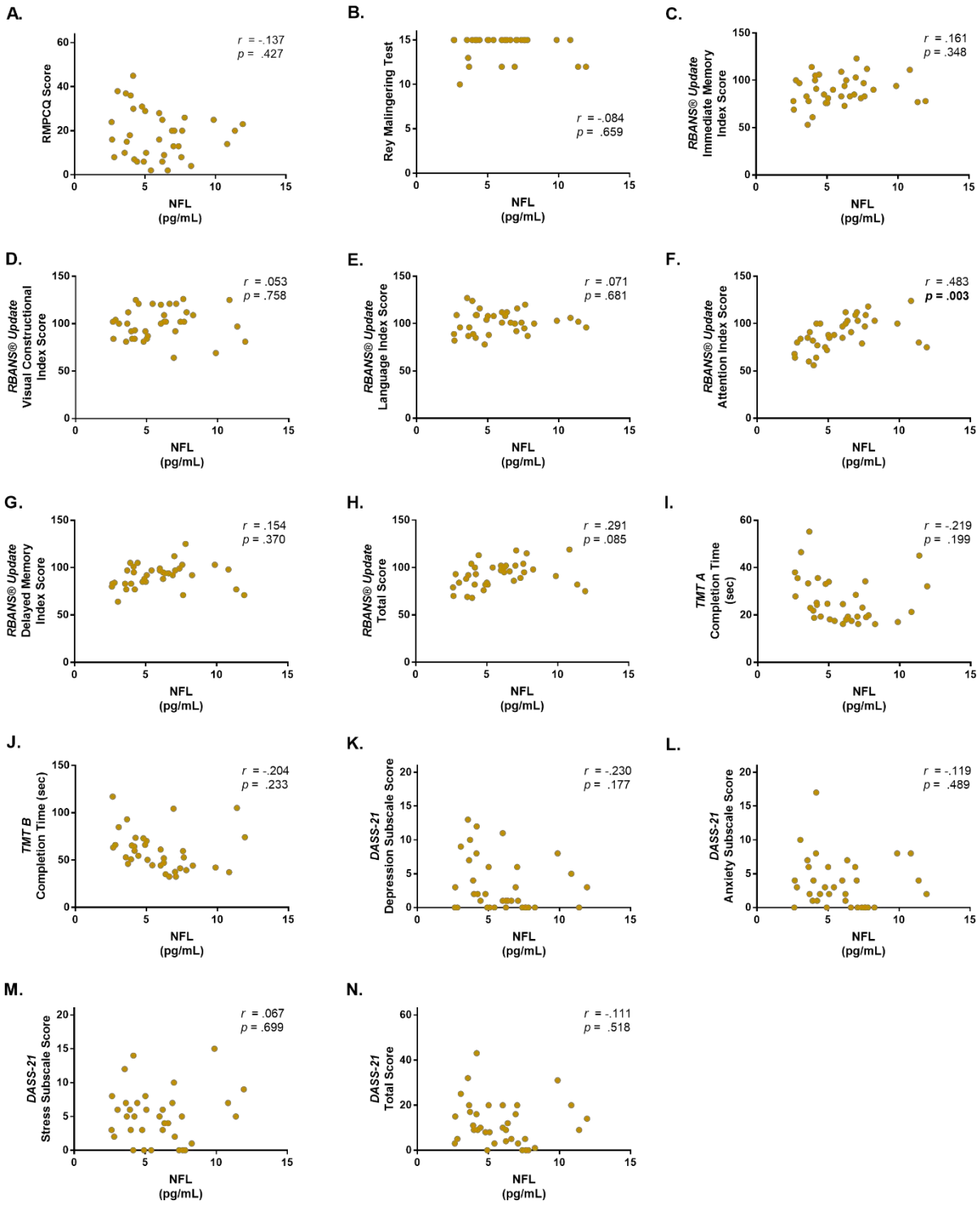
Supplementary Table 5. Descriptive statistics for blood-based biomarker concentrations and performance on neuropsychological outcomes measures as assessed in the acute stages of injury of mTBI individuals.

	n	Mean	Standard Deviation	Median	Range	Skewness	Kurtosis
Blood based Biomarkers							
GFAP	32	720.60	1177.27	197.00	63.40 – 5519	2.955	9.515
NFL	36	5.92	2.42	5.71	2.63 – 11.94	.845	.303
NSE	36	8418.31	14470.00	4281.00	1702.00 – 88813.00	5.186	29.084
Neuropsychological measures							
RMPCQ	36	18.33	11.12	17	2 - 45	.489	-.487
RMT	30	14.27	1.41	15	10 - 15	-1.689	1.698
RBANS® Update Immediate Memory	36	89.42	15.46	87.50	53 – 123	-.004	-.131
RBANS® Update Visual Constructional	36	100.03	16.35	101	64 – 126	-.134	-.651
RBANS® Update Language	36	101.83	11.81	101.5	78 – 127	.031	-.431
RBANS® Update Attention	36	89.33	16.87	88	56 – 124	-.024	-.618
RBANS® Update Delayed Memory	36	91.33	12.32	93	64 – 125	.129	.636
RBANS® Update Total Score	36	92.36	13.25	93	68 – 119	.095	-.403
Trails Making Test Form A *(seconds)*	36	26.06	9.68	23.05	16.17 – 55.20	1.238	1.159
Trails Making Test Form B *(seconds)*	36	59.40	21.02	53.73	32.50 – 117.00	1.082	.850
DASS-21 Depression	36	3.33	3.96	1.50	0 – 13	1.093	-.014
DASS-21 Anxiety	36	3.58	3.67	3	0 – 17	1.579	3.711
DASS-21 Stress	36	5.08	3.82	5	0 – 15	.717	.523
DASS-21 Total Score	36	12.03	10.01	9.50	0 – 43	1.120	1.413

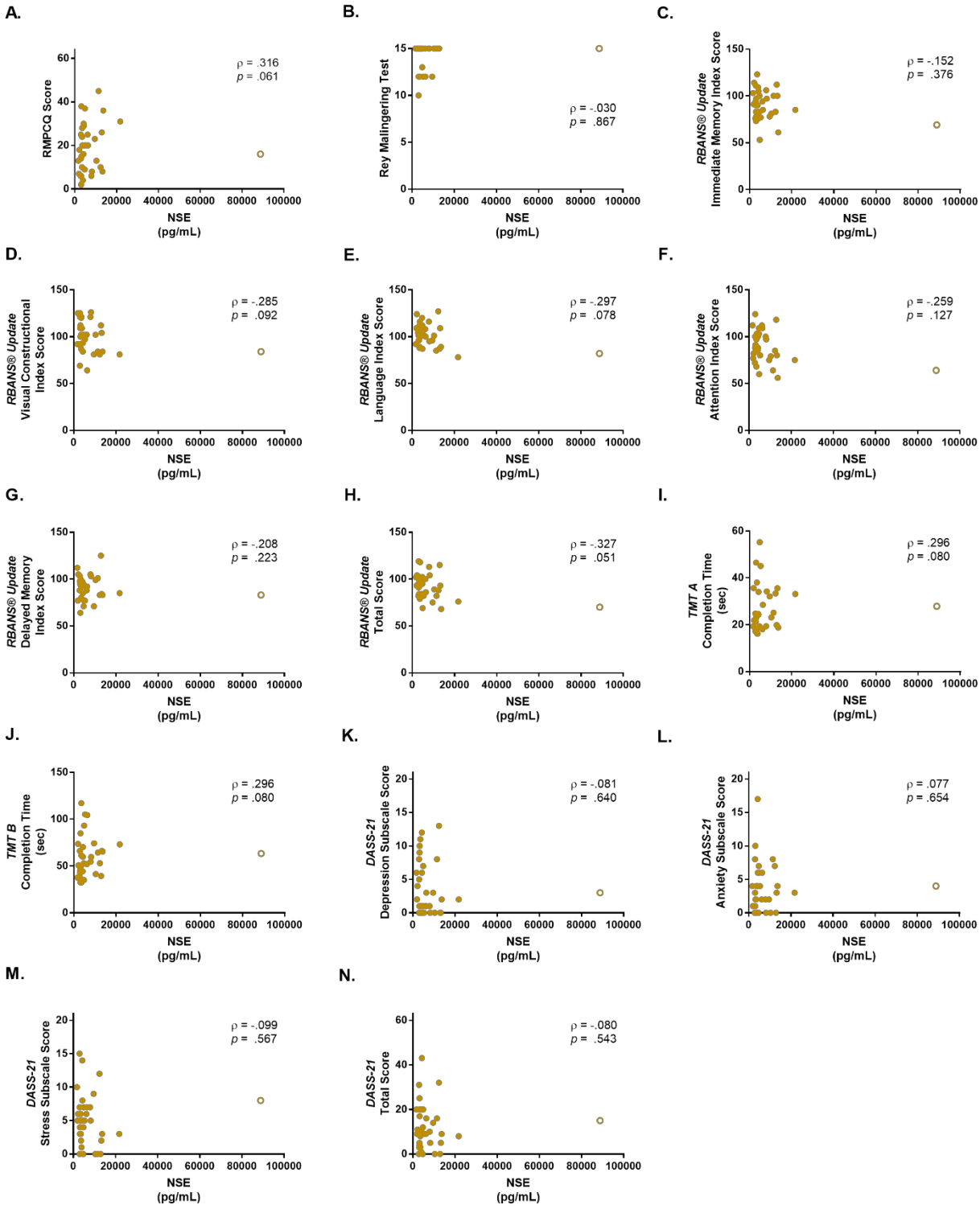
Abbreviations: GFAP: Glial Fibrillary Acidic Protein; *NFL:* Neurofilament- Light; *NSE:* Neuron Specific Enolase; *RMPCQ:* Rivermead Post-Concussion Symptoms Questionnaire; *RMT:* Rey Malingering Test: *RBANS*: Repeatable Battery for the Assessment of Neuropsychological Status; *DASS-21*: Depression Anxiety Stress Scales- 21 item version.



Supplementary Figure 5a. Scatterplots depicting the association between mTBI patient GFAP concentrations and acute performance on neuropsychological measures of cognition and affect. Outliers were identified using *Tukey's Outlier Detection Method* and are represented by hollow circles. Spearman correlations (ρ) were performed as GFAP data was not normally distributed, and outliers were included in the analyses presented. Neuropsychological outcomes assessed were as follows: (**A**) Rivermead Post-Concussion Symptoms Questionnaire *(RMPCQ)*; (**B**) Rey Malingering Test; (**C**) Repeatable Battery for the Assessment of Neuropsychological Status *(RBANS®)* Update Immediate Memory Index score; (**D**) *RBANS®* Update Visual Constructional Index score; (**E**) *RBANS®* Language Index score; (**F**) *RBANS®* Attention Index score; (**G**) *RBANS®* Delayed Memory Index score; (**H**) *RBANS®* Total Index score; (**I**) Trail Making Test Form A *(TMT-A)*; (**J**) Trail Making Test Form B *(TMT-B)*; (**K**) Depression Anxiety Stress Scales- 21 item *(DASS-21)* Depression subscale score; (**L**) *DASS-21* Anxiety subscale score; (**M**) *DASS-21* Stress subscale score; (**N**) *DASS-21* Total score.



Supplementary Figure 5b. Scatterplots depicting the association between mTBI patient NFL concentrations and acute performance on neuropsychological measures of cognition and affect. Data were screened for outliers using *Tukey's Outlier Detection Method* prior to undertaking analyses. No outliers were identified. Pearson correlations (*r*) were performed. Neuropsychological outcomes assessed were as follows: (**A**) Rivermead Post-Concussion Symptoms Questionnaire *(RMPCQ)*; (**B**) Rey Malingering Test; (**C**) Repeatable Battery for the Assessment of Neuropsychological Status *(RBANS®)* Update Immediate Memory Index score; (**D**) *RBANS*® Update Visual Constructional Index score; (**E**) *RBANS*® Language Index score; (**F**) *RBANS*® Attention Index score; (**G**) *RBANS*® Delayed Memory Index score; (**H**) *RBANS*® Total Index score; (**I**) Trail Making Test Form A *(TMT-A)*; (**J**) Trail Making Test Form B *(TMT-B)*; (**K**) Depression Anxiety Stress Scales-21 item *(DASS-21)* Depression subscale score; (**L**) *DASS-21* Anxiety subscale score; (**M**) *DASS-21* Stress subscale score; (**N**) *DASS-21* Total score.



Supplementary Figure 5c. Scatterplots depicting the association between mTBI patient NSE concentrations and acute performance on neuropsychological measures of cognition and affect. Outliers were identified using *Tukey's Outlier Detection Method* and are represented by hollow circles. Spearman correlations (ρ) were performed, as NSE data was not normally distributed, and outliers were included the analyses presented. Neuropsychological outcomes assessed were as follows: (**A**) Rivermead Post-Concussion Symptoms Questionnaire *(RMPCQ)*; (**B**) Rey Malingering Test; (**C**) Repeatable Battery for the Assessment of Neuropsychological Status *(RBANS®)* Update Immediate Memory Index score; (**D**) *RBANS®* Update Visual Constructional Index score; (**E**) *RBANS®* Language Index score; (**F**) *RBANS®* Attention Index score; (**G**) *RBANS®* Delayed Memory Index score; (**H**) *RBANS®* Total Index score; (**I**) Trail Making Test Form A *(TMT-A)*; (**J**) Trail Making Test Form B *(TMT-B)*; (**K**) Depression Anxiety Stress Scales- 21 item *(DASS-21)* Depression subscale score; (**L**) *DASS-21* Anxiety subscale score; (**M**) *DASS-21* Stress subscale score; (**N**) *DASS-21* Total score.

4 *CREST* Protocol

Gozt, A., Hellewell, S.C., Thorne, J., Thomas, E., Buhagiar, F., Markovic, S., van Houselt, A., Ring, A., Arendts, G., Smedley, B., van Schalkwyk, S., Brooks, P., Iliff, J., Celenza, A., Mukherjee, A., Xu, D., Robinson, S., Honeybul, S., Cowen, G., Licari, M., Bynevelt, M., Pestell, C., Fatovich, D., & Fitzgerald, M. (2021). Predicting outcome following mild traumatic brain injury: Protocol for the longitudinal, prospective, observational concussion recovery (CREST) cohort study. *BMJ Open*, *11*(5): e046460

BMJ Open

Predicting outcome following mild traumatic brain injury: protocol for the longitudinal, prospective, observational Concussion Recovery (*CREST*) cohort study

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ABSTRACT

Introduction Mild traumatic brain injury (mTBI) is a complex injury with heterogeneous physical, cognitive, emotional and functional outcomes. Many who sustain mTBI recover within 2 weeks of injury; however, approximately 10%–20% of individuals experience mTBI symptoms beyond this 'typical' recovery timeframe, known as persistent post-concussion symptoms (PPCS). Despite increasing interest in PPCS, uncertainty remains regarding its prevalence in community-based populations and the extent to which poor recovery may be identified using early predictive markers.

Objective (1) Establish a research dataset of people who have experienced mTBI and document their recovery trajectories; (2) Evaluate a broad range of novel and established prognostic factors for inclusion in a predictive model for PPCS.

Methods and analysis The Concussion Recovery Study (*CREST*) is a prospective, longitudinal observational cohort study conducted in Perth, Western Australia. *CREST* is recruiting adults aged 18–65 from medical and community-based settings with acute diagnosis of mTBI. *CREST* will create a state-wide research dataset of mTBI cases, with data being collected in two phases. *Phase I* collates data on demographics, medical background, lifestyle habits, nature of injury and acute mTBI symptomatology. In *Phase II*, participants undergo neuropsychological evaluation, exercise tolerance and vestibular/ocular motor screening, MRI, quantitative electroencephalography and blood-based biomarker assessment. Follow-up is conducted *via* telephone interview at 1, 3, 6 and 12 months after injury. Primary outcome measures are presence of PPCS and quality of life, as measured by the Post-Concussion Symptom Scale and the Quality of Life after Brain Injury questionnaires, respectively. Multivariate modelling will examine the prognostic value of promising factors.

Ethics and dissemination Human Research Ethics Committees of Royal Perth Hospital (#RGS0000003024), Curtin University (HRE2019-0209), Ramsay Health Care (#2009) and St John of God Health Care (#1628) have approved this study protocol. Findings will be published in peer-reviewed journals and presented at scientific conferences.

Strengths and limitations of this study

- Concussion Recovery Study (*CREST*) is a prospective, longitudinal cohort study recruiting adult participants who have experienced mild traumatic brain injury (mTBI) *via* hospital emergency departments and community-based pathways in Perth, Western Australia.
- A primary strength of *CREST* is the establishment of a clinical research dataset of mTBI in Western Australia and documentation of variable recovery trajectories, for which there is currently limited data.
- Another asset of *CREST* is the investigation of novel and established preinjury predictive factors, blood-based biomarkers, neuropsychological tests, exercise tolerance, vestibular/ocular function and advanced neuroimaging outcome measures with the aim of generating a predictive model from this 'suite' of factors that may be useful for identifying individuals at risk of experiencing delayed recovery following mTBI.
- A primary limitation of this study may be loss to follow-up and resulting missing data points.
- Other limitations include possible selection bias on the basis of geographic location or injury severity, and sample-size constraints pertaining to predictive modelling.

Trial registration number ACTRN12619001226190.

INTRODUCTION

Mild traumatic brain injury (mTBI), also known as concussion, accounts for approximately 80% of all traumatic brain injuries occurring both in Australia and worldwide.[1] mTBI is characterised by a rapid, transient change in neurological function[2 3] accompanied by numerous signs and symptoms, the most frequent of which are headache, neck pain, dizziness, difficulty concentrating and alterations in mood and sleep.[4] mTBI sequelae can be broadly classified into physical, cognitive, emotional and sleep-related domains,[5] although the clinical presentation of mTBI is known to vary considerably between individuals,[6] significantly hampering development of reliable prognostic tools.

The prevailing notion of mTBI recovery trajectory implies that symptomatic resolution can be expected within approximately 2 weeks of injury.[7–10] However, it is increasingly realised that recovery is complex and multifactorial,[11] and this recovery trajectory which has been previously defined in the literature pertaining to young sportspeople may not necessarily reflect recovery across age, sex and socioeconomic status. It is frequently cited that 10%–20% of individuals who sustain a mTBI will experience symptoms at least 1 month following injury,[12] known as persistent post-concussion symptoms (PPCS).[13] Determining the true prevalence of PPCS has been complicated by the lack of consistent follow-up across studies and the non-specific nature of the condition.[14] The multitudes of documented ramifications stemming from PPCS have contributed to its status as an emergent public health issue. PPCS may profoundly impact an individual's ability to carry out activities of daily living, and can result in functional consequences including delayed or reduced ability to return to work,[15 16] study[17] and playing sport,[18] as well as impaired satisfaction and quality of life.[19–22] Furthermore, PPCS has been linked with heightened use of healthcare services,[23–25] making it an under-recognised economic burden.

It is not currently possible to identify which individuals will experience delayed recovery at the time of mTBI diagnosis, nor is there a consensus on how to manage patients who experience such a debilitating constellation of symptoms. The ability to predict who will develop PPCS would be of great benefit. From a clinical perspective, a prognostic model would assist with decision-making and management of patient expectations about their recovery. Importantly, it would enable the provision of personalised healthcare to patients by facilitating triage to the most appropriate forms of treatment according to individual needs *before* symptoms become chronic, thereby potentially resulting in improved patient outcomes. Researchers would also benefit from prognostic models, which could be used to enrich clinical trials for evidence-based treatments, which aim to prevent or ameliorate the effects of PPCS or other late-stage conditions associated with mTBI, such as chronic traumatic encephalopathy[26–31] or Alzheimer's disease.[32–34]

A plethora of studies have been conducted assessing biomarkers and other factors for their capacity to predict outcome following mTBI. However, variations in study methodologies have resulted in inconsistent results reported in the literature,[35 36] and many of the studies conducted to date have been limited to investigating only one type or at best a small subset of prognostic factors.[37] Demographics and injury-related characteristics are among the most frequently examined variables, partly because of the convenience with which they can be extracted from medical records. Factors including female sex,[38–41] previous history of mTBI[42 43] and prein-jury mental health issues[41 43–48] have all been flagged as potential predictors of PPCS, while others such as age,[49] educational status,[40 42 50] loss of consciousness[35 48 50 51] and (post-traumatic) amnesia[35 42 52–54] are contentious and require further and more thorough investigation.

Reports of poor cognitive function following mTBI has led to the investigation of individual performance on neuropsychological tests as a potential predictor of PPCS. A heightened risk of PPCS has been found among individuals who perform poorly on post-mTBI tests of executive function,[54] memory[38 55–57] and psychomotor function[53]; however, the overall fidelity with which neuropsychological measures alone can prognosticate PPCS has been called into question given that individual performance can be influenced by extraneous factors such as age, prior education and socioeconomic status.[58–61] Consequently, efforts have turned towards identifying and examining other markers of PPCS.

Blood-based biomarkers are one viable option that has been embraced by the research community, as they can be a relatively inexpensive and rapid way of assessing the physiological mechanisms that underpin conditions of interest. To date, a vast array of candidate biomarkers pertaining to cellular structural or functional damage as well as the biochemical and molecular secondary injury cascades have been investigated for their ability to predict outcome after traumatic brain injury.[62–64] While biomarkers such as S100B[65] and the combination of glial fibrillar acidic protein (GFAP) and ubiquitin C-terminal hydrolase-L1 (UCH-L1)[66] have been proposed to assist with clinical decision making processes relating to traumatic brain injury, studies specifically assessing the relationship between fluid biomarkers and clinical outcome following mTBI have generally yielded small or variable effects.[67]

More recently, a host of neuroimaging techniques (e.g. MRI,[68] CT,[69] PET[70]) and physiological biomarkers (e.g. exercise tolerance,[71] vestibular/ocular function,[72] psychomotor responses[73]) have also been identified as having the potential to serve as objective markers of PPCS; however, investigations into their prognostic capabilities have yielded inconsistent results and/or been relatively limited, and thus their utility remains to be ascertained. Similarly, the potential for personal predispositions

(e.g. resilience,[74] coping style[75]) to influence outcome following injury has also been acknowledged, but more research is needed to elucidate the extent of involvement.

Considering that a single predictive variable is unlikely to be the 'silver bullet' that predicts outcome at the level of the individual,[35] it is not altogether surprising that research is yet to accurately identify which individuals will experience PPCS. It is increasingly recognised that a more fruitful approach would draw from multiple assessment elements for multivariate prognostic modelling to better calibrate the risk of poor clinical outcomes.[35] No study to date has successfully developed a prediction model that is targeted specifically for prediction of individual patient outcomes following mTBI.[35 76] Efforts to develop validated and pragmatic tools for use in a clinical and/or research context have been impeded by considerable variation between studies and use of suboptimal methodologies across studies.[12 76] Common limitations identified include small and/or selected sample sizes (often resulting from the use of a single centre), recruitment of participants beyond the acute injury period or across a wide postinjury timespan, inconsistencies in definition and measurement of PPCS as well as variable follow-up time points.[35 76 77] Furthermore, prognostic models arising from retrospective study cohorts often encounter additional issues including poor data quality, missing data, minimal use of validated symptom scoring scales and lack of standardised acute evaluations.[77]

The Concussion Recovery Study (*CREST*) is a large, cross-institutional study conducted in Perth, Western Australia (WA), developed with the aim of identifying individuals that are at an increased risk of developing PPCS. Approximately 2.4 million people reside in WA, of which 79% live within the capital city of Perth[78]; the most isolated capital city in the world. The greater Perth area extends a distance of over 125 km, occupies an area of 6418 km^2,[79] and is served by 10 Emergency Departments (EDs: one private and nine public, of which one is maternity and one is child/adolescent exclusively).

CREST is collecting longitudinal data in two phases and uses a multivariate, 'suite-based' approach that incorporates demographics, injury-related characteristics, neuropsychological assessment, blood-based biomarkers, MRI, quantitative electroencephalography (qEEG), exercise tolerance and vestibular/ocular function to develop an evidence-based acute predictive model for PPCS. The study hypothesises that a suite of preinjury factors and outcome measures that are assessed during the early presentation period may be used to predict those at risk of experiencing PPCS compared with those who recover within a typical timeframe. It is predicted that a combination of these outcome measures will provide superior discriminatory capacity relative to any single marker used in isolation.

OBJECTIVES

The primary objectives of *CREST* are:

1. To establish a large-scale clinical research dataset of adults experiencing mTBI in Western Australia, in order to observe the typical pattern of recovery from mTBI and determine the incidence of PPCS within the Western Australian context.
2. To identify a suite of preinjury factors and outcome measures during the early presentation period that may be used to predict those at risk of experiencing PPCS compared with those who recover within a typical timeframe.

The secondary objective of the *CREST* study is todetermine the feasibility of recruiting a large cohort of participants with mTBI from a variety of sources (e.g. EDs, general practitioners (GPs), and community sporting groups), as this widespread collection of community mTBI data has not previously been conducted to this scale in Australia to date.

METHODS AND ANALYSIS

Patient and public involvement

A *Community Conversation* was held in August 2018 involving clinicians and general community members with and without a history of mTBI. The conversation took form of a thematic exploration of current management considerations for mTBI, assessment measures, long-term prognosis and symptomatology and contributing factors to recovery. This public consultation highlighted the need for research to determine the predictors for poor outcomes following mTBI and growing interest in combining screening tools, radiological scans and biological markers for predictive purposes. This stakeholder group shaped the design of the study by highlighting the importance of recruiting participants from the wider community, in addition to clinical populations. The clinicians shaped the *CREST* study's multimodal research design. Several individuals who participated in the *Community Conversation* assisted with recruitment strategies and dissemination of information, although there were not asked to assess the burden of the time required to participate in the research. Interested members of the group will be consulted at the conclusion of the study to guide dissemination of findings.

CREST aims to capture a broad cross-section of community mTBI resulting from a variety of different injury mechanisms (e.g. assault, falls, sports, transport accidents, workplace incidents). Enrolment into *CREST* is open to individuals aged 18–65 years who have sustained a medically diagnosed mTBI within the last 7 days. Box 1 details additional inclusion and exclusion criteria for *Phases I* and *II* of the study. Eligibility criterion for referral to the study are straightforward in design given that in addition to traditional medical-based pathways, the study aims to recruit participants from the general community, who may have a varied understanding of mTBI. We aim to enrol n=500 participants in *Phase I* of the study.

Box 1 Inclusion and exclusion criteria for Phase I and Phase II for the Concussion Recovery Study *(CREST)*

Phase I

Inclusion criteria

- Aged 18–65 years
- Mild traumatic brain injury (mTBI) within 7 days days
- Diagnosed with mTBI by medical practitioner

Exclusion criteria

- Significant history of pre-existing conditions that would interfere with outcome assessment and follow-up (e.g. substance abuse/alcohol abuse, homelessness, terminal illness)
- Significant debilitating pre-existing diagnosed mental health disorder that would interfere with neuropsychological and possibly blood biomarker outcome measures, or ability to contact for follow-up (e.g. schizophrenia, bipolar disorder).
- Significant pre-existing neurological condition, which may interfere with ability to complete outcome measures or follow-up (e.g. stroke, dementia)
- Pre-existing cognitive impairment (e.g. intellectual disability), which may interfere with ability to undertake neuropsychological examination
- Non-English speakers or individuals with poor English language skills
- Prisoners in custody or people known to be involved in illegal activity
- Head injury deemed to be entirely due to primary seizure
- Pregnancy

Phase II

Inclusion criteria

In addition to Phase I inclusion criteria

- Willing and able to attend the Curtin University and Perron Institute for Neurological and Translational Sciences research tenancies located at the Ralph and Patricia Sarich Neuroscience Research Institute within 7 days days of date of injury, and Sir Charles Gardiner Hospital (SCGH) for MRI within 9 days days of injury.

Exclusion criteria

In addition to Phase I inclusion criteria

- Significant other physical trauma that would interfere with physical and/or biochemical outcome assessments and follow-up (e.g. lower limb injuries that would compromise balance or exercise bike testing, or cause changes in blood biomarkers)
- Any pre-existing heart conditions or other medical conditions that may compromise ability to complete an exercise tolerance test
- Epilepsy or history of seizure
- Meets exclusion criteria to undertake MRI, which can be any of the following:
  - Has cardiac pacemaker or pacing wire *in situ*
  - Has metal surgical clips or staples of any kind (particularly aneurysm clips) *in situ*
  - Has lap band surgery
  - Has electronic inner ear implants (bionic ears)
  - Has metal fragments in eyes (past or present)
  - Has electronic stimulators
  - Has implanted pumps
  - Has metal pins or rods in bones
  - Has an IUCD fitted
  - Has shrapnel, bullets or foreign bodies
  - Is pregnant
  - Has braces
  - Has embolisation coils*
  - Unable to lie flat*

Note: *: item not strictly listed as an exclusion criterion but screened for as part of routine practice at the Sir Charles Gairdner Hospital MRI department. IUCD: Intrauterine contraceptive device

Participant recruitment pathways

Recruitment occurs across multiple pathways including major *WA Health* hospital EDs located throughout the Perth metropolitan area (see figure 1), GPs, sports physicians, allied health professionals, community/amateur and semi-professional sporting clubs, as well as self-referral to the study. Participants sign a *Participant Referral Form* (PRF; see online supplemental document 1) consenting for their contact details to be released to the study research team at the medical practitioner's premises (e.g. hospital ED or GP), as further described below. Participants are emailed or provided with a written copy of their verbal consent and the participant information sheet at the conclusion of the enrolment interview. Furthermore, *Phase II* participants also receive written documentation of informed consent when they attend the Research Hub, prior to undertaking any of the testing components.

Hospital ED pathway

Staff at hospital EDs screen for individuals presenting with mTBI for eligibility. Individuals may be considered for *CREST* if they provide a description of an incident likely to have resulted in a mTBI, with accompanying symptoms that can be attributed to that injury as defined by the World Health Organization (WHO).[80] Prospective participants must also describe at least one of the following, as described by the *American Congress of Rehabilitation Medicine*[3] and Theadom *et al*.[81]

1. Alteration in mental state at the time of the incident. If present, loss of consciousness must not exceed 30 min in duration.
2. Neurological symptoms (e.g. headache, dizziness, fogginess) that may or may not be transient.
3. Memory loss for events immediately before or after the accident. If present, the duration of post-traumatic amnesia must be less than 24 hours.
4. No significant findings on acute brain CT scan, or CT scan not required/performed.

Following the identification of individuals that meet the above criteria, clinicians or research staff assist prospective participants to fill out the PRF—which contains the individuals' date of birth, date of injury and contact details. The PRF functions as a *Permission-to-Contact* form that permits the hospital to release the participants' contact details to the *CREST* research team. Completed PRFs are emailed or faxed through to a dedicated email address, and *CREST* research team members then use a dedicated mobile telephone number to contact participants within 7 days following the date of injury noted on the PRF.

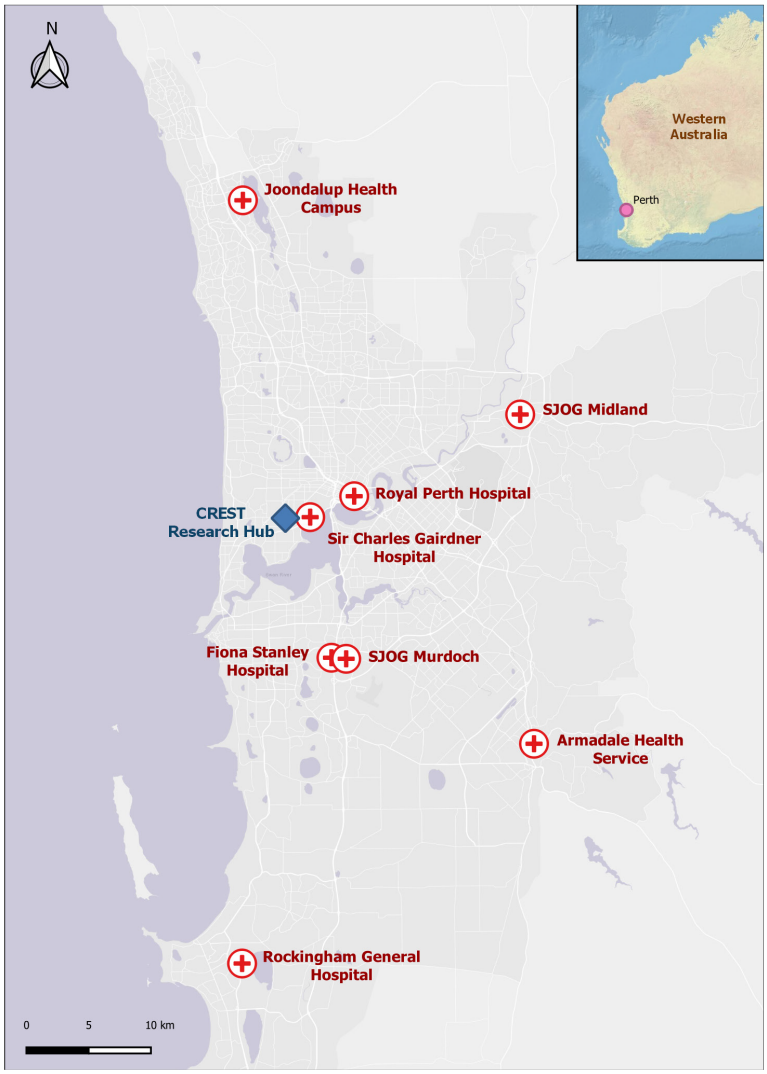

Figure 1 Map showing location of hospital emergency departments (red crosses) throughout the Greater Perth Area from which prospective Concussion Recovery Study (*CREST*) participants are recruited, relative to the location of the *CREST* Research Hub (blue diamond). SJOG, Saint John of God Hospital.

Community pathways

In addition to recruiting individuals from Hospital EDs, *CREST* is also recruiting from the general community. The community-based pathway can be broadly categorised into the following three recruitment streams: (1) *GP/sports physicians and allied health professionals*, (2) *Community Sports Groups* and (3) *self-referral*. Recruitment of prospective participants *via* the community pathways largely mirrors that of the hospital ED pathway.

GPs, sports physicians and allied health professionals

Private GP practices, sports physicians and allied health professionals within the Perth metropolitan area have been informed about the *CREST* study, either by direct in-person approach or by digital communication (e.g. advertisement in professional association newsletters/mailing lists, social media). In this pathway, medical practitioners screen for individuals meeting the above criteria presenting at their practices. Details of interested participants are forwarded *via* email or fax to the *CREST* Research Team using the PRF.

Community sports groups

Physiotherapists, athletic trainers and medics at sports clubs approached by the *CREST* research team screen for prospective participants using the aforementioned

criteria. If a player experiences a suspected mTBI at training or on game day, they are informed of the *CREST* study by the attending first aid personnel, who provide the prospective participant with a copy of the PRF and direct them to seek medical confirmation of mTBI. Should they receive a diagnosis of concussion and wish to participate in the study, individuals can self-refer to the study by contacting the *CREST* Research Team themselves *via* telephone, email or website (https://concussion-study.com.au/), or by requesting their attending medical professional to forward the PRF to the *CREST* research team on their behalf.

Self-referral

Individuals from the general community who have sustained an mTBI may participate in the study *via* self-referral, and can do so by directly contacting the *CREST* Research Team *via* telephone, email, fax or website. Individuals recruited using this pathway are asked to provide the name of the medical professional who diagnosed them with an mTBI. In the event that prospective participants have not yet sought medical attention by the time they make contact with the research team, individuals are requested to first seek medical confirmation of mTBI. If prospective participants are able to meet this request and make contact with the research team within 7 days of date of injury, they remain eligible for study enrolment.

Study design

CREST is a prospective, longitudinal observational cohort study, which follows participants over the course of 1 year after their mTBI. Individuals who do not develop PPCS serve as controls, which is in line with the study's second primary objective of identifying factors that may be able to discriminate between individuals who do and do not follow a typical recovery trajectory following mTBI. The study comprises of two parts, referred to as *'Phase I'* and *'Phase II'*, respectively, and follow-ups conducted at multiple time points. This study design was primarily adopted to maximise recruitment efforts. Very little research has been conducted in WA with respect to mTBI, and this two-part approach will help foster greater inclusivity and representation by allowing individuals to partake in the research despite the tyranny of distance. This is particularly pertinent to individuals residing in rural and regional areas of WA, whom can be under-represented in research studies. The inclusion of this demographic may also provide insights into otherwise unknown factors that may influence recovery following mTBI. Figure 2 provides a graphical depiction of study design.

To assess the influence of potential biases, a minimal screening log records basic demographic characteristics of individuals who are referred to the study but do not meet eligibility criteria or decline participation. Furthermore, data being collected as part of *Phase I* will elucidate any differences in the characteristics of individuals who do and do not opt to participate in *Phase II*.

Phase I

Phase I comprises a semi-structured telephone interview, which is conducted within 7 days of date of injury. This time frame was selected as it encompasses the acute to subacute period following injury, and is prior to anticipated resolution of symptoms in those who experience typical recovery. During this telephone call, information pertaining to demographics, injury-related characteristics, acute post-mTBI clinical care, and medical background, exercise habits and experience of mTBI symptomatology is collected. *Phase I* typically takes 30 min to complete. This includes time required to explain the aims and procedures of the study and acquire verbal consent over the telephone, all of which take place prior to collection of data from the participant. Further detail about the data acquired in *Phase I* can be found in table 1 below.

Phase II

Phase II has been designed to serve as a comprehensive in-person battery of tests, which is also completed within 7 days of date of injury for the reasons stated above. Testing takes place at the Curtin University and Perron Institute for Neurological and Translational Science tenancies, which are both located on the Queen Elizabeth II Medical Centre (QEIIMC) campus in Nedlands (Perth, Western Australia). During this session, qEEG is performed, a blood sample is taken, and neuropsychological, exercise tolerance and vestibular/ocular function testing is conducted. *Phase II* testing typically takes 2.5–3 hours to complete.

MRI is also performed as part of *Phase II* testing. This takes place at the Department of Radiology at Sir Charles Gardiner Hospital located on the QEIIMC campus. Due to the scheduling requirements of the scanner that is being used for the purposes of the study, the MRI is often performed separately to the other *Phase II* components, generally taking place afterhours or on weekends. To accommodate for scanner availability, *CREST* participants may be scanned up to 9 days following the date that they sustained their mTBI.

Follow-up

Regardless of whether participants opt to complete *Phase I* only, or both *Phase I* and *Phase II*, they are followed-up by telephone interview at 1, 3, 6 and 12 months post-injury. To ensure consistency with follow-up timeframes, the following variations are being adhered to:

- 1 month follow-up is completed at 30 days±4 days from date of injury
- 3 month follow-up is completed at 90 days±7 days from date of injury
- 6 month follow-up is completed at 180 days±14 days from date of injury
- 12 month follow-up is completed at 360 days±30 days from date of injury

The purpose of the follow-up telephone interviews is to document each participant's recovery experience following their mTBI. Thus, at each follow-up time point,

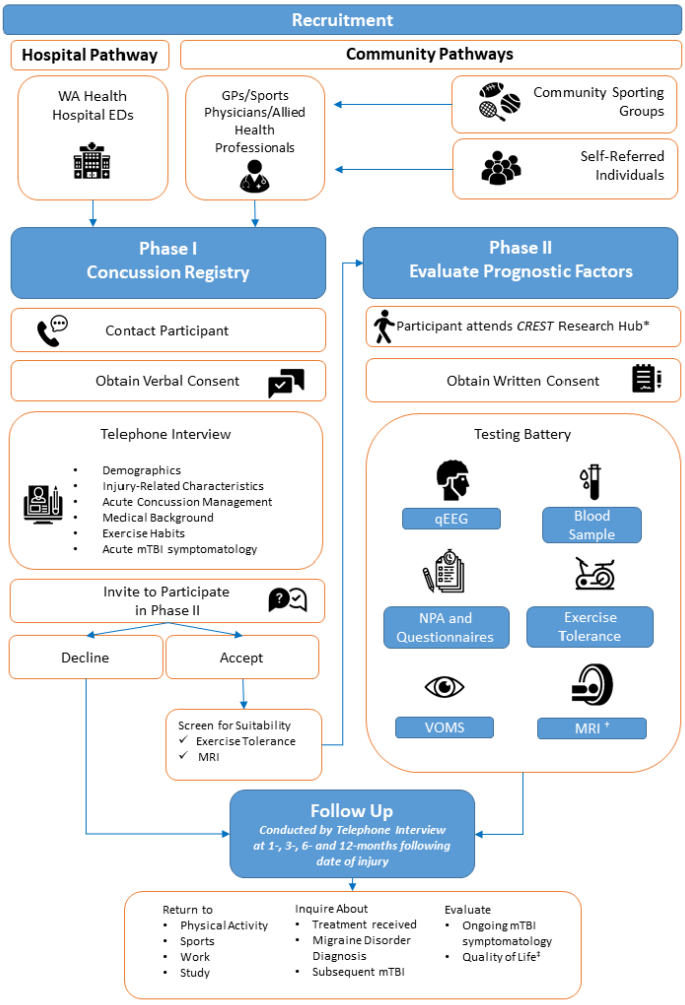

Figure 2 Flow diagram of the Concussion Recovery Study (*CREST*) study design. Participants are recruited *via* Hospital Emergency Department (ED) or community-based pathways using a dedicated *Participant Referral Form (PRF)*. Following the receipt of a completed *PRF*, either by email or fax, a member of the *CREST* research team uses a dedicated mobile telephone number to contact prospective participants. During this phone call, interested participants are briefed on the study aims and procedures, and verbal consent is obtained to participate in the study. Following this, the *Phase I* semistructured telephone interview is conducted and on its conclusion participants are asked if they also wish to participate in *Phase II* of the study. If interested, the *CREST* research team member completes a telephone screen to assess the participant's eligibility to undertake the additional components of *Phase II*. If a participant is deemed eligible, a testing session is organised at the *CREST* Research Hub. Both *Phase I* and *Phase II* components are conducted within 7 days of a participant sustaining an mild traumatic brain injury (mTBI). All participants are followed-up by telephone interview at 1, 3, 6 and 12 months following the date of injury. *Note:* * Comprises the Curtin University and Perron Institute for Neurological and Translational Science tenancies, which are located at Queen Elizabeth II Medical Centre, Nedlands (Perth, Western Australia); †: MRI may be conducted up to 9 days following participant's mTBI; ‡: quality of life is assessed using the QOLIBRI-OS at 3, 6 and 12-month follow-ups only. qEEG, quantitative electroencephalography; VOMS, Vestibular/Ocular Motor Screening Test; WA, Western Australia.

information is collected about a number of functional outcomes that may also be predicted. More specifically, these include the individual's return to physical activity, sport, work and study (if applicable). During the follow-up telephone interviews, participants are also queried about whether or not they have (1) received or are currently seeking any ongoing allied health, alternative or medical treatments for their mTBI (e.g. physiotherapy, psychotherapy, chiropractic or other medical treatment), (2) been diagnosed with a migraine disorder subsequent

Table 1 *Phase I* semistructured telephone interview/questionnaire components

***Phase I* telephone interview/questionnaire components**	
Demographics	Age, sex, height, weight, contact details, next of kin, nominated GP, highest level of completed education
Circumstances of injury	Description of mechanisms of injury (e.g. sport, non-sport), whether other injuries were sustained during the incident resulting in the mTBI, compensation/litigation status, site/s of impact, loss of consciousness (presence/absence, duration), amnesia (presence/absence, nature: anterograde and retrograde, duration), experience neck pain, presence of seizures or fits following the mTBI, estimated amount of alcohol consumed prior to incident (in standard drinks)
Acute post-mTBI clinical care	Details of where medical attention was sought (i.e. ED, GP, First Aid personnel), CT scan performed or not.
Medical background	Number of previous concussions, including the date and duration of recovery for the most recent concussion, previous whiplash injury (how many in total, date of most recent); whether participants have ever been diagnosed with epilepsy, seizure disorder, migraine or other headache disorder, mental health disorder, sleep disorder, learning disorder: for each of these health conditions, participants are also asked whether they are currently receiving treatment for this disorder (namely, medication and dosage), whether they take prescribed medication on a regular basis (i.e. anti-inflammatory, blood thinners, pain medication, other)
Exercise habits	Exercise on a regular basis (number of times per week, type of exercise: strength training, cardiovascular exercise, sport)
Acute mTBI symptomatology	*PCSS*

ED, Emergency Department; GP, general practitioner; mTBI, mild traumatic brain injury; PCSS, Post Concussion Symptom Scale.

to the mTBI and (3) sustained another mTBI since the injury that they were enrolled in the study for. Furthermore, the participant's experience of ongoing mTBI symptomatology is ascertained using the *Post Concussion Symptom Scale-22 Item version (PCSS)*[82 83] at each follow-up time point, while quality of life is being measured using the short form of the *Quality of Life after Brain Injury*[84] (QOLIBRI-OS) at the 3, 6 and 12 month follow-ups.

Study completion

Individual participation in the study is considered to be complete at the 12-month follow-up. At no point is a participant considered to be discontinued (i.e. the study participants are not required to complete all of the follow-up interviews). Research team members attempt to contact participants at each of the four individual follow-up time points, regardless of whether or not data was collected for the preceding follow-up time point. A participant is considered to be *'lost to follow-up'* when contact cannot be made with a participant within the follow-up variations stated above, but only for the individual time point in question. Inability to contact participants at follow-up does not preclude participants from participating in any subsequent follow-ups. Unsuccessful attempts to contact participants are recorded by research team members in a study log. In the event that a participant contacts the research team on their own accord outside of the corresponding follow-up time point variations, such as that which may occur when a participant is responding to a research team member's unsuccessful attempt to contact them *via* telephone or email, data is collected for that time point in the interest of maintaining rapport with the participant; however, this protocol deviation is noted by research team in the participants REDCap profile and the data collected will not be included in any data analyses.

Data collection

Phase I

In *Phase I*, a semi-structured interview is conducted via telephone to collect data on participant demographics, circumstances of injury, acute post-mTBI clinical care, medical background, exercise habits and experience of acute mTBI symptomatology. This information is collected using a combination of custom-designed metrics and validated instruments (see table 1).

Phase II

qEEG

EEG acquisition is conducted using a 19-channel Electro-cap (Electro-Cap International, Eaton, Ohio, USA) and a Mitsar amplifier (Mitsar, St Petersburg, Russia), with quantitative and low resolution electromagnetic tomography analysis (LORETA) conducted using NeuroGuide software (Applied Neuroscience, Florida, USA), which has been extensively validated in the literature, including within populations with mTBI.[85 86] For scalp EEG recording, the participant's head circumference is measured and fitted with an appropriately sized Electro-cap, with all electrodes connected using the standard 10–20 system (see online supplemental figure 1). Each scalp electrode is prepared by parting the hair and filling it with electroconductive gel (Electro-Gel,

Electro-Cap International, Eaton, Ohio, USA). EEG activity is recorded from 19 scalp electrodes and impedance kept below 10 k , using a linked ears montage, where the ear lobes act as a reference. Resting state data is recorded for 10 minutes, with 5 minute *eyes open* and *eyes closed* condition blocks.

Approximately 60 seconds of artefact-free data will be selected using NeuroGuide software (Applied Neuroscience), and individual's activity will be compared with the software's normative database (N=727). This comparison will provide a Traumatic Brain Injury Index score using a TBI Discriminant Index,[86] indicating the severity of the person's TBI ranging from zero to ten (normal=0, mild=1 to<3, moderate=3–5, severe ≥5). LORETA analysis and NeuroNavigator software (Applied Neuroscience, Largo, Florida, USA) will be used to identify areas of dysfunction within networks of interest.

Blood-Based Biomarkers

Trained research assistants obtain a 20 mL blood sample from non-fasting participants by venepuncture. Whole blood is collected into BD Vacutainer ethylenediaminetetraacetic acid and serum (SST) blood collection tubes, and rested at room temperature for approximately 30 min before centrifugation at 3000 rpm for 10 min at 4°C. Samples are then aliquoted into 250 µL vials and put into long-term storage at −80°C until analysis. Blood samples will be analysed by a variety of methods with the intent of quantifying novel and established fluid biomarkers that are associated with mTBI pathophysiology. In particular, protein biomarkers pertaining to neuronal and glial structure and function (e.g. GFAP, UCH-L1), microRNAs, genetic signatures, phenomics and metabolomics will be investigated. An additional whole blood sample is examined using a haematology panel (Mindray BC-2800 Vet Auto Hematology Analyzer; Shenzhen, China) to investigate differences in blood components.

Neuropsychological Assessment and Questionnaires

Participants undergo a brief neuropsychological assessment, which is conducted by trained research team members who have a postgraduate qualification in psychology, under the supervision of a clinical neuropsychologist (CP). The ability to assess a broad range of cognitive domains and executive functions known to be affected by mTBI in a timely manner was the primary driver for the selection of tests comprising the neuropsychological testing battery. More specifically, the *Repeated Battery for the Assessment of Neuropsychological Status Update (RBANS Update)*[87] is being used to measure immediate and delayed memory, visuospatial constructional skills, language and attention, while the *Trail Making Test Forms A* and *B*[88] are being used to measure components of executive function. Effort is also measured using the *Rey Memory Test*.[89] In addition, participants complete a battery of questionnaires to assess mTBI symptomatology (*PCSS*)[82 83], psychological distress (*Depression Anxiety and Stress Scales-21 item version*[90] and *Brief Symptoms Inventory-18 item version*[91]), resilience (*Brief Resilience Scale*[92]) and coping style (*Utrecht Coping List*[93 94]). The neuropsychological assessment and questionnaires are both completed in a private room, and in accordance with standard neuropsychological testing arrangements, with administration time typically taking 30–40 minutes.

Buffalo Concussion Bike Test

Participants undergo exercise tolerance testing using the *Buffalo Concussion Bike Test* as outlined by Haider *et al*,[95] which involves graded exertion on a recumbent bicycle ergometer (Monark RT2, Monark Exercise, Vansbro, Sweden). Prior to conducting the test, participants are screened using the *Physical Activity Readiness Questionnaire*[96] to assess for pre-existing cardiac issues or increased risk for cardiopulmonary disease, orthopaedic issues or injuries that may limit their ability to cycle, as well as other medical issues that may impede their ability to complete the exercise test safely. Participants are then asked to rate their current symptoms at rest on a 0 to 10 point *Visual Analogue Scale (VAS)*, and the test is not conducted if their score is 5/10 or more at rest. Heart rate (HR) at rest is determined after 5 minutes of quiet sitting using a Polar OH1+ armband (Polar Electro Oy, Kempele, Finland). During the test, the participant is asked to maintain a set workload as calculated by a predetermined formula based on body weight.[95] Exercise intensity is increased every 2 minutes by increasing the required workload. HR, rating of perceived exertion (RPE) and symptom exacerbation are also monitored and documented at the end of each stage. RPE is determined using a modified Borg scale, which records an individual's subjective level of exertion on a scale of 6–20,[97] and symptom levels on a *VAS* of 0–10 are also recorded. The criteria for ceasing the test include: (1) symptom exacerbation of more than two points from the pre-exercise value (including an increase in current symptoms or the appearance of a new symptom), (2) voluntary exhaustion as ascertained by an RPE exceeding 17, (3) judgement by the researcher that the participant is displaying visible signs of distress or (4) a request by the participant to stop the test. The participant's HR at cessation of the test is recorded as the 'HR threshold'.

Vestibular/Ocular Motor Screening (VOMS) Assessment

The *VOMS* assessment is a targeted test used to identify vestibular and/or ocular motor dysfunction following mTBI as described by Mucha and colleagues.[98] Briefly, the *VOMS* involves examining horizontal and vertical smooth pursuits, horizontal and vertical saccades, near point convergence (measured in centimetres) and visual motor sensitivity. Symptoms (namely headache, dizziness, nausea and fogginess) are monitored prior to the commencement of the test, as well as after the completion of each task, to determine the effect of each component on symptom exacerbation. Symptoms are recorded as a score on a *VAS* ranging from 0 to 10, and the test is ceased if symptoms increase by three points.

Table 2 List of Concussion Recovery Study MRI sequences and their associated purpose

Sequence	Purpose
T_1- weighted magnetisation-prepared rapid gradient echo	Grey and white matter morphometry Anatomical reference
Susceptibility-weighted imaging	Quantitative susceptibility mapping
Resting state functional MRI	Brain connectivity Correlation with qEEG findings
Pseudo-continuous arterial spin labelling	Cerebral blood flow
Diffusion-weighted imaging	White matter microstructure

qEEG, quantitative electroencephalography.

Any abnormal findings or provocation of symptoms is considered a 'positive' test, and a potential indicator of vestibular/ocular system dysfunction. The *VOMS* takes approximately 5–10 minutes to complete.

Magnetic Resonance Imaging

MRI acquisition

MRI is conducted using a Philips Ingenia 3T Multi Transmit Wide Bore Scanner (Philips Healthcare, Best, The Netherlands) equipped with a 32-channel head coil. The imaging protocol takes approximately 50 minutes to compete and comprises standardised sequences as outlined in table 2.

MRI data analysis

Custom-built automated data processing pipelines will be constructed in Python under the Nipype framework[99] on Linux (Ubuntu 18.04 Bionic Beaver distribution) and deployed using Jupyter Notebook.[100] Raw DICOM data are concerted to NIfTI format and stored for analysis according to the Brain Imaging Data Structure (BIDS[101]) recommendations.

Brain morphometry

T1-weighted data will be processed using FreeSurfer image analysis software (http://surfer.nmr.mgh.harvard.edu/), from which volumetric and cortical thickness measurements will be extracted. Data may also be explored using voxel-based morphometry via SPM12 (https://www.fil.ion.ucl.ac.uk/spm/) in MATLAB (MathWorks, Natick, Massacheusetts, USA).

Quantitative susceptibility mapping

SWI images will be preprocessed for quantitative susceptibility mapping (QSM) using the MEDI toolbox (http://pre.weill.cornell.edu/mri/pages/qsm.html) in MATLAB. This preprocessing toolbox includes removal of phase inconsistencies, estimation of frequency offset, phase unwrapping, and background field removal using projection onto dipole fields, followed by Morphology enabled dipole inversion. Reconstructed QSM images will be explored for iron and calcium concentration using a region of interest (ROI)-based approach.

Resting state functional MRI

Images will be preprocessed using ANTS, FreeSurfer, SPM and aCompCor. Standard preprocessing methods will be employed, including despiking, slice time and motion correction, spatial normalisation to the MNI template, temporal normalisation, linear regression and bandpass filtering. Data will be explored using network connectivity and graph theoretic analysis.

Pseudo-continuous Arterial Spin Labelling (pCASL) images will be used to quantify cerebral blood flow (CBF) using the BASIL toolkit in FSL (https://asl-docs.readthedocs.io/en/latest/index.html), with preprocessing including kinetic-model inversion using a Bayesian algorithm, calculation of the magnetisation of arterial blood, and registration to MNI space. Data will be probed for both global and ROI-based analyses of CBF.

Diffusion MRI

Diffusion MRI image preprocessing will leverage FMRIB Software Library (FSL; http://www.fmrib.ox.ac.uk/fsl) and MRtrix software, with a pipeline including skull stripping, Gibbs deranging, correction for motion and eddy currents and susceptibility artefacts and bias field correction. Constrained spherical deconvolution will be used to estimate the white matter fibre Orientation Distribution Function. Outputs will be registered to MNI space for voxel-based exploration of white matter alteration via tract-based spatial statistics[102] alongside ROI-based analysis for diffusion MRI metrics.

Clinical notification

All MRI scans are reported by a neuroradiologist with medically relevant incidental findings communicated to the participant's nominated GP.

General data management plan

CREST's study design requires data collection using various media, including electronic and paper formats. Data acquired electronically (e.g. *Phase I* telephone interview) are being entered directly into a secure, encrypted REDCap[103 104] database hosted by Curtin University, effectively serving as a standardised case form. Paper copies of participant's personal information (e.g. PRF, results from *Phase II* components) are stored securely in a locked filing cabinet at the research office, and are also digitised and uploaded to REDCap for storage. Imaging data (i.e. qEEG, MRI) are being organised according to BIDS and are stored on a secure, cloud-based storage platform also provided by Curtin University, as well as on securely stored physical hard drives for long-term storage.

On enrolment into the study, all participants are assigned a unique identification number, and all data that are collected from participants are identified by this number. A master list containing select identifying information is securely stored on an encrypted server, and is available only to authorised research staff. All identifiable information accrued for the purpose of the research study is treated as strictly confidential, and will only be disclosed with permission from participants, or as required by law. In line with WA Health guidelines, all research data will be retained for at least 7 years.

Data analysis plan

PCSS diagnosis

PPCS will be diagnosed using the PCSS. This questionnaire is listed as a National Institute of Neurological Disorders and Stroke-Common Data Element, although there are no definitive rules for implementing a threshold for determining the presence of PPCS. As described in Alla *et al*,[105] we will be applying a threshold of six or more for males and seven or more for females on the PCSS. Diagnosis of PPCS will be made at 3 months post injury, and will be revisited independently at the 6 and 12 months follow-ups.

Statistical analysis plan

This is the first registry of its kind in WA. There is limited existing data from which to extrapolate power for calculations. Nevertheless, *Phase I* is considered to be appropriately powered to detect known potentially predictive indicators from preinjury and demographic factors. Our data analysis plan of analysing modalities separately will ensure that *Phase II* is sufficiently powered to detect particularly promising differences. It is acknowledged that only a select number of variables can be included in the multivariate model, and these will be identified using regression analyses. Only those that are identified to be most promising based on these analyses will be included in the final multivariate model.

Baseline characteristics will be compared using χ^2 tests for categorical variables and t-tests for continuous variables, with respect to outcome (PPCS or no PPCS). In order to identify suitable indicators, each type of outcome measure will be analysed separately, and the most promising measures identified. For example, each MRI modality being investigated will be analysed separately, and statistical analyses will be conducted on outcomes relevant to each modality (e.g. concentrations in regions of interest for particular brain structures in QSM images will be quantified and compared in individuals who are 'diagnosed' with PPCS and those who are deemed to have recovered). Receiver operating characteristic analysis will be used to determine a discriminate index to separate PPCS from typical recovery. Standard regression modelling will be used to build best-performing prediction models for each of the outcomes of interest, using principal component analysis to identify the most promising predictive indicators to include in the model. The most predictive outcomes for each modality will be identified and can be used in multivariate modelling combining the most promising outcomes from the multiple modalities. Multiple measures of model performance including calibration and discrimination as well as novel measures employing reclassification tables and net reclassification improvement will be used to establish the best and most parsimonious prediction model. This could help define criteria for further validation studies in future.

Missing data will be handled on a case-by-case basis and appropriate approaches will be implemented under the guidance of a biostatistician. The study purpose is to identify predictors of PPCS at various time points post-injury. An advantage of such an approach is that if certain follow-up time points are missed, analysis can still proceed.

Ethics and dissemination

Ethics approval for the study has been directly obtained from the Human Research Ethics Committees (HRECs) at all of the institutions involved in the study, or where applicable, reciprocal approval has been granted. Informed verbal consent is obtained from all participants over the telephone as part of enrolment into the study, before data is collected in *Phase I*. Participants are provided with a copy of their verbal consent and study information documentation *via* email following the *Phase I* interview. Written consent is also sought from those participants partaking in *Phase II* prior to the undertaking of any testing components. All data and samples are managed entirely anonymously with the exception of the required information for follow-up telephone calls. There are few significant risks to the participants in this study, and for those that have been identified, appropriate protocols have been devised which have been approved by the HRECs. Participants can withdraw from the study at any time and this will not have any impact on their clinical care. Data contributed to the study can also be withdrawn on request. The results of this study will be published in peer-reviewed journals and presented at local, domestic and international scientific meetings. No identifiable information will be published, unless permission has been obtained from participants to do so.

DISCUSSION

Relative to studies previously conducted in the field, two main advantages distinguish the *CREST* study by design to provide superior insight into the recovery trajectory of individuals sustaining an mTBI. First: *CREST* is recruiting widely from a number of different clinical and community-based sources, with scope to recruit from regional/rural and remote areas in future. Not only will this facilitate the simultaneous

observation of recovery trajectories associated with a variety of different mTBI injury mechanisms, but it will also provide insight into whether some factors may be more salient for recovery following mTBI due to different causal mechanisms. This unique recruitment approach will also provide much needed data regarding the circumstances under which mTBI occurs within WA as well as the incidence and prevalence of both mTBI and PPCS that may ensue, for which data are significantly limited. Second: *CREST* uses an extensive testing battery that comprises a broad range of both novel and established predictors of PPCS. This in itself is significant for several reasons: first and foremost, such an approach will enable the evaluation of previously identified factors in a novel, community based cohort that has been followed-up over a prolonged period of time. Furthermore, it features several novel techniques (e.g. QSM, qEEG, metabolomics, proteomics) that have received limited attention and others (e.g. exercise tolerance) that have been investigated only in specific populations (e.g. adolescent athletes), expounding the utility of such methods. The systematic approach adopted by *CREST* in which data is being collected also creates a fertile setting for the examination of novel or poorly investigated relationships between different clinical parameters predictive of poor outcome (e.g. congruency between qEEG and rs-fMRI; ASL and exercise tolerance), and provides opportunity for economic evaluation of diagnostic and prognostic methods from both the healthcare and consumer perspectives. Taken together, this research has the potential to empower clinicians and researchers alike by identifying factors that may contribute to the development of an optimal 'suite' of rapidly deployable predictive variables for the early identification of PPCS risk. It also has the potential to assist with the early identification of patients at risk of experiencing PPCS and enable timely patient-centred treatment, and thereby help to reduce the personal, economic and societal burden of mTBI.

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Predicting Outcome Following Mild Traumatic Brain Injury: Protocol for the Longitudinal, Prospective, Observational Concussion Recovery (*CREST)* Cohort Study

Gozt, AK et al.

Supplementary information

1. Document 1. Participant site referral form.
2. Figure 1. The 10-20 International system of EEG electrode placement.
3. Table 1. MRI scan parameters.

CURTIN HEALTH INNOVATION RESEARCH INSTITUTE | Curtin University | perron institute for neurological and translational science

CREST Concussion REcovery STudy

Participant Referral Form

Patient Name: **DOB:** / /

Phone: **Date of Injury:** / /

Email:

I consent to _____ (*Name of Healthcare Provider*) providing my details above to the Concussion Study team and to a member of that team contacting me to discuss the Concussion Study in more detail.

Patient Signature: **Date:**

MEDICAL PRACTITIONER TO COMPLETE THIS SECTION

Referring Doctor or Healthcare Provider Details

Name:

Practice Details or Stamp:

Signature: **Date:** / /

Key Participant Selection Criteria:

Identifying potential participants: To determine if a concussion has occurred, potential participants may be considered for this study if they provide a description of an incident likely to lead to a traumatic brain injury, with accompanying neurological signs and symptoms which can be attributed to that injury, as defined by the World Health Organisation. Participants must also describe **at least one** of the following, as described by the American Congress of Rehabilitation Medicine and Theadom and colleagues:

1. Any period of loss of consciousness (Were you "knocked out")?
2. Alteration in mental state at the time of the accident (Were you dazed, disoriented or confused? Did you "see stars" at the time of injury?)
3. Any memory loss for events immediately before or after the accident (Do you have any memory loss around the time of injury - before or after?)
4. Any neurological deficits (eg headache, dizziness, fogginess) that may or may not be transient?

Please forward the completed form to:

concussionstudy@curtin.edu.au

or

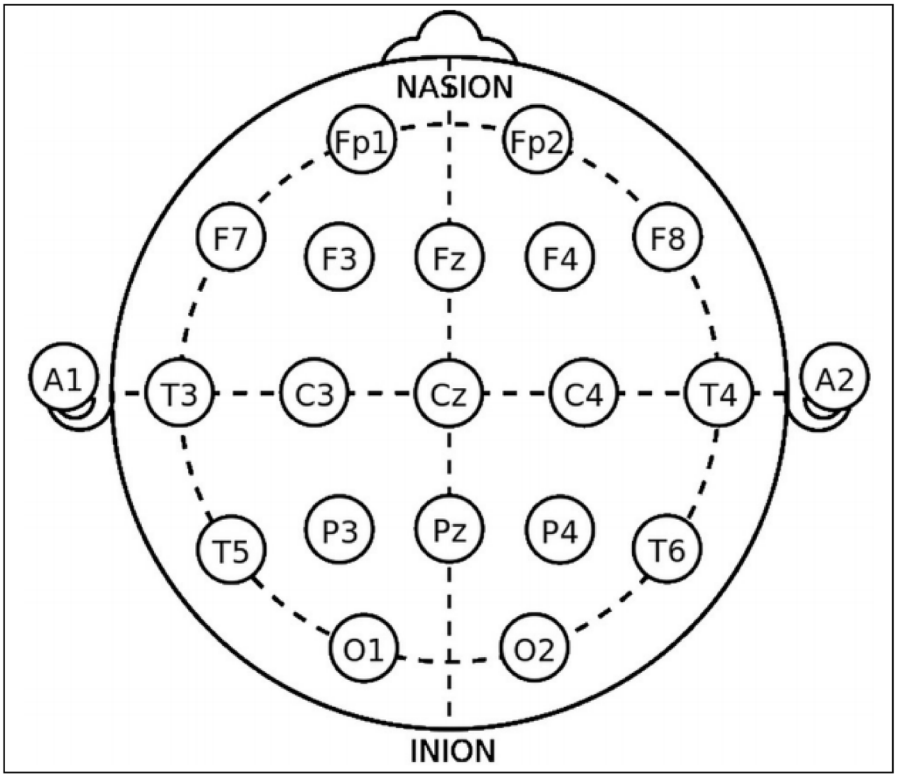
Secure e-fax 08 6270 5470

Thank you very much for your participation!

This study has Ethics Approval through Royal Perth Hospital Human Research Ethics Committee (#RGS0000003024) and Curtin University (HRE2019-0209). Please contact the Curtin research team on 0466 526 849 if you have any further questions.

Master Participant Referral Form V4 06/02/2020

Supplementary Figure 1. The 10-20 International system of EEG electrode placement.



Note: Figure adapted from Rojas G, Alvarez C, Montoya C, et al. Study of resting-state functional connectivity using EEG electrodes position as seed. *Front Neurosci*;12 doi:10.3389/fins.2018.00235 [published Online First 24 April 2018]

Supplementary Table 1. MRI scan parameters.

Sequence	**T1**	**3D FLAIR**	**3D SWI**	**rs-fMRI**	**pCASL**	**DTI**
Orientation	Transverse	Sagittal	Transverse	Transverse	Transverse	Transverse
Voxel size (mm)	1 x 1 x 1	1 x 1 x 1	0.7 x 0.7 x 1.4	3 x 3 x 3	3.75 x 4 x 8	2 x 2 x 2
TR (ms)	6.01	4,800	33.4	3,000	4,064	4,694
TE (ms)	2.7	302	5/4 echoes Δ7.7	30	11.17	113
TI (ms)	-	1600	-	-	-	-
Flip angle (deg)	8	90	10	90	90	90
Phase FOV (mm)	256	182.5	220	216	240	224
Matrix size	256 x 256	252 x 252	301 x 301	69 x 69	88 x 88	110 x 110
# slices	175	365	110	46	15	60
Fat suppression	no	no	no	yes	yes	yes
b-values (sec/mm^2) [directions]	-	-	-	-	-	0, 1500 [32]
Time (min)	6:19	3:31	9:40	7:42 [a]	4:45[b]	11:36

Note: [a]: 150 dynamics; [b]: Post-label delay 1800ms

Introduction to Series Two: QSM Narrative Review, Pipeline Development and Example Analysis

Series Two of this thesis is comprised of a narrative review and an original investigation chapter. This series centres on QSM, its emerging applications within the field of TBI and its capacity to elucidate the biological mechanisms that underpin or may contribute to PPCS.

The first study in this series is a narrative review, which has been published in the journal *Neuroscience*. The primary aim of this review was to provide a comprehensive overview of the different ways in which QSM has been utilised in TBI research. With no other reviews known to be published on this topic to date, this timely review is broad in scope to provide the reader with ample context to illustrate how QSM enables us to better understand the pathophysiology of TBI. As such, the review commences with a succinct introduction to TBI, the role of iron in the brain and its homeostasis, as well as the accumulation of iron in the brain and its implications for neuropathology. It then provides a brief overview of neuroimaging in TBI, and how brain iron can be imaged by harnessing the properties of magnetic susceptibility. This includes a brief outline of the fundamental components and steps required to acquire and process MRI data in order to generate QSM images without going into excessive technical details that have been documented by others. Most importantly, the narrative review summarises the current state of the literature in which QSM has been used to investigate a host of pathophysiological changes that are associated with TBI. Specifically, investigations have assessed for alterations in tissue and venous magnetic susceptibility alterations, and venous structure following TBI, the presence of cerebral microhaemorrhages following injury as well as the detection of calcifications in the chronic stages of TBI. Each pathophysiological change covered in the review is accompanied by a brief theoretical background, which brings together both biology and physics, to better contextualise how QSM can be used to detect the respective changes. Where applicable, the use of QSM in a prognostic capacity within the current literature has also been reported. Finally, the narrative review concludes with a discussion of the outstanding gaps in the literature, including methodological limitations and considerations for improving comparability and reproducibility in future research within area of TBI. Of particular note is the potential application of new developments in anatomical segmentation software to facilitate increasingly specific and precise estimation of susceptibility-related changes.

The second study of this series presents two elements pertaining to the analysis of QSM data. Firstly, it describes the methodological pipeline that will be used to process and analyse the QSM

data being collected as part of the *CREST* research project, described in Chapter 4. Drawing upon insights gained from the narrative review presented in Chapter 5, this pipeline will ultimately facilitate detailed analysis of QSM data being collected, which may potentially be useful for the prediction of PPCS. More specifically, the first section of Chapter 6 describes the pipeline, as well as select optimisation and troubleshooting steps, in a narrative format. The methodological details include the code that is used to generate susceptibility maps. The level of detail presented is considered to be pertinent given the formative stage of this work and emerging nature of the technology.

Secondly, the chapter presents a novel investigation using available data collected from *CREST* participants and the described computational pipeline. Due to the small number of participants recruited to date, the central tenet of the thesis surrounding the prediction of PPCS could not be examined, as doing so would be considered an interim analysis that could potentially confound the study. As such, an examination of the relationship between self-reported history of previous mTBI and the formation of calcium deposits within the thalami was conducted as an alternative. Research to date has found that individuals who have previously sustained a mTBI may be at greater risk of experiencing PPCS following subsequent instances of mTBI, although it is not entirely clear why this may be the case. A potential mechanism for this is the accumulation of calcium deposits within the thalami. Located deep within the brain, the thalamus is a bilateral GM structure that contains many groups of nuclei. It is known to be particularly vulnerable to the biomechanical forces of mTBI and sequelae that accompany injury. Thus, it may be that the presence of calcium deposits here could partially account for differences in outcome following subsequent instances of mTBI. Due to the limited sample size, investigation was confined to the level of the thalamus as a whole due to the multiple comparisons that would be required for an analysis at a subregion level. While the investigation did not reveal any statistically significant differences in the mean tissue magnetic susceptibility within the left and right thalami between the examined sample of *CREST* participants with and without a self-reported history of previous mTBI, they did provide interesting insights that may be useful for guiding future studies.

5 QSM Narrative Review

Gozt, A., Hellewell, S., Ward, P.G.D., Bynevelt, M., & Fitzgerald, M. (2021). Emerging Applications for Quantitative Susceptibility Mapping in the Detection of Traumatic Brain Injury Pathology. *Neuroscience*.

NEUROSCIENCE

NEUROSCIENCE FOREFRONT REVIEW

A. Gozt et al. / Neuroscience 467 (2021) 218–236

Emerging Applications for Quantitative Susceptibility Mapping in the Detection of Traumatic Brain Injury Pathology

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Abstract—Traumatic brain injury (TBI) is a common but heterogeneous injury underpinned by numerous complex and interrelated pathophysiological mechanisms. An essential trace element, iron is abundant within the brain and involved in many fundamental neurobiological processes, including oxygen transportation, oxidative phosphorylation, myelin production and maintenance, as well as neurotransmitter synthesis and metabolism. Excessive levels of iron are neurotoxic and thus iron homeostasis is tightly regulated in the brain, however, many details about the mechanisms by which this is achieved are yet to be elucidated. A key mediator of oxidative stress, mitochondrial dysfunction and neuroinflammatory response, iron dysregulation is an important contributor to secondary injury in TBI. Advances in neuroimaging that leverage magnetic susceptibility properties have enabled increasingly comprehensive investigations into the distribution and behaviour of iron in the brain amongst healthy individuals as well as disease states such as TBI. Quantitative Susceptibility Mapping (QSM) is an advanced neuroimaging technique that promises quantitative estimation of local magnetic susceptibility at the voxel level. In this review, we provide an overview of brain iron and its homeostasis, describe recent advances enabling applications of QSM within the context of TBI and summarise the current state of the literature. Although limited, the emergent research suggests that QSM is a promising neuroimaging technique that can be used to investigate a host of pathophysiological changes that are associated with TBI.

Key words: quantitative susceptibility mapping, QSM, MRI, Iron, traumatic brain injury, concussion.

INTRODUCTION

The role of iron is of increasing interest in the pathogenesis of traumatic brain injury (TBI), with recognition of iron as both a mediator of secondary injury processes and iron dysregulation and accumulation as a downstream pathological event. The visualisation of iron *in vivo* is possible with MRI, however, it is only recently that the technology has been developed to quantify iron concentration using the advanced neuroimaging quantitative susceptibility mapping (QSM). These advances have sparked a surge in interest in imaging iron in TBI, with QSM applied to examine tissue susceptibility, characterise venous susceptibility and structural alterations, and investigate tissue calcifications. In this article we provide an overview of TBI and an introduction to the function and homeostasis of iron in the brain, and review the current state of the literature applying QSM to the field of TBI.

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Abbreviations: AUC, Area under the curve; BBB, Blood–brain barrier; BESS, Balance error scoring system; $CMRO_2$, Cerebral metabolic rate of oxygen consumption; DMT1, Divalent metal iron transporter 1; DWI, Diffusion weighted imaging; ETPT, Elapsed time post-trauma; Fe^{2+}, Ferrous iron; Fe^{3+}, Ferric iron; GM, Gray matter; GRE, Gradient recalled echo; HARPERELLA, Harmonic phase removal using the Laplacian operator; MRI, Magnetic resonance imaging; NMDA, N-methyl-D-aspartate; OEF, Oxygen extraction fraction; PET, Positron emission tomography; PDF, Projection onto dipole fields; SAC, Standardized assessment of concussion; SCAT-3, Sports concussion assessment tool- 3rd edition; SHARP, Sophisticated harmonic artefact reduction for phase data; SvO_2, Venous oxygen saturation; SWAN, Susceptibility weighted angiography; SWI, Susceptibility weighted imaging; SWI-p, Susceptibility weighted imaging-phase; T_1WI, T_1 weighted imaging; T_2 FLAIR, T_2 fluid attenuated inversion recovery; Tim2, T cell immunoglobulindomain and mucin-domain protein 2; ROC, Receiver operating characteristic analysis; ROS, Reactive oxygen species; RPQ, Rivermead post-concussion symptoms questionnaire; TBI, Traumatic brain injury; QSM, Quantitative susceptibility mapping; WM, White matter.

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OVERVIEW OF TBI

TBI can be difficult to typify due to the broad spectrum of mechanisms, aetiologies, symptoms and external factors involved. Most commonly defined as mechanical injury to the head from external forces, TBI exists on a spectrum from mild TBI (mTBI, often referred to as concussion) to severe TBI, with gross physiological scores such as the Glasgow Coma Scale used as an initial classifier of severity. Increasing recognition has also been given to the importance of 'subconcussion' (i.e. impacts to the head which do not elicit symptoms), particularly when experienced repeatedly as is most frequently seen in contact sports (Bailes et al., 2013; Lasry et al., 2017).

The number of TBIs occurring each year is estimated at between 295 and 369 per 100,000 persons internationally (Nguyen et al., 2016; James et al., 2019) though this number is likely conservative as it only accounts for those seeking medical treatment for their injuries. Globally, falls and road traffic accidents are the most common causes of non-fatal TBI (James et al., 2019), although the age at which TBIs are most likely to occur varies with aetiology, with falls most common in paediatric and geriatric populations and road traffic accidents and sporting injuries more frequent in young adults (Dewan et al., 2016). Males have been previously thought of as more likely to sustain TBI, however this is changing with the rise and retention of sport participation among females (Casey et al., 2019) and increasing recognition of predominance of females in geriatric falls (Gardner et al., 2018). Geriatric populations account for the highest incidence of emergency presentations, hospitalisations and deaths (Gardner et al., 2018), although they are comparatively the least frequently studied population. Perhaps the most frequently studied group is those experiencing sports-related TBI, the incidence for which has been estimated at between 3.5 and 31.5 per 100,000 in hospital-based settings (Theadom et al., 2020). In contrast, estimates for community-based studies are much higher at 170/100,000 when accounting for those who sought care outside the hospital setting, or not at all (Theadom et al., 2014). Sports-related TBIs occur most often on the mild end of the spectrum (Theadom et al., 2020), most frequently as a result of soccer, rugby, gridiron and Australian rules football codes, cycling/vehicular sports and equestrian (Theadom et al., 2020).

Disparities in TBI incidence have emerged between high- and low-income countries, with lower income countries having greater case numbers of TBI alongside reduced access to specialty care and rehabilitation. As a consequence, patients are more likely to die following severe TBI in low and middle income countries (de Silva et al., 2009), and are more likely to have poorer outcomes long-term (Samanamalee et al., 2018).

TBI can be classified according to numerous approaches, however, in preclinical and clinical research it is typically conceptualised according to the pathophysiology and evolution of injury cascades (Saatman et al., 2008). In this approach, TBI is said to consist of two distinct events: a *primary* or *mechanistic injury* that occurs at the time of the accident, and an ensuing multidimensional series of complex, self-propagating biochemical injury cascades, which are often collectively referred to as *secondary injury* (Adams and Graham, 1994; Werner and Engelhard, 2007).

Primary injury refers to the physical damage inflicted on cerebral tissues (i.e. neurons, glia and blood vessels) as a consequence of its displacement within the skull (Prins et al., 2013). Primary injury can be aggravated by a number of acute local systemic insults (hypotension, hypoxia, hyponatremia, hypocapnia, haemorrhage) and neurotoxic pathways, leading to secondary brain injury (Saatman et al., 2008; Morganti-Kossmann et al., 2019). Morphologically, primary injury is classified as being either *focal* or *diffuse,* which often co-occur but to different extents. Focal TBI results from mechanical impacts between the brain and the skull, and often presents as defined intraparenchymal lesions and haemorrhages. Diffuse TBI, on the other hand, is a global pathology that predominantly affects white matter (WM) subjected to stretching and shearing forces, which result from rapid changes in inertia that may occur at time of injury (Adams et al., 1983; Saatman et al., 2008).

Immediately following the occurrence of TBI, a number of dynamic physiological processes are instigated within the damaged tissue, which continue to evolve over the course of minutes to months after initial impact (Walker and Tesco, 2013). These include, but by no means are limited to, aberrant neurotransmitter release (i.e. glutamate excitotoxicity: Faden et al., 1989), depolarisation and disturbances in ionic homeostasis (Gentile and McIntosh, 1993), changes in cerebral blood flow (Yuan et al., 1988), oedema (Marmarou, 1994), energy failure (Yoshino et al., 1991), mitochondrial dysfunction (Xiong et al., 1998), blood–brain barrier (BBB) dysfunction (Neuwelt et al., 2008), oxidative stress (Awasthi et al., 1997), dysmyelination (Payne et al., 2012), and the initiation of neuroinflammatory responses (Morganti-Kossmann et al., 2010). The pathophysiological mechanisms may occur simultaneously, and can have additive or synergistic consequences for morbidity and mortality following TBI. For more recent reviews on TBI pathophysiology (Werner and Engelhard, 2007; Barkhoudarian et al., 2011; Prins et al., 2013; Karve et al., 2016; Fehily and Fitzgerald, 2017; Puntambekar et al., 2018).

OVERVIEW OF BRAIN IRON

Iron is an essential trace element most commonly recognised for its ability to bind to oxygen *via* the haemoglobin complex, and its role in oxygen transport throughout the body (Pantopoulos et al., 2012; Abbaspour et al., 2014). Beyond this role, iron is also an important cofactor in a host of biological processes, including energy production, cellular metabolism, growth and differentiation, and gene expression (Daglas and Adlard, 2018). Iron is primarily acquired through diet as heme or non-heme forms (Abbaspour et al., 2014). More specifically, heme iron refers to iron that is bound to heme cofactors within proteins (e.g. haemoglobin), whereas non-heme iron is not bound to heme groups (e.g. free

iron, or that which is bound to proteins such as ferritin and transferrin). In biological systems, iron generally exists in an insoluble trivalent cationic *ferric* (Fe^{3+}) or soluble divalent cationic *ferrous* (Fe^{2+}) state. Being a transition metal, iron actively participates in electron transfer/redox reactions that catalyse the generation of reactive oxygen species (ROS), which can ultimately result in oxidative stress-induced cellular toxicity (Graf et al., 1984; Núñez et al., 2012; Daglas and Adlard, 2018). As such, tight regulation of iron is essential for the prevention of toxic events that result in tissue damage.

Within the central nervous system, iron is abundant and fundamental for a number of physiological processes including neuronal development, myelination, neurotransmitter synthesis and synaptic plasticity (Ke and Qian, 2007; Ward et al., 2014). Along with other biometals, it is found in neurons, astrocytes and microglia, but is most abundant in oligodendrocytes (Connor et al., 1990; Connor and Menzies, 1996; Burdo et al., 1999; Beard and Connor, 2003; Badaracco et al., 2010; Angelova and Brown, 2015). This apparent selectivity is likely to be driven by the combination of the extensive role oligodendrocytes play in myelination, and the fact that iron is crucial for myelination and myelin maintenance (Connor and Menzies, 1996).

Brain iron homeostasis, distribution, age-related changes and implications for neuropathology

Peripherally circulating iron is bound to the iron transport protein transferrin, which has a hydrophilic nature that prevents its passage across the BBB into the brain parenchyma (Moos et al., 2007). Hence, the entry of iron into the brain requires active transport to traverse the BBB (Abbott et al., 2006; Rouault and Cooperman, 2006). Many details regarding exactly how iron crosses the BBB and enters the CNS remain uncertain (Ke and Qian, 2007; Crichton et al., 2011). However, it is generally accepted that the uptake of iron into the brain occurs at the BBB interface in a multi-step transcellular process that is mediated by the expression of transferrin receptors, which in turn is likely to be regulated by neurons in response to the iron status of the CNS (Rouault and Cooperman, 2006; Mills et al., 2010). Different neural cell types are also suspected of having unique ways of acquiring iron and maintaining its homeostasis but these are yet to be clarified (Crichton et al., 2011; Zucca et al., 2011).

Iron found in systemic circulation typically enters the brain *via* endocytosis of transferrin/transferrin receptors, which are highly expressed on the luminal surface of vascular endothelial cells of the BBB. Iron may also enter the brain as non-transferrin bound iron or as a low molecular weight complexes (e.g. ascorbate, ATP, citrate), although this is thought to occur to a lesser extent (Burdo and Connor, 2003; Ke and Qian, 2007; Moos et al., 2007; Ward et al., 2014; Daglas and Adlard, 2018). The slightly acidic pH environment of endosomes causes Fe^{3+} to detach from transferrin, which is in turn reduced to Fe^{2+} by ferric reductase (Ward et al., 2014). Subsequent to this, Fe^{2+} is released out of the endosome and into the cytoplasmic iron pool by a process largely thought to involve divalent metal ion transporter 1 (DMT1). Whether or not this particular transporter is expressed in the endothelial cells of the BBB is a contentious subject (Burdo et al., 2001; Siddapa et al., 2002; Moos and Morgan, 2004), and other mechanisms by which iron separates from transferrin that do not involve DMT1 (e.g. mucolipin 1; Dong et al., 2008) have been suggested. Following its release into the cytosol, iron is exported from the abluminal surface of BBB vascular endothelial cells and into the interstitial fluid of the CNS by the transmembrane protein, ferroportin (Mills et al., 2010; Crichton et al., 2011; Ward and Kaplan, 2012; Ward et al., 2014). Ferroportin-dependent efflux is also thought to be aided by ceruloplasmin; a copper containing α2-globulin protein that is found abundantly in its glycophosphosinositide-linked form within astrocytic end-foot processes that surround brain microvessels (Klomp et al., 1996). More specifically, ceruloplasmin helps regulate iron export at the cellular level by prolonging the presence of ferroportin on the cell surface (De Domenico et al., 2007; Ayton et al., 2014) and rapidly converting Fe^{2+} to Fe^{3+} *via* its ferroxidase activity (Attieh et al., 1999; Jeong and David, 2003).

Once in the interstitial fluid of the CNS, Fe^{2+} is taken up by neurons, astrocytes, microglia, and oligodendrocytes. This most likely occurs with the help of DMT1 and ferroportin, which have both been found to varying degrees on each of these types of cells. However, additional mechanisms of iron uptake specific to individual cell types have also been identified (Mills et al., 2010; Crichton et al., 2011). For example, oligodendrocytes have been found to express ferritin binding *Tim 2* receptors, while neurons and microglia express transferrin receptors that facilitate the uptake of transferrin-bound iron (Ward et al., 2014). Iron is then stored within cells, which is thought to help circumvent oxidative damage (Oshiro et al., 2011; Angelova and Brown, 2015). Within oligodendrocytes, iron is mostly present as ferritin and transferrin (Connor et al., 1990; Connor and Menzies, 1995), while the form of iron stored in astrocytes and microglia is yet to be defined (Badaracco et al., 2010).

Given the constant influx of iron into the brain, it stands to reason that there must also be a way for the brain to dispose of excess iron in order to maintain homeostasis. While very little is known about how this occurs, it is speculated that transferrin-bound iron may leave the CNS and return to systemic circulation by entering the venous system *via* granulations of the arachnoid membrane (Bradbury, 1997; Rouault, 2001; Rouault and Cooperman, 2006). It is also hypothesised that endothelial cells of the BBB facilitate export of elemental iron from the brain interstitium to systemic circulation, however, evidence for the direct export of iron from the CNS is currently lacking (Rouault, 2001).

The brain contains significant amounts of iron; however, its distribution is not uniform (Angelova and Brown, 2015). Most likely underpinning this heterogeneous distribution are differences in oxidative metabolism and neurotransmitter concentration (Burdo and Connor, 2003). The highest levels of iron in the brain parenchyma are found within the deep grey nuclei. Collectively, these structures are extensively involved in processing and inte-

gration of information, and are highly interconnected to themselves as well as to cortical gray matter (GM) (from which they receive and then transmit back signals), and thus they require extensive levels of neurotransmitter production (Eskreis-Winkler et al., 2017). Since iron serves as a cofactor for neurotransmitter synthesis, high levels of iron are found accordingly in the basal ganglia, substantia nigra, red nucleus and subthalamic nucleus (Dexter et al., 1991).

Iron concentration is not static throughout the lifespan, with age-related increases noted in otherwise healthy individuals (Ashraf et al., 2018). This accumulation is structurally specific, with MRI and tissue post-mortem examinations revealing age-related progressive increases in the deep GM of the basal ganglia (globus pallidus, caudate nucleus, putamen) and substantia nigra (Pfefferbaum et al., 2009; Ramos et al., 2014), with more conservative increases in the cerebellum, frontal, occipital and temporal lobes. In contrast, iron levels appear to increase only modestly in the pons and medulla of the brainstem, in which the overall concentrations are less than 20% of that in the deep GM (Ramos et al., 2014). Immunohistochemical analysis of deposition in iron-sequestering neuromelanin neurons has also confirmed these findings, with linear iron increases in neurons of the substantia nigra and putamen (Zecca et al., 2001, 2008) and stable concentrations of iron in neurons of the locus coeruleus of the pons (Zecca et al., 2001).

Iron accumulation in otherwise healthy elderly populations has been linked to age-related declines in cognitive performance (Ghadery et al., 2015), while the predominance of iron in brain structures of motor control suggest a role for iron accumulation in age-related slowing of motor function and cognitive decline (Pfefferbaum et al., 2009; Spence et al., 2020). Of note, the structures in which iron accumulates are typically the most affected by abnormally high iron concentration in inflammatory diseases of ageing and neurodegeneration, suggesting that iron dysregulation is a key contributor to disease and degeneration-related functional decline. Indeed, abnormal iron deposition and accumulation is a notable common feature of Alzheimer's disease (Lane et al., 2018; Liu et al., 2018), Friedreich's ataxia (Waldvogel et al., 1999; Koeppen et al., 2012; Ward et al., 2019), Huntington's disease (Muller and Leavitt, 2014; Chen et al., 2019) and Parkinson's disease (Graham, 2000; Berg et al., 2008).

The importance of iron in TBI pathophysiology is increasingly acknowledged, and evidence of abnormal iron in the brain following TBI is emerging. Primary TBI may cause acute bleeds which appear as lesions, sub-, intra- or extradural hematomas, in which free heme may be converted into its bioactive products, with iron accumulation as a consequence (Chang et al., 2005). There have now been several studies noting accumulation of non-heme iron chronically after TBI, particularly in the deep GM where structure-specific elevated concentration have been found in the thalamus, hippocampus, substantia nigra and globus pallidus. These findings have been correlated to cognitive dysfunction (Raz et al., 2011; Lu et al., 2015), suggesting that elevation of iron may exacerbate cognitive symptoms. As mentioned above, excess iron is also noted in several neurodegenerative diseases, and has recently been linked to accelerated cognitive decline in a large post-mortem clinical sample (Ayton et al., 2020).

While the role of excess iron in TBI pathology is yet to be fully elucidated, iron dysregulation is now thought to be an additional source of secondary injury, which may drive TBI pathology in the acute stages of injury though a number of mechanisms. Perhaps most importantly, excess iron present as Fe^{2+} can react with hydrogen peroxide to generate soluble hydroxyl or lipid alkoxy radicals as key reactive oxygen species (ROS) in a process known as the Fenton reaction. These ROS exacerbate cellular pathology by causing lipid damage and membrane permeabalization. Iron-dependent accumulation of ROS also mediates ferroptosis, a newly recognised form of non-apoptotic cell death which occurs after TBI (Kenny et al., 2019; Tang et al., 2020) and in other neurodegenerative disorders (Masaldan et al., 2019; Yan and Zhang, 2020). Excessive iron is also a key contributor to oxidative stress after TBI (Nisenbaum et al., 2014), prompting particular generation in microglia (Yauger et al., 2019) and interplay with neuroinflammatory processes (Daglas and Adlard, 2018). Chronically, iron may also encourage the phosphorylation of tau and formation of neurofibrillary tangles (Nisenbaum et al., 2014).

Overview of neuroimaging in TBI

Recent advances in neuroimaging technology have enabled insight into microstructural pathology *in vivo*, with potential TBI-specific applications in injury diagnosis and management as well as disease monitoring and outcome prediction. However, conventional computed tomography (CT) and MRI lack this sensitivity and cannot be used for such applications in their standard clinical capacity, with high-resolution MRI predominantly utilised in the research domain due to high cost and lengthy scan times. Advances in technology are gradually lessening the burden of these factors, with uptake expected as scan times are reduce, signal-to-noise ratio improves and image processing packages are simplified. At present, CT scans are the most common neuroimaging modality for TBI patients, with many hospitals worldwide implementing the Canadian CT Head Rule (Stiell et al., 2001) to determine the presence of gross pathological features such as lesions or skull fractures.

MRI has several advantages over CT and other neuroimaging modalities, given that it does not require exposure to radiation and has superior capacity to discriminate and map brain tissue on the basis of physiological properties. MR sequences leverage these physiological differences to characterise tissue on the basis of water (diffusion MRI), blood (arterial spin labelling, time-of-flight and perfusion imaging), fat (fat saturation imaging) or iron (susceptibility weighted imaging and quantitative susceptibility mapping: QSM).

Imaging brain iron in vivo by harnessing magnetic susceptibility properties

Magnetic susceptibility is an intrinsic, dimensionless electromagnetic property of bulk material or tissue that determines how a substance will interact with and distort an applied magnetic field (Schenck, 1996; Shmueli, 2020). In short, the magnetic susceptibility is related to the electron configuration of atoms (Schenck, 1996; Duyn and Schenck, 2017). As reviewed in Shmueli (2020) and Ravanfar et al. (2021), paramagnetic substances (e.g. most biological forms of iron and copper) have a positive susceptibility with magnetisation that increases in proportion with the applied magnetic field strength, while diamagnetic substances (e.g. water, myelin, calcifications) have a negative susceptibility which decreases magnetisation with increasing field strength. Most biological tissue is weakly diamagnetic in nature with susceptibility values akin to water. Leveraging these natural magnetic properties, MRI protocols have been developed to be sensitive to the paramagnetic and diamagnetic substances in tissue using field distortions to differentiate brain tissue based upon these substances (Haacke et al., 2004; Schweser et al., 2010; Liu et al., 2017). Major contributors to the detectable changes in magnetic susceptibility across the brain are myelin (weakly diamagnetic) and iron-containing molecules (mostly strongly paramagnetic). The greatest contribution to tissue magnetic susceptibility among all iron compounds found in the brain is ferritin-bound non-heme iron; free and transferrin-bound iron only contribute modestly/minimally to the measured mean tissue susceptibility (Liu et al., 2015; Deistung et al., 2017; Duyn and Schenck, 2017). Other iron-containing complexes that constitute main sources of magnetic susceptibility include neuromelanin (particularly true for deep GM structures such as the substantia nigra pars compacta and the locus coeruleus) (Eskreis-Winkler et al., 2017; Ravanfar et al., 2021), deoxyhemoglobin (Jain et al., 2012), and hemosiderin (Eskreis-Winkler et al., 2017), the latter of which may occur in as a result of pathogenic events.

Susceptibility weighted imaging (SWI) is a technique that has been predominantly used in the brain to examine venous oxygen saturation and determine the presence of iron or calcifications (Wu et al., 2009; Liu et al., 2017). SWI is generated by application of gradient-echo (GRE) pulse sequences as an alternative to more conventional spin-echo sequences (Haacke et al., 2009). The technical development and implementation of GRE sequences for SWI are beyond the scope of this article, however comprehensive review can be found in Duyn, (2013) and in companion papers by Haacke et al., (2009) and Mittal et al., (2009). Developments in SWI have led to implementation of 3D acquisitions allowing for thinner slices, smaller voxel sizes, reduction of artefacts and reduced scan time, which have paved the way for clinical uptake to determine iron and/or calcifications in brain tissue (Kirsch et al., 2009; Halefoglu and Yousem, 2018). SWI sequences generate magnitude and filtered phase images, which are processed separately and combined together to suit a radiological examination. It is the filtered phase maps which distinguish diamagnetic and paramagnetic substances as opposite signal intensities, and these are used to accentuate the signal loss (Haacke et al., 2009; Duyn, 2013). Directionality of signal (i.e. bright or dark) differs between MR manufacturers, so care must be taken in image interpretation. All major MR vendors now produce SWI sequences under proprietary trade names, with SWI adopted by Siemens, SWAN (susceptibility weighted angiography) offered by GE, and SWIp (SWI-phase) an equivalent Philips sequence. SWI has served as the precursor to the nascent technique QSM.

A brief outline of QSM acquisition and post-processing

The generation of a QSM image involves several steps, which can be briefly summarised as follows. First, magnitude and phase images are acquired using a T2*-weighted gradient echo sequence. The magnitude image is used to create a mask of the brain to provide the volume of interest. The phase image is unwrapped (e.g. using Path-based (Jenkinson, 2003) or Laplacian-based (Schofield and Zhu, 2003) approaches) to remove jumps of $\sim 2\pi$ that appear in the raw phase image or fitted field map. Background phase filtering is then performed on the unwrapped phase image within the masked region in order to remove the phase contribution from the background sources. Background field can be removed using a number of approaches, including high-pass spatial filtering, projection onto dipole fields (PDF: de Rochefort et al., 2010; Wharton et al., 2010; Liu et al., 2011), SHARP processing and its variants (Schweser et al., 2011; Wu et al., 2012), and the HARPERELLA (Li et al., 2014) algorithm. Lastly, a tissue magnetic susceptibility map is obtained by applying regularization methods to solve the ill-posed inverse problem (i.e. dipole inversion). For more detailed information about each of these steps, the reader is directed to Liu et al., (2015), Robinson et al., (2017), Schweser et al. (2017b), Langkammer et al., (2018), and Vinayagamani et al., (2021).

An overview of the current literature in which QSM analysis has been utilised in TBI

The application of QSM within the field of TBI is in a nascent state, with very few clinical and preclinical studies having been conducted to date. Amongst the current literature, QSM has predominantly been used to investigate a number of distinct post-injury pathophysiological changes in mTBI. These include changes in brain tissue magnetic susceptibility (Brett et al., 2020; Gong et al., 2018; Koch et al., 2018, 2020; Weber et al., 2018; Zivadinov et al., 2018; Lin et al., 2017), venous susceptibility (Chai et al., 2017) and venous structure (Liu et al., 2019). Furthermore, QSM can reveal post-injury cerebral microhaemorrhages (Liu et al., 2016) and calcium deposits (Schweser et al., 2017a). Each of these 'types' of pathophysiological changes has been investigated to varying extents, both in terms of the number of individual studies conducted as well as the post-injury phase at which that they were examined, and this review will cover each in turn. Given

that the pathophysiological processes associated with TBI manifest over time and are highly dynamic, time post-injury is an important factor that needs to be taken into consideration in efforts to observe iron-associated changes using QSM. TBI-recovery is often divided into 3 phases: *acute*, *subacute* and *chronic*. The exact duration of each phase can vary according to study specifications, and often take into account an individual's age, location and severity of TBI, and hence numerous definitions have been reported in the literature (Elbin et al., 2014; Toshkezi et al., 2018; Chung et al., 2019; Muftuler et al., 2020). In the following section, we describe the available clinical literature in which QSM has been utilised in examination of TBI.

TBI-related tissue magnetic susceptibility alterations

Within the context of mTBI, QSM has most often been used to assess the magnetic susceptibility of brain tissue post-injury. Following TBI, the magnetic susceptibility of WM and GM may be altered in response to co-occurring pathophysiological processes, such as dysmyelination, accumulation of iron and calcium, and gliosis (Chary et al., 2021). Furthermore, it is likely that observable changes are underpinned by different processes at different phases of injury. While research is limited, it appears that tissue magnetic susceptibility of WM initially increases following injury (but may normalise by the chronic stages), whereas GM tissue magnetic susceptibility appears to decrease. Increases in WM tissue magnetic susceptibility in the acute stages of injury have been attributed to oedema (Koch et al., 2018, 2020) and/or loosening of the myelin sheath (i.e. myelin decompaction; Weber et al., 2018), both of which are known to occur following mTBI. Findings from animal and human diffusion MRI studies appear to corroborate these hypotheses (Song et al., 2003, 2005; Sun et al., 2006; Aung et al., 2013; Lancaster et al., 2016). In the latter stages of injury, increases in WM susceptibility may be better accounted for by demyelination and iron accumulation (Koch et al., 2020; Chary et al., 2021). On the other hand, iron and calcium accumulation have been suggested as possible mechanisms underpinning observed decreases in GM tissue magnetic susceptibility following injury, however, further investigation is required to better understand the role and relative salience of these individual processes at different post-injury phases. Overall, the findings reported amongst the studies conducted to date have been mixed, with some studies reporting changes in tissue susceptibility subsequent to injury (Lin et al., 2017; Koch et al., 2018, 2020) while others have not (Gong et al., 2018; Weber et al., 2018; Zivadinov et al., 2018; Brett et al., 2020) (Table 1).

One of the earliest preliminary descriptions of altered tissue magnetic susceptibility following mTBI was reported in a published conference abstract by Lin et al., (2017). In this work, a cohort of 16 mTBI patients were imaged within 2 weeks of injury and compared to 23 healthy adult controls. Significant group differences were found in the susceptibility of several WM tracts, with mTBI-related increase in susceptibility observed in the left superior and posterior corona radiata, left superior longitudinal fasciculus, left posterior thalamic radiation, left retrolentricular part of the internal capsule and bilateral posterior limb of internal capsule. Significant GM susceptibility changes were also noted bilaterally thalamus, although the direction of susceptibility change was not specified.

Changes in tissue magnetic susceptibility have also been reported in two studies by Koch and colleagues. In Koch et al., (2018), QSM was used to investigate longitudinal changes in WM and GM tissue magnetic susceptibility following sport-related concussion. In this study, a cohort of injured (n = 28) and matched (n = 28) male, high-school and collegiate football players were imaged within 24 hours after injury, followed by further examination at 8 days and 6 months post-injury. Results echoed that of Lin et al., (2017), identifying a number of WM tracts of interest with increased susceptibility in concussed athletes at the acute and subacute periods. These effects were most pronounced at the subacute time point and appeared to resolve over time, showing no significant difference from controls at the 6-month visit. No statistically significant group differences were observed in the susceptibility of any of the GM regions investigated at any of the three time points examined. More recently, Koch et al., (2020) used QSM to further explore acute changes to tissue susceptibility in a cohort of 78 concussed male football athletes who were imaged within 48 hours of injury. In contrast to their previous work and that of Lin et al., (2017), this study examined tissue susceptibility changes in *global* WM and GM compartments created using masks from regional neurological segmentations extracted from atlases. Relative to uninjured athlete controls (n = 75), tissue susceptibility was increased in the WM compartment and decreased in the sub-cortical GM in the injured athlete group.

Weber et al., (2018) used QSM to investigate changes in tissue magnetic susceptibility in several regions of interest that had previously been observed by their group to have reduced myelin water fraction at two weeks post-concussion (Wright et al., 2016). In their former study, the authors measured the magnetic susceptibility of a number of voxels-of-interest located within the splenium of the corpus callosum, right posterior thalamic radiation, left superior corona radiata, left superior longitudinal fasciculus, and left posterior limb of the internal capsule in a mixed-sex subgroup of ice-hockey athletes (n = 11, 6 females) who had sustained a concussion during play over the course of a single season. In this study, QSM and diffusion imaging was performed at baseline and 3- (n = 8), 14- (n = 10) and 60-days (n = 9) days following injury. While the results of the statistical analyses did not indicate a significant change in magnetic susceptibility over time, a transient increase in WM tissue magnetic susceptibility appeared to occur at 3 and 14 days post-injury. This is in contrast to the reported diffusion metrics, which remained the same across the four time points examined. Taken together, the authors suggested that their findings reflected alterations in myelin sheath structure caused by decompaction, as opposed to myelin degradation or fragmentation, stemming from mTBI.

Similarly, Brett et al., (2020) used QSM as part of a multi-modal magnetic resonance imaging study that

Table 1. Summary of white and gray matter susceptibility changes observed across studies

	Lin et al., (2017)[a]	Koch et al., (2018)[b]	Weber et al., (2018)[c]	Koch et al., (2020)[b]	Gong et al., (2018)[c]	Zivadinov et al., (2018)[d]
Time points examined in study	**Acute-Subacute;** Within 2 weeks post-injury	**Acute-Subacute-Chronic;** 24 hours; 8 days; 45 days post-injury	**Acute-Subacute-Chronic;** Baseline; 72 hours; 2 weeks; 2 months	**Acute;** 48 hours post-injury	**Pre- and Post-Season;** Median 2 days prior first contact practice; Median 10 days after season	**Chronic;** Not specified
White Matter ROIs Investigated						
Global white matter compartment		↑ L (24hr; 8d)		↑		
Anterior thalamic radiation		↑ L (24hr)				
Posterior thalamic radiation	↑ L		*			
Corticospinal tract						
Corpus Callosum (splenium)			*			
Cingulum (cingulate gyrus)						
Cingulum (hippocampus)		↑ R (8d; 6m)				
Superior Corona Radiata	↑ L		*			
Posterior Corona Radiata	↑ L					
Forceps major						
Forceps minor		↑ (24hr; 8d)				
Inferior fronto-occipital fasciculus		↑ B (8d)				
Inferior longitudinal fasciculus		↑ B (8d)				
Retrolentricular part of Internal Capsule	↑ L					
Posterior Limb of Internal Capsule	↑ B		*			
Superior longitudinal fasciculus	↑ L	↑ L (8d) ↑ R (24hr; 8d)	*			
Superior longitudinal fasciculus (temporal)		↑ L (8d) ↑ R (24hr; 8d)				
Uncinate fasciculus						
Gray Matter ROIs Investigated						
Global gray matter compartment				↓		
Total deep gray matter						
Thalamus	B[†]					
Caudate						
Putamen						
Globus Pallidus						
Hippocampus						
Amygdala						
Accumbens						

[a] Comparison with healthy adults
[b] Comparison with matched athlete controls
[c] No comparison group
[d] Comparison with matched non-contact sport athletes
B, Bilateral; d, days; hr, hours; ROI, Region of Interest; L, left hemisphere; m, month; R, right hemisphere; ↑: increase in magnetic tissue susceptibility; ↓: decrease in magnetic tissue susceptibility; *: ROI of fixed volume located within this structure; †: direction of observed change not specified

Not studied
Statistically significant difference observed
No statistically significant difference observed
Gray: ROI not studied; Orange: statistically significant difference observed in ROI; Blue: ROI examined but no statistically significant difference observed

aimed to investigate the relationship between prior concussion history and contact sport exposure with metrics of WM microstructure and macrostructure. In this study, data were examined from a cohort of 121 high school and collegiate football and non-contact sport athletes who had not recently experienced a concussion (within the past 6 months), who were recruited originally as controls for a parent study (Chin et al., 2016). In addition to a baseline assessment, participants also attended multiple in-season neuroimaging visits that corresponded to the schedule of the parent study (i.e. < 48 h, 8 days, 15 days, and 45 days post-injury). WM was examined in two ways: firstly, as a global compartment, as per Koch et al., (2020), and secondly as individual ROIs in *post hoc* analyses, which were identical to those investigated by Koch et al., (2018). Linear mixed-effects models were used to examine the association of years of contact sport exposure (which served as a proxy measure for repetitive head impacts) and prior concussion across multiple metrics of WM properties, which included total WM volume, diffusion tensor imaging, diffusion kurtosis imaging, and QSM. Specific to QSM findings, a significant inverse association was observed between the tissue magnetic susceptibility of the global WM compartment and cumulative years of contact sport exposure but not concussion history. Subsequent *post hoc* analyses in which statistical models were fit for individual WM ROIs identified statistically significant negative associations between cumulative years of contact sport participation and tissue magnetic susceptibility in nine ROIs. Namely, these were the left cingulum (hippocampus), forceps major, forceps minor, left and right inferior fronto-occipital fasciculi, left and right inferior

longitudinal fasciculi, left superior longitudinal fasciculus and the left superior longitudinal fasciculus (temporal). As noted by the authors, the negative relationship between cumulative sport exposure and WM tissue magnetic susceptibility that was observed in their study contrasts with previous reports of increased WM tissue magnetic susceptibility occurring in the acute stages of injury. This suggests that QSM metrics may be differentially affected by the acute effects of concussion and the chronic effects of contact sport exposure, however, further investigation is required.

In a study investigating microstructural alterations associated with subconcussive head impacts, Gong et al., (2018) used QSM to measure changes in tissue magnetic susceptibility in deep and cortical GM over a single season of high school football. In this study, 16 athletes who experienced subconcussive head impacts, but not concussion, underwent MR scans and cognitive assessment pre- and post-season, with a median of 2 days (range 2–6 days) before the first contact practice and a median of 10 days (range 6–17 days) after the season, which lasted 4 months. In contrast with their diffusion kurtosis imaging results, which appeared to indicate the presence of significant microstructural changes following subconcussive head impact, no statistically significant differences were observed between pre- and post-season magnetic susceptibility values in any of the GM regions of interest examined. While the precise mechanisms of subconcussive injury are yet to be established and it remains to be elucidated if and how they might compare to those of mTBI, the authors concluded that microstructural changes in subconcussive head injury are unlikely to involve iron.

In a study also looking exclusively at deep GM structures, Zivadinov et al. (2018) used QSM to investigate differences in tissue magnetic susceptibility between a cohort of retired contact athletes (n = 21) and age matched non-contact sport athlete controls (n = 21). Although it was acknowledged that participants were likely to have experienced concussion, the authors had no reliable means of quantifying the number of concussions experienced by participants and thus could not control for this in the study. No information was provided on when neuroimaging was conducted relative to athlete retirement for either athlete groups, although a chronic time point was implied. Overall, no statistically significant group differences were found in any of the regions of interest examined, which comprised total deep GM, thalamus, caudate, putamen, globus pallidus, hippocampus, amygdala, or nucleus accumbens.

With only two studies having been conducted to date, investigations into the utility of tissue magnetic susceptibility as a clinical correlate are very limited. Nevertheless, the existing circumstantial evidence supports the notion that tissue magnetic susceptibility may be useful for predicting outcome following mTBI.

In addition to investigating longitudinal changes is tissue magnetic susceptibility, Koch et al. (2018) examined the relationship between acute (i.e. 24 h) tissue susceptibility and individual performance on several clinical measures that are commonly used in sports-concussion research and management. Namely, these were the Sports Concussion Assessment Tool-3rd Edition (SCAT-3), Standardized Assessment of Concussion (SAC), and the Balance Error Scoring System (BESS). While no statistically significant correlations were observed between the acute tissue magnetic susceptibility and the SCAT-3, SAC, or BESS within the injured group, a positive relationship was observed between acute tissue susceptibility within several WM regions and return-to-play duration. More specifically, at the individual subject level, susceptibility increases in the global WM compartment, right cingulate (hippocampus), left inferior fronto-occipital fasciculus, right inferior longitudinal fasciculus, left superior longitudinal fasciculus, right superior longitudinal fasciculus, and the left superior longitudinal fasciculus (temporal), were associated with a relatively longer time away from playing sport. Interestingly, the observed changes in tissue susceptibility appeared to persist beyond those detected on clinical outcome measures, which suggests that there may be a discrepancy between the timing of biological and functional recovery.

Similarly, following their examination of tissue magnetic susceptibility changes in the acute (48 hour) post-injury period, Koch et al. (2020) conducted preliminary investigations into the prognostic utility of QSM. A statistically significant correlation between the tissue magnetic susceptibility of global subcortical GM and self-reported symptom duration was identified within a sub-group of concussed athlete participants who experienced symptoms for at least 1 week after injury (n = 39). Furthermore, results of receiver operating characteristic (ROC) analyses, which were conducted to evaluate the specificity and sensitivity of the QSM and clinical metrics, found a univariate model constructed using subcortical GM compartment tissue susceptibility values to have a reasonable validation area under the curve (AUC) performance of 0.78 when predicting symptoms lasting longer than two weeks. These findings suggest that acutely measured QSM MRI metrics show promising prognostication capabilities.

TBI-related venous susceptibility alterations

The brain requires a continuous supply of oxygen to maintain normal functioning and tissue integrity (Zauner et al., 2002). While only 2% of the body's total mass, the brain consumes 20% of the body's total oxygen supply (Rolfe and Brown, 1997; Gallagher et al., 1998; Magistretti and Pellerin, 1999; Zauner et al., 2002; Rodgers et al., 2016). These unique energy requirements are met almost entirely by the oxidative metabolism of glucose, which is delivered to the brain *via* blood circulating in the cerebrovascular system. Therefore, any interruptions to cerebral blood flow that result in an impaired oxygen supply can have devastating consequences for the brain.

Following TBI, there is an acute decoupling between cerebral metabolic demand and blood supply that is accompanied by a host of abnormal vascular functioning and hemodynamic responses. This results in an imbalance between cerebral oxygen delivery and consumption, which is reflected in aberrant metabolic rates of oxygenation at the brain tissue level and causes

further damage to injured neuronal cells (Werner and Engelhard, 2007; Kou et al., 2016). While few studies have directly examined blood oxygenation in TBI patients, reports suggest that cerebral oxygenation is altered across all severities of TBI. More specifically, cerebral oxygenation has been observed to be reduced following TBI (Dings et al., 1996; Daugherty et al., 2004; Bhambhani et al., 2006) and may persist for up to 7 days post-injury (Cote et al., 2006). Furthermore, transient increases in haemoglobin oxygen saturation have been observed in animal models of moderate TBI (Armstead and Kurth, 1994).

Given that the brain is highly dependent on oxygen to meet its metabolic needs, measures of oxygen utilisation can be used to gain insight into brain tissue viability and function, as well as any changes that may occur following adverse pathophysiological events, such as TBI. Cerebral metabolic rate of oxygen consumption ($CMRO_2$) and oxygen extraction fraction (OEF) are two key physiological parameters of cerebral blood oxygenation, however, these are most accurately determined using positron emission tomography (PET) imaging, which is costly and requires administration of radioactive isotopes (Liu and Li, 2016). Efforts to develop MRI-based methods of measuring brain oxygenation *in vivo* have made it possible to infer parameters of interest for brain metabolism. One measure that has recently garnered increased attention is venous oxygen saturation (SvO_2).

SvO_2 is an indirect index of global oxygen supply-to-demand (Chai et al., 2018), and may complement direct measures of oxygen metabolism such as $CMRO_2$. SvO_2 is typically measured in large cerebral veins, ranges between 60–75% in healthy individuals (Perlmutter et al., 1987; McCormick et al., 1991; Marchal et al., 1992; Ibaraki et al., 2008; Krishnamurthy et al., 2014; Fan et al., 2016; Jog et al., 2016; Kudo et al., 2016), and is influenced by local differences in tissue metabolism and blood flow (Mergenthaler et al., 2013). Discrepancies between metabolic demand and arterial blood supply due to TBI or accompanying secondary insults (such as ischemia and hypoxia) can result in reduced brain tissue oxygen saturation. This in turn will be reflected as abnormally low blood oxygenation within draining veins (Kou et al., 2016). Observations of decreased venous oxygenation saturation may indicate that a specific brain area has unmet energy needs or altered energy demands resulting from changes in function, and is thus particularly vulnerable to damage (Doshi et al., 2015).

QSM, which uses iron in haemoglobin as a contrast mechanism, can be used to image veins and approximate SvO_2 in a non-invasive manner. The magnetic susceptibility of haemoglobin is highly sensitive to oxygenation (Pauling and Coryell, 1936). Deoxygenated haemoglobin (also referred to as *deoxyhemoglobin* in the literature) has unpaired electrons, and is thus paramagnetic (Reichenbach et al., 1997). Conversely, oxygenated haemoglobin *(oxyhemoglobin)* has a weak and negative susceptibility (i.e. diamagnetism) as it has no unpaired electrons. Veins contain a higher proportion of deoxyhemoglobin relative to other vascular structures and are thus visible on susceptibility maps. Since venous susceptibility depends linearly on deoxyhemoglobin concentration (Jain et al., 2012) and the volume fraction of red blood cells in whole blood (i.e. haematocrit; Weisskoff and Kiihne, 1992) it is possible to measure SvO_2 and infer venous oxygenation using QSM (Fan et al., 2014; Xu et al., 2014; Hsieh et al., 2016, 2017).

To date, only one QSM study has examined venous oxygenation following TBI. Chai et al., (2017) used QSM to measure the susceptibility of 13 major cerebral veins to explore potential differences in regional SvO_2 in a cohort of 48 individuals presenting to a hospital ED with mTBI in the acute-to-subacute stage of injury and a community sample of 32 healthy controls. In this study, the authors categorised mTBI patients into axonal injury ($n = 16$) and non-axonal injury groups ($n = 32$), which was determined by the presence or absence of abnormalities found in any of the other MRI sequences (T_1WI, T_2 FLAIR, DWI or SWI) that were performed as part of the imaging protocol. Of the veins examined, the only statistically significant difference observed in susceptibility between the three groups was in the straight sinus, with the axonal injury and non-axonal injury patient groups both having lower susceptibility relative to healthy controls ($p < 0.001$). According to the authors, this may indicate cerebral SvO_2 is increased within this particular region in patients following mTBI. Further examination of the susceptibility of the straight sinus did not reveal a statistically significant difference between the axonal injury and non-axonal injury groups. While the authors suggested this latter finding may be accounted for by differences in the time post-injury that imaging was conducted for the axonal injury (110.13 h ± 105.93) and non-axonal (51.0 3 h ± 39.26) groups, it is important to note that their statistical evaluation did not indicate these imaging time points to be significantly different from each other. Regardless, the broad range of stages of injury and recovery in each group at the different time points in this cross-sectional study make direct comparison of SvO_2 challenging.

In addition to examining changes in venous susceptibility, Chai et al., (2017) also performed correlation analyses to examine the relationship between the susceptibility of the selected major cerebral veins, elapsed time post-trauma (ETPT), and post-concussion symptom scores measured by the Rivermead Post-Concussion Symptoms Questionnaire (RPQ). The diagnostic efficiency of susceptibility to discriminate mTBI patients from healthy controls was also analysed. Examination into the relationship between susceptibility of the major veins and ETPT revealed a statistically significant moderate correlation between the susceptibility of the straight sinus and ETPT in the non-axonal injury group, but not the axonal injury group. Given that the authors state that mTBI injury was believed to be more severe amongst axonal injury patients due to the presence of wide-spread lesions in in WM tracts, this distinction could potentially reflect different, or additional, pathological mechanisms that are at play in the axonal injury group, which in turn may influence the rate of SvO_2 recovery. Furthermore, a moderate negative correlation was

observed between the susceptibility of the straight sinus and RPQ scores reported by axonal injury patients, but not non-axonal injury patients. This finding may suggest that oxygen consumption may be reduced amongst individuals who experience a relatively more 'severe' mTBI, as reflected by an increased cerebral SvO_2, which in turn could result in a manifestation of post-concussion symptoms, such as headaches, dizziness etc. No other correlations were observed between the susceptibility of other cerebral veins and ETPT or RPQ scores in the axonal injury or non-axonal injury groups ($p > 0.05$). Results of a ROC analysis found that the sensitivity, specificity, and AUC values for the discrimination between mTBI patients and healthy controls using the susceptibility of the straight sinus at a cut-off value of 298 ppb to be 88%, 69%, and 0.84, respectively, which led the authors to propose that the susceptibility of the straight sinus could be used as a biomarker to differentiate mTBI patients from healthy controls as well as monitor the progress of mTBI.

Changes in venous structure following TBI

The cerebral vascular system is vulnerable to the biomechanical insult that occurs in TBI (Kou et al., 2015). Damage to cerebral vasculature can result from both primary and secondary injury mechanisms (Kenney et al., 2016), and may manifest as a number of pathological responses including microbleeds, local perfusion reduction, and changes in oxygen metabolism, all of which themselves can instigate or exacerbate other secondary injury consequences (Doshi et al., 2015). Venous blood vessel walls are mainly composed of collagen and contain little elastic tissue or smooth muscle. As such, they are thinner than the robust vessels of the arterial system, and are more vulnerable to damage at the same force of impact (Haacke et al., 2013). Veins located on the brain surface constitute the most vulnerable part of the outer cerebrovascular system, and insults to these superficial veins can also result in damage to the deeper veins that drain into them (Haacke et al., 2013). Trauma-induced vascular injury remains an under investigated area in TBI research, however, the sensitivity of QSM to deoxyhemoglobin in venous blood provides an excellent opportunity to analyse venous structure in a non-invasive manner.

To date, only one study has investigated structural changes to the cerebral vasculature following TBI with QSM. Liu et al., (2019) used QSM, in addition to SWI, to characterise the overall state of venous structures at the chronic injury stage in a cohort of military service members with severe ($n = 14$), moderate ($n = 38$), or mild ($n = 58$) TBI and controls ($n = 37$). Venous segmentation was performed using a multiscale vessel enhancement filter on SWI images and a threshold approach on QSM images. Results of the SWI imaging indicated that only patients with severe TBI had significantly higher segmented venous volumes relative to controls, whereas QSM imaging revealed statistically significantly increased segmented venous volumes relative to controls in the whole brain for severe ($p = 0.01$), moderate ($p = 0.008$), and mild ($p = 0.042$) TBI, relative to controls. Somewhat similar outcomes were observed when data were separately analysed in the left (severe $p = 0.01$; moderate $p = 0.038$, relative to controls) and right hemispheres (severe, $p = 0.001$; moderate $p = 0.013$, mild, $p = 0.027$, relative to controls). The authors suggested numerous explanations for the observed increases in venous volume, which included increased venous stasis, enlarged or damaged veins, extravasated blood, and/or microthrombosis.

Cerebral microhaemorrhages in TBI

Cerebral microhaemorrhages, also known as cerebral microbleeds, are small, focal intracerebral haemorrhages that are most likely caused by structural alteration in the small blood vessels of the brain (Martinez-Ramirez et al., 2014). Cerebral microhaemorrhages appear as small, hypointense circular (petechial) or elliptical (linear) lesions ranging from 2-10 mm in size on GRE and SWI images (Greenberg et al., 2009; Wardlaw et al., 2013; Lee et al., 2018; Griffin et al., 2019). Histopathologically, they represent an accumulation of hemosiderin-containing macrophages, which possess paramagnetic properties that allow them to be detected using T2* gradient-recalled echo (GRE) and susceptibility weighted imaging (SWI) sequences (Lee et al., 2018; Nael et al., 2020). In TBI, cerebral microbleeds have been associated with diffuse axonal injury (Scheid et al., 2003; Liu et al., 2014), and are frequently observed to occur at the gray-white matter junction (Liu et al., 2014; Huang et al., 2015a). This is partly due to the differential biomechanical responses of the two tissue types to the forces associated with TBI, which also make it particularly susceptible to diffuse axonal injury, and in part due to differences in venous drainage at the GM/WM boundary (Liu et al., 2014; Irimia et al., 2018). Given that the GM/WM interface is at times superficial (e.g. superior frontal gyri, middle temporal gyri) and at other times deep (e.g. insulae, cingulate gyri) relative to the scalp, the location of cerebral microhaemorrhages can be widespread and difficult to anticipate across individuals affected by TBI (Irimia et al., 2018).

Cerebral microhaemorrhages are a common finding in the acute to subacute stages of TBI (Perel et al., 2009; Wu et al., 2010; Liu et al., 2016) and their presence has been linked to injury severity and clinical outcome (Huang et al., 2015b). While some studies have found the number, location, type and volume of cerebral microhaemorrhages to correlate with injury severity and clinical outcome, others have not (Toth et al., 2016; Lawrence et al., 2017). Keeping in mind that an optimal timeframe for image acquisition has not yet been established, the discrepancy in reported findings may be explained by differences in time elapsed between injury and when imaging was conducted, which ranges from days to years (Lawrence et al., 2017). Cerebral microhaemorrhages are commonly believed to persist over time (Haller et al., 2018). According to conventional wisdom, the brain has no mechanism by which it can remove hemosiderin once it is formed (Liu et al., 2016). Thus, it is believed to remain present indefinitely in the brain and can serve as an indicator of prior haemorrhage (Liu et al., 2016). Investigations into the detection and evolution of cerebral

microhaemorrhages in the chronic stage of injury are currently severely limited, although it is acknowledged that having the ability to monitor the evolution of microhaemorrhages could provide unique insight into disease progression and/or recovery.

To date, no study has utilised QSM to investigate cerebral microhaemorrhages in the acute or subacute stages of TBI, however, QSM was used by Liu and colleagues (Liu et al., 2016) to monitor the longitudinal evolution of cerebral microhaemorrhages in the chronic stages of injury amongst a subset of $n = 13$ military service members with TBI of mixed severity (mild TBI $n = 3$; moderate TBI $n = 7$; severe TBI $n = 3$). Results of the study indicated a statistically significant decrease in both the number and susceptibility cerebral microhaemorrhages between the two imaging occasions, which were conducted on average 205 (standard deviation = 226, median = 128) and 270 (standard deviation = 144; median = 252) days post-injury, respectively. According to the authors, this suggests that hemosiderin products in the brain may continue to undergo subtle evolution in the chronic stages of TBI, although they acknowledged that further investigation in other populations and with larger cohorts is required.

Detecting calcifications in chronic TBI

Dystrophic calcifications are abnormal deposits of calcium that accumulate in areas of degenerated and necrotic tissue (Casanova and Araque, 2003). Considered to be a pathological sequelae of brain damage, they have been observed to occur in a number of neurological disease states (Aggarwal et al., 2018), including TBI (Makariou and Patsalides, 2009; Grech et al., 2012). The mechanisms underlying brain tissue mineralisation are poorly understood. Previously, the formation of calcium deposits in the brain have been thought to be primarily driven by localised increases in intracellular calcium concentrations within areas of apoptotic or necrotic cell death (Kim, 1995); however, more recent evidence highlights the roles of microglial and astroglial activation (Gayoso et al., 2003) and iron accumulation (Harder et al., 2008; Aggarwal et al., 2018; Snyder-Keller et al., 2020) in the calcification process. The precise role of dystrophic calcifications in TBI is also unclear. Currently, they are presumed to be functionally inert (Lehto et al., 2019), and are hypothesised to serve as a protective buffer against the presence of excess free calcium ions in brain tissue (Rodríguez et al., 2000; Mäkinen et al., 2008; Lehto et al., 2012). Following TBI, dystrophic calcifications are most commonly detected in the chronic stages of injury, where they appear as well-developed entities (Pierce et al., 1998; Lehto et al., 2012; Laitinen et al., 2015). Histological studies indicate that calcium salts may also be present as small and diffuse granules in the earlier stages of injury (Gayoso et al., 2003).

Contrasting with the strong paramagnetic properties of iron, calcium has a diamagnetic susceptibility that also lends itself to being investigated using QSM (Yamada et al., 1996; Schweser et al., 2010; Liu et al., 2015). Despite this, the occurrence of dystrophic calcifications following TBI is yet to be extensively investigated using QSM. Preliminary results have been reported in a published conference abstract by Schweser et al. (2017b) who used QSM to ascertain the presence of calcium deposits within the thalamus amongst a cohort of middle-aged, retired football and ice-hockey male professional contact-sports athletes ($n = 22$), professional athlete controls ($n = 20$) and normal controls ($n = 25$; non-professional, no history of playing contact sports). While the authors did not specify when imaging was conducted relative to retirement, a chronic timeframe was implied. The thalamus, which was the sole region of interest in the study, was selected based upon previous findings that suggested calcium deposition in this structure may be related to the activation of N-methyl-D-aspartate (NMDA)-receptors following concussion (i.e. mild traumatic brain injury) (Choe, 2016). Calcifications were manually traced by a trained image analyst according to criteria set by the authors. Results of the study indicated a statistically significant group difference, with thalamic micro-calcifications being identified in 23% of contact-sports athletes, 10% of athlete controls, and none in the normal controls. Interestingly, all contact-sport and athlete controls in which micro-calcifications were identified were ice-hockey players and runners/triathletes, respectively. All lesions were located in the medial nuclear group of the thalamus, although one athlete control was also found to have lesions in the anteroventral and reticular nuclei of the thalamus. The authors also noted that the lesions could not be identified on magnitude images, which they suggested implied a diffuse microscopic distribution of calcium (possibly intracellular), as opposed to a solid agglomeration. Overall, the authors concluded that the observed presence of thalamic calcification years after acute concussions indicates that calcium deposits are persistent over time, however, it must be noted that the authors did not specify how they established previous history of concussion in the cohort. While further research is required, the capacity of QSM to detect calcifications and differentiate them from microhaemorrhages suggests a potential role for QSM in prognostic and theranostic monitoring of TBI.

FUTURE DIRECTIONS

Although limited, the emergent literature suggests that QSM is a promising neuroimaging technique that can be used to investigate a host of pathophysiological changes that are associated with TBI. Further research is required to realise the full potential of QSM within the context of TBI, with notable gaps in the literature yet to be addressed.

The capacity for greater anatomic specificity in brain imaging software is particularly amenable to the application of QSM. For example, the recent development of thalamic segmentation in Freesurfer (Iglesias et al., 2018) has for the first time allowed detailed automated examination of thalamic nuclei *in vivo* (Fig. 1). The thalamus appears to be particularly vulnerable to the effects of TBI (Maxwell et al., 2006; Lutkenhoff et al., 2013, 2020; Grossman and Inglese, 2016; Glushakov et al., 2018; Moe et al., 2018), however, findings of iron

accumulation are inconsistent in this structure and may be confounded by the diamagnetic influence of diffuse calcifications. It may well be that iron accumulates in some thalamic nuclei and not others after TBI, which has now been demonstrated to be the case in multiple sclerosis (Hagemeier et al., 2013; Burgetova et al., 2017). Additionally, segmentation of key structures such as the hippocampus and amygdala (Fig. 1) may also facilitate greater insight into the extent and location of iron-related damage within these specific brain areas following TBI, as well as if any variation may account for differences in the specific types and duration of symptoms that individuals experience after injury. While evidence of iron-related changes within these two structures – which are closely associated with memory and emotion respectively- has been previously noted in QSM studies of individuals affected by Alzheimer's Disease (Acosta-Cabronero et al., 2013; Kim et al., 2017; Kan et al., 2020), we are yet to examine these relationships in structural or substructural detail in TBI.

Methodological limitations associated with QSM have been discussed in detail elsewhere (Haacke et al., 2015; Wang and Liu, 2015; Deistung et al., 2017; Jung et al., 2020; Vinayagamani et al., 2021), however, particularly pertinent to TBI research is the ability to differentiate iron from calcium deposits. For example, given the opposing magnetic properties of iron and calcium, intra-voxel pseudo-normalisation may result in a failure to detect significant changes. Future technological advances should focus on developing ways to better differentiate the relative contribution of specific ions to signal, possibly utilizing threshold-based methodologies. Post-mortem or animal studies may also provide scope to investigate and validate relative contribution to signal. Furthermore, cerebral microhaemorrhages have been observed to occur following TBI, however, they are also a common finding amongst healthy

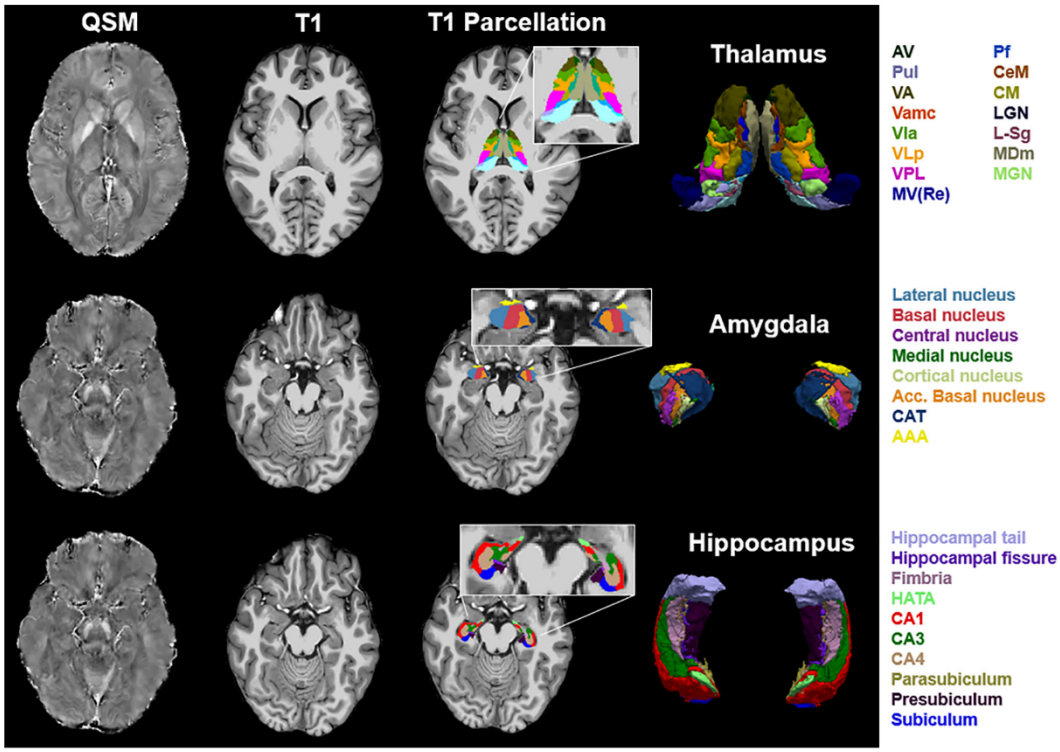

Fig. 1. Example QSM image, anatomical T1-weighted image, substructural parcellation and corresponding 3D render of thalamic nuclei, amygdala nuclei and hippocampal subfields. Example images acquired on Phillips Ingenia 3 T, with QSM processed using the MEDI toolbox (http://pre.weill.cornell.edu/mri/pages/qsm.html) with iField correction. Parcellation was performed using FreeSurfer (https://surfer.nmr.mgh.harvard.edu/), with 3D renders of structural parcellations generated in FreeSurfer's Freeview, with rotated to demonstrate segmentation of morphological features. Images are drawn from a 21 year old female 10 days post-mTBI as part of the CREST Concussion Recovery Study (trial registration: ACTRN12619001226190; ethics approval number: RGS0000003024). Images are provided for illustrative purposes only. No conclusions are drawn regarding pathology associated with the brain injury. Thalamus: AV, anteroventral subnucleus; Pul, pulvinar nucleus; VA, ventral anterior nucleus; Vamc, ventral anterior thalamic nucleus; Vla, ventral lateral nucleus anterior; VLp, ventral lateral nucleus posterior; VPL, ventral posterolateral nucleus; MV(Re), medioventral nucleus (reuniens nucleus); Pf, parafascicular nucleus; CeM, central medial nucleus; CM, centromedian nucleus; LGN, lateral genticulate nucleus; L-Sg, supragenticulate nucleus; MDm, mediodorsal medial magnocellular nucleus; MGN, medial genticulate nucleus; Amygdala: CAT, corticoamygdaloid transition; AAA, anterior amygdaloid area; Hippocampus: HATA, hippocampal amygdala transition area; CA1-4, cornu ammonis regions 1–4. Note: additional segmentations not visible in the figure have been omitted from the legend.

older adults. QSM may be able to shed light on their clinical significance in healthy populations, and to distinguish what is typical of healthy ageing and what may be considered as pathological hallmark of TBI. Few studies have examined TBI-related iron dyshomeostasis and accumulation in females. This is important to explore given both the rise of female partcipation in contact sport, and the frequency with which anaemia presents in females (World Health Organization, 2017).

QSM is resolved in a non-local fashion, i.e. the magnetic susceptibility of a voxel is inferred from the pattern of magnetic field perturbations that surround it. This is akin to measuring pebbles being dropped into a river from the size of the ripples across the entire surface. For this reason, the resolution and coverage of MRI sequences can significantly impact QSM images (Karsa et al., 2019). Cerebral veins are particularly susceptible to these impacts, and may also be confounded by orientation and size relative to voxels (Li et al., 2012; Hsieh et al., 2015; McDaniel et al., 2017; Ward et al., 2017). Care should be taken to control for these factors within-groups before comparing TBI and controls, especially when attempting to precisely distinguish cerebrovascular volume (Liu et al., 2016) and oxygenation (Chai et al., 2017).

Lastly, in the interest of enabling comparability and reproducibility amongst studies, future research within the TBI space should align with defining criteria for a TBI diagnosis and the standardization of timing for examination and investigation post-injury. This will also enable a more accurate estimation of the role of iron in TBI pathogenesis over time. Comparability and reproducibility will also be improved by adherence to MRI reporting standards (e.g. COBIDAS; Nichols et al., 2017) and the emergence of standardized QSM processing methods (Langkammer et al., 2018).

DECLARATIONS OF INTEREST

Melinda Fitzgerald is the Chief Executive Officer of the charitable organization *Vision TBI (Ltd.)*, trading as *Connectivity - Traumatic Brain Injury Australia*.

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6 QSM Pipeline Development and Example Analysis

6.1 Introduction

This chapter presents a detailed exposition of the analysis pipeline that will be used to analyse QSM data that are being collected for the *CREST* study, which was introduced in Chapter 4 of this thesis. Due to the novel nature of this analysis pipeline, the method development is presented in narrative format and includes screenshots of the scripts that are being used at each stage of the analysis, together with representative images of the resultant outputs. This chapter concludes with an example analysis in the form of a brief report, in which the developed pipeline has been applied to real-world *CREST* data as a proof-of-concept. Examining the hypothesis that prior mTBI may be associated with changes in magnetic susceptibility within the thalamus (a subcortical GM structure), this novel investigation examines whether these alterations can be detected using QSM.

6.2 QSM Pipeline Overview

The reconstruction of susceptibility maps (i.e. QSM images) entails several distinct computational steps, which are deeply rooted in a complex theoretical background that interweaves physics and mathematical algorithms. The workflow that produces a QSM image is illustrated in Figure 12 below, and can be summarised as follows. First, a T2*-weighted GRE sequence is performed on an MRI scanner to acquire a magnitude and phase image. The *magnitude image* is used to create a mask of the brain, which is required to delineate the volume of interest, while the *phase image* is unwrapped using Laplacian-based[800], path-following[801,802], region-growing[803,804], or deep-learning[805,806] approaches in order to remove jumps of ~2π, which appear as black and white bands in the raw phase image or fitted field map. The mask is applied to the *unwrapped phase image*, and background phase filtering is subsequently performed to remove phase contribution from background sources. Background field removal can be achieved *via* a number of different approaches, which include High-Pass Spatial Filtering, Projection onto Dipole Fields (PDF)[807–809], Sophisticated Harmonic Artifact Reduction for Phase Data (SHARP) processing and its variants[810,811] and the HARmonic (background) PhasE REmovaL using the LAplacian operator (HARPERELLA)[812] algorithm. Finally, regularization methods such as Calculation Of Susceptibility through Multiple Orientation Sampling (COSMOS)[813], Morphology Enabled Dipole Inversion (MEDI)[814], Homogeneity Enabled Incremental Dipole Inversion (HEIDI)[815], or emerging deep neural network reconstruction techniques such as DeepQSM[816], are used to solve the ill-posed inverse problem (i.e. dipole inversion) and construct the susceptibility map.

For studies involving multiple participants, a few additional steps are required in order to perform group-level analyses. Specifically, once QSM images have been generated, it is also necessary to construct a common space in which all individuals can be compared, and to segment the QSM image into anatomical regions of interest[817]. The latter facilitates the estimation of magnetic susceptibility within specific regions of the brain from the susceptibility maps and can be performed either manually by the researcher or automatically using additional neuroimaging software, usually *via* a T_1-weighted scan that has been registered to QSM space[817].

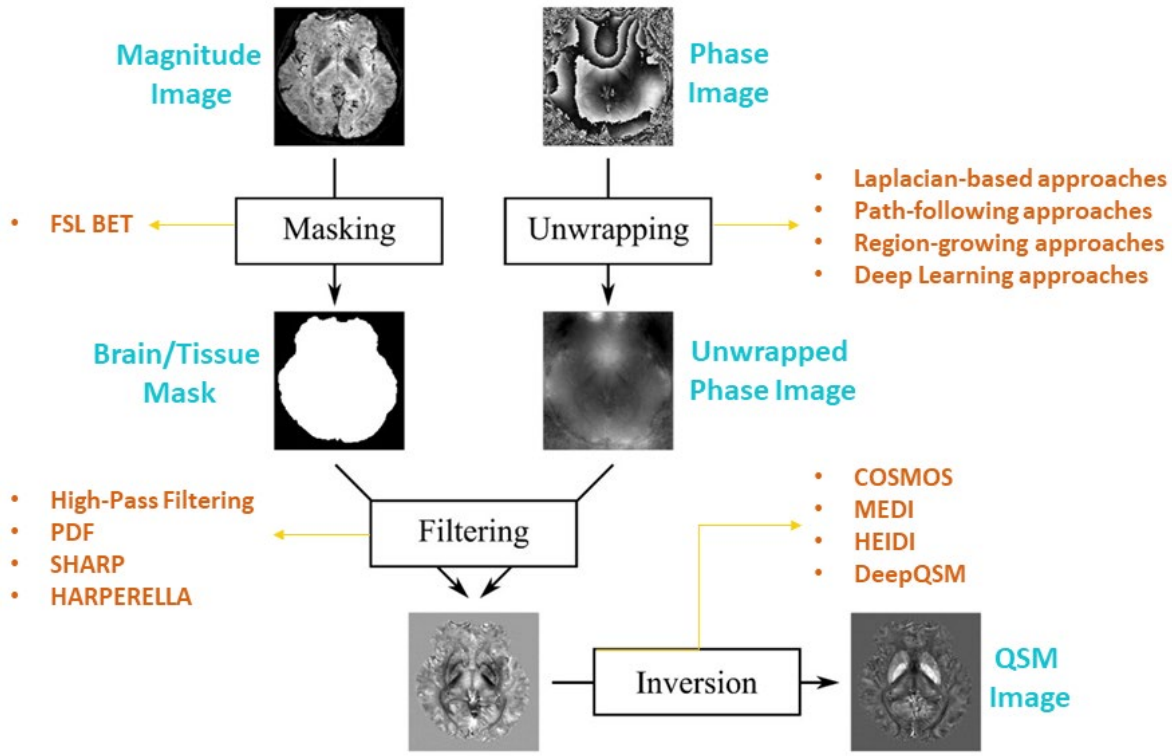


Figure 12. QSM image generation pipeline. This flow chart illustrates the workflow that results in the generation of a QSM image. Beginning at the MRI scanner, a T2* gradient recalled echo sequence is performed to procure a *magnitude* and *phase image*. The *magnitude image* is used to generate a *signal mask* for the volume of interest, while the *phase image* is unwrapped to create an *unwrapped phase image*. The *mask* and *unwrapped phase image* are combined and subsequently undergo a filtering process to remove background phase. Lastly, regularization methods are then applied to solve the ill-posed inverse problem and generate the QSM image. As is indicated in the figure, a number of approaches can be used to execute the *Masking*, *Unwrapping*, *Filtering* and *Inversion* steps of the pipeline, each of which is associated with a unique set of strengths and limitations, which in addition to the research context, often guide the researcher's selection. *Abbreviations*: FSL BET: FMRIB Software Library Brain Extraction Tool; PDF: Projection onto Dipole Fields; SHARP: Sophisticated Harmonic Artefact Reduction for Phase Data; HARPERELLA: HARmonic (background) PhasE RemovaL using the LAplacian operator; COSMOS: Calculation Of Susceptibility through Multiple Orientation Sampling; MEDI: Morphology Enabled Dipole Inversion; HEIDI: Homogeneity Enabled Incremental Dipole Inversion. Figure adapted from Liu et al., (2015)[818].

6.3 *CREST* QSM Pipeline Development

A challenge to using cutting edge and nascent technology like QSM is the substantial amount of bespoke work and background knowledge required to apply it correctly. For example, modern MRI *via* an out-of-the-box vendor console with in-built reconstruction software is comparatively easier than building your own array coils, reconstruction software and user interface, which was necessary in the early days of MRI research.

To analyse QSM data that are being collected as part of *CREST*, a modified pipeline has been compiled in *MATLAB* (MathWorks®, Natick, Massachusetts, USA), which uses original materials sourced from the MEDI Toolbox (http://pre.weill.cornell.edu/mri/pages/qsm.html). More specifically, the pipeline includes an additional piece of code (i.e. modification) to account for a digital signal processing error known to occur for QSM data collected using *Philips* MRI systems. The following subsections detail this work, outline the design decisions made, and provide guidance for subsequent researchers to replicate this complex process. Such efforts are critical for supporting reproducibility and the path to clinical adoption of QSM. The custom software has been organised into two separate *scripts* (i.e. collections of code), which have been titled *Prep_Data* and *Run_QSM*, respectively, and will be referred to as such throughout this chapter.

6.3.1 Prep_Data Script and Output

The purpose of the *Prep_Data* script (detailed in Figure 13) is to process MRI data obtained directly from the MRI scanner and generate the individual components that are required to produce a QSM image, specifically the magnitude and phase components. The script begins with setting up input and output directories where data (which has already been converted to the Neuroimaging Informatics Technology Initiative (NIfTI: *.nii*) processing format) are sourced from and saved, respectively. Meta-data associated with the MRI sequence performed that are critical to the QSM reconstruction are also sourced at this stage.

```
function Prep_Data(nifti_dir, output_dir)
%PREP_DATA: Pre-Processing Script
% Use this script to create all the components needed to generate a QSM image

%Set-up input and output directories
nifti_dir = 'ParticipantData/CREST_XXXX';
output_dir = 'Prep_Data_CREST_XXXX_iFieldCorrection';

% Meta data from JSON file
files = dir(sprintf('%s%s*SWI*_real.json',nifti_dir,filesep));  in_hdr =
jsondecode(fileread(strcat(files(1).folder,filesep,files(1).name)));  B0_strength =
in_hdr.MagneticFieldStrength; % (Tesla)
B0_dir = [0 0 1];  CF = 127.745071*1E6; % (Hz)
TE = zeros(1,in_hdr.EchoTrainLength);

% Meta data from single input file
temp_file = dir(sprintf('%s%s*SWI*_real.nii',nifti_dir, filesep));
in_temp = load_nii([nifti_dir filesep temp_file(1).name]);
matrix_size = size(in_temp.img);
voxel_size = in_temp.hdr.dime.pixdim(2:4); % (mm)
out_nii = in_temp; iField = zeros([matrix_size numel(TE)]);

for i=1:numel(TE)
       re_file = dir(sprintf('%s%s*SWI*_e%d_real.nii',nifti_dir, filesep,i));
       in_re = load_nii([nifti_dir filesep re_file(1).name]);
       im_file = dir(sprintf('%s%s*SWI*_e%d_imaginary.nii',nifti_dir, filesep,i));
       in_im = load_nii([nifti_dir filesep im_file(1).name]);
       iField(:,:,:,end-i+1) = complex(in_re.img,in_im.img);
       json_file = dir(sprintf('%s%s*SWI*_e%d_real.json',nifti_dir, filesep,i));
       in_hdr =
       jsondecode(fileread(strcat(json_file(1).folder,filesep,json_file(1).name)));
       TE(i) = in_hdr.EchoTime;
end
delta_TE = TE(2)-TE(1);
```

Figure 13. Code for setting up QSM pipeline input and output directories and sourcing of meta-data.

Following this initial set-up step, the *Prep_Data* script proceeds to generate components *I-VI* described below and concludes by performing background field removal using the PDF approach *(*component *VII)*.

I. Magnitude image

The *magnitude image* (Figure 14) is collected as part of the MRI sequence that is performed on the scanner. Being an anatomical scan, magnitude images are similar in appearance to a T_1-weighted image but show a greater level of detail, particularly within the subcortical areas, due to their spin-density-like contrast. *Magnitude images* are primarily required to create *brain masks*, although they may also be used to detect pathologies, such as microhaemorrhages, and in subsequent stages of analysis involving the generated susceptibility maps.

```
% Calc Magnitude
iMag = sqrt(sum(abs(iField).^2,4));
out_nii.img = iMag;
save_nii(out_nii,[output_dir filesep 'Mag.nii.gz']);
```

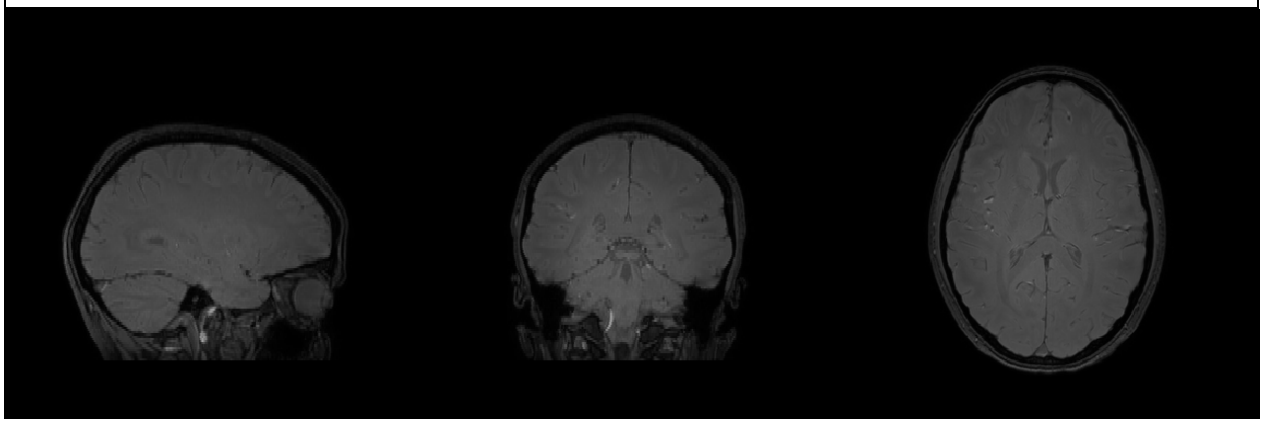


Figure 14. Code for generating the magnitude image and representative image of the resultant output.

II. Brain Mask

A signal mask (i.e. *brain mask*- see Figure 15) is generated to specify the volume of interest (i.e. the brain), which contains a reliable phase signal for the background field removal and dipole inversion steps. To achieve this, the skull and eyes need to be removed (i.e. "*stripped*") from the *magnitude image* to leave only the brain. In this instance, the pipeline uses the *Brain Extraction Tool* (BET)[819] from the FMRIB Software Library (FSL) (http://www.fmrib.ox.ac.uk/fsl) to perform this task.

```
% Calculate brain mask
Mask = BET(iMag,matrix_size,voxel_size);
out_nii.img = Mask;
save_nii(out_nii,[output_dir filesep 'Mask.nii.gz']);
```

Figure 15. Code for calculating the brain mask using the FMRIB *FSL BET* tool and representative image of the resultant output.

III. Estimate of R2 Decay*

Next, the *R2* decay* is estimated from the magnitude data (see Figure 16). *R2** is the inverse of the T2* relaxation rate, and reflects dephasing due to local magnetic field inhomogenieties[820]. *R2** serves an alternative measure for estimating iron load in brain tissue[821] and can also be used to identify the presence of pathologies, such as micro-haemorrhages, as well as for manual inspection during quality control stages of the analysis.

```
% Estimate R2s decay
R2s = arlo(TE(end:-1:1), abs(iField));
out_nii.img = R2s.*Mask;
save_nii(out_nii,[output_dir filesep 'R2s.nii.gz']);
```

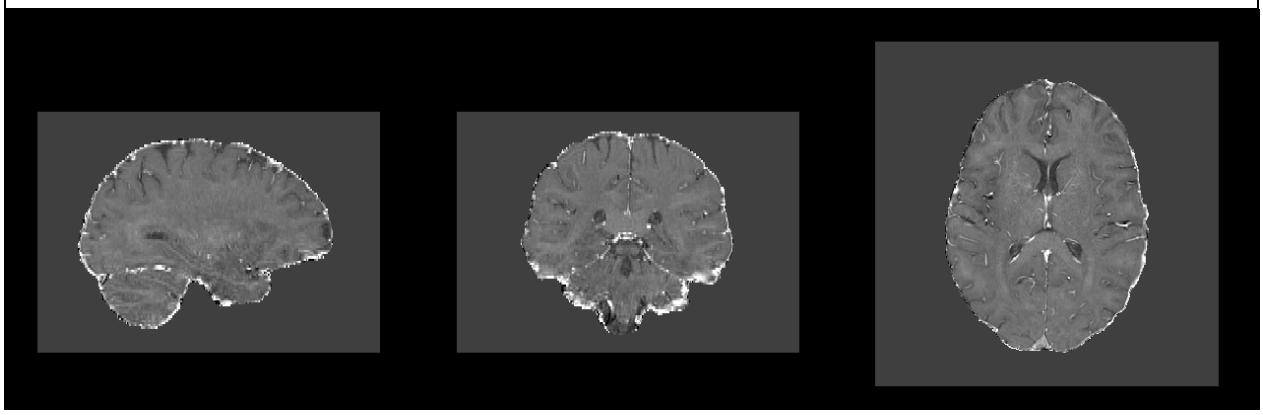


Figure 16. Code for calculating an estimate *R2* decay* and representative image of the resultant output.

IV. Tissue Mask

The brain mask, which provides a gross estimate of what is and is not brain tissue in the acquired MRI scan, may require a more conservative (smaller) estimate to ensure a volume of interest with sufficient quality for QSM processing. This process generates an output referred to as the *Tissue Mask* (see Figure 17). The importance of this step to optimise the QSM image is discussed in *Section 6.4.1* below.

```
% Generate Tissue Mask
Mask = imerode(Mask,ball(3));
out_nii.img = Mask;
save_nii(out_nii,[output_dir filesep 'Tissue_Mask.nii.gz']);
```

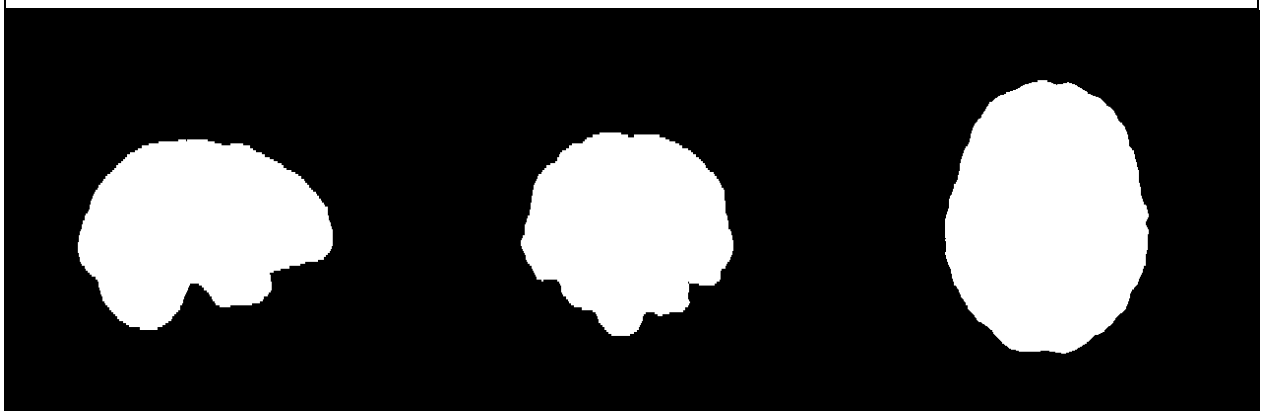


Figure 17. Code for calculating the *Tissue Mask* and representative image of the resultant output.

V. Raw Phase Image

A *Raw Phase Image* (Figure 18) is then calculated from the four echoes that are performed during the MRI scan sequence. *Raw phase images* are characterised by the presence of distinct black and white bands called '*wraps*', which represent slowly changing "macro"-susceptibility gradients that result from inhomogenieties in the magnetic field, as well as distortions occurring at the air and bone interfaces. The magnetic susceptibility differences from these features are orders of magnitude larger than the features of interest to us (e.g. small differences in tissue iron concentration, and overshadow them on the images).

```
% Calc Raw Phase from Echos
[iFreq_raw N_std] = Fit_ppm_complex(iField);
out_nii.img = iFreq_raw;
save_nii(out_nii,[output_dir filesep 'Raw_Phase.nii.gz']);
```

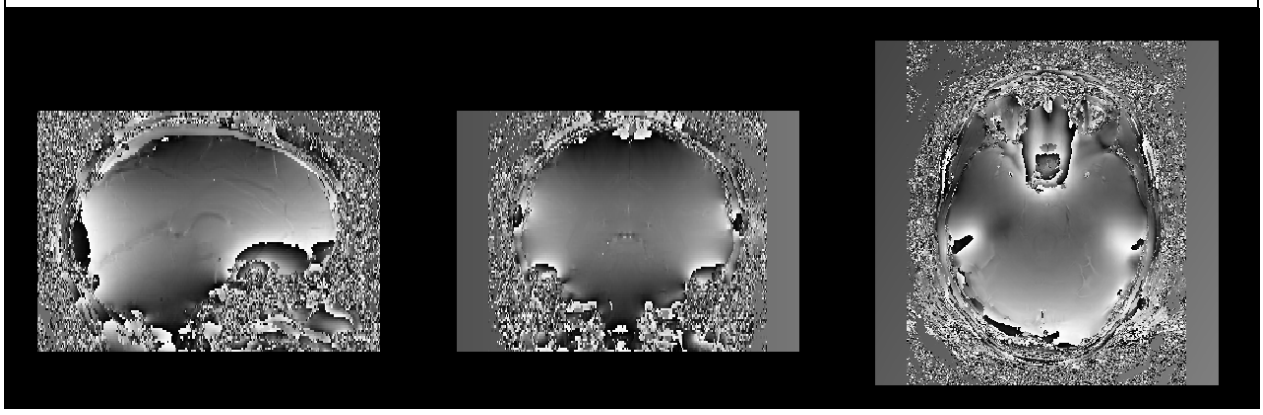


Figure 18. Code for calculating the Raw Phase Image and representative image of the resultant output. Note that wraps can be seen in areas of air-tissue interfaces, such as the sinuses and ear canals.

VI. Unwrapped phase image

It is critical to carefully model and remove the larger sources of magnetic field distortions in order to focus on the smaller ones of interest. To achieve this, the *raw phase image* must first undergo a process known as '*unwrapping*'. More specifically, this process involves removing ~2π aliasing of the phase data (i.e. the *wraps*) in order to obtain a more accurate estimate of the true magnetic field perturbation. A Laplacian-based approach has been implemented in the presented pipeline (Figure 19).

```
% Unwrap phase
iFreq = unwrapLaplacian(iFreq_raw, matrix_size, voxel_size);
out_nii.img = iFreq;
save_nii(out_nii,[output_dir filesep 'Unwrapped_Phase.nii.gz']);
```

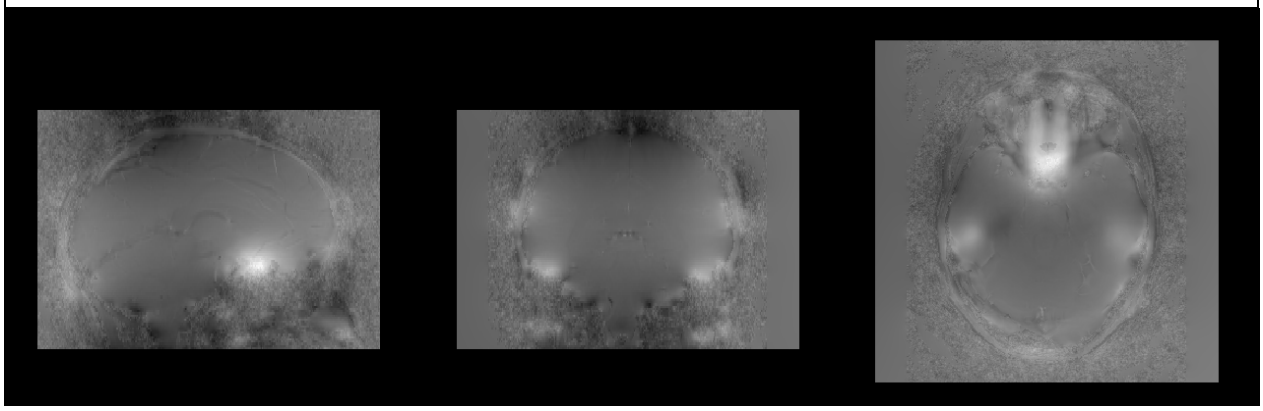


Figure 19. Code for unwrapping the Raw Phase Image and representative image of the resultant output.

VII. Background Field Removal

Lastly, the *Prep_Data* script sees the removal of background components of phase, which may arise from sources such as magnetic field inhomogeneity, and interfaces between air-tissue and air-bone. This process is necessary in order to extract the local magnetic field induced by local sources of susceptibility. To achieve this final step, background field removal is performed on the *unwrapped phase image* using the PDF approach (Figure 20).

```
% Background field removals using Projection onto Dipole Fields
RDF = PDF(iFreq, N_std, Mask, matrix_size, voxel_size, B0_dir);
out_nii.img = RDF;
save_nii(out_nii,[output_dir filesep 'Tissue_Phase.nii.gz']);
save([output_dir filesep 'RDF.mat'],'RDF','iFreq','iFreq_raw', ...
'iMag','N_std','Mask','matrix_size','voxel_size','delta_TE',...
'CF','B0_dir','R2s');
End
```

Figure 20. Code for performing Background Field Removal.
Note there is no output image associated with this step, however, a matrix is generated (not presented).

6.3.2 *Run_QSM* Script and Output

The second of the two scripts that constitute the pipeline, the *Run_QSM* script (detailed in Figure 21), produces a susceptibility map (QSM image), which is the subject of further analysis using additional neuroimaging analytical software. Notably, the script utilises the MEDI[814] approach, which incorporates anatomical information available from the *magnitude image* and implements L1 (least absolute error) regularization to complete the *Field-to-Susceptibility Inversion*. This step estimates the underlying susceptibility distribution from the background removed, unwrapped and masked phase image (i.e. determines the underlying magnetic susceptibility of brain tissue) in parts per million (ppm).

The problem of field-to-susceptibility inversion is considered ill-posed (i.e. there may be multiple plausible solutions). A further challenge to solving the ill-posed inversion is that all MRI images have an inherent amount of noise. Thus, the algorithm may produce an extravagant solution in an attempt to explain the noise (which is random in nature). The MEDI approach overcomes this by *i)* using the magnitude image to provide the general shape to the brain structure, and *ii)* a regularization parameter that encourages a 'smooth' solution to discourage over-fitting.

The L1 regularization parameter, lambda (λ), determines the smoothness (i.e. resolution) of the reconstructed susceptibility map[822] by adjusting data fidelity[823]. Lower values of λ place a strong constraint on the data fidelity (i.e. noise) but may not effectively supress streaking artifacts, and *vice versa*[823]. Thus, in adjusting the λ value, a compromise is made between data fidelity/noise amplification and streaking artifacts. The MEDI toolbox default value of λ=1000 has been implemented in the *Run_QSM* script. The MEDI approach was selected based on its relative popularity within the existing literature, and the available computing power.

```
%Run_QSM: Script to generate a QSM image

addpath(genpath());
folders={'Prep_Data_CREST_XXXX_iFieldCorrection'};

for i=1:numel(folders)
Prep_Data(strcat(folders{i}, filesep, 'CREST_XXXX'),strcat(folders{i},
filesep));
end

base_dir = pwd;
lambda=[1000];

for j=1:numel(lambda)
for i=1:numel(folders)

cd([base_dir filesep folders{i}])
QSM = MEDI_L1('lambda',lambda(j));
fprintf('Running QSM with L=%d in %s\n',lambda(j), pwd)
in_temp = load_nii('R2s.nii.gz');
out_nii = in_temp;
out_nii.img = QSM;
save_nii(out_nii,sprintf('QSM_L%d_iFC.nii.gz',lambda(j)));

end
end
```

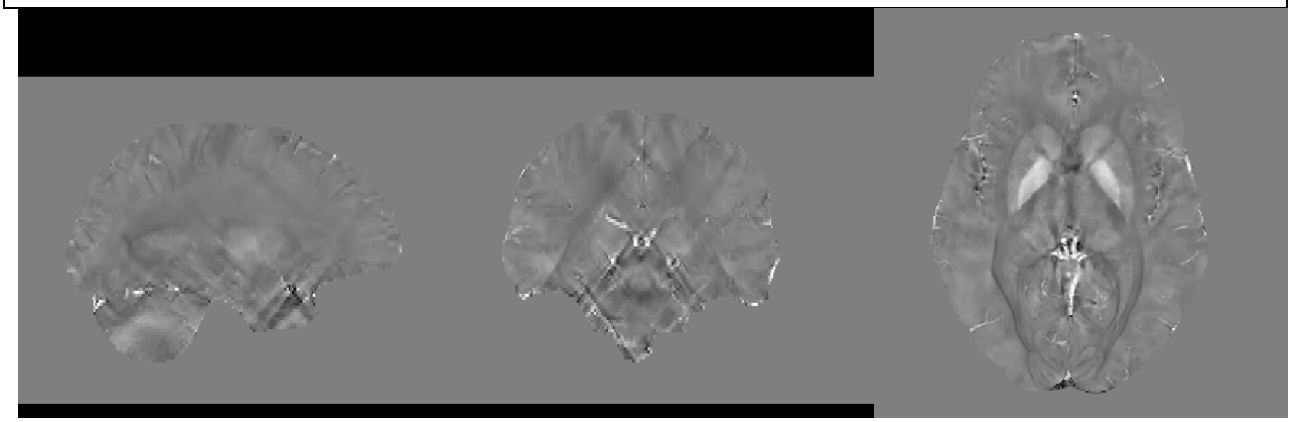


Figure 21. Code for constructing a QSM image and representative image of resultant output. *Note:* $\lambda = 1000$

6.4 Pipeline Optimisation and Troubleshooting

The following section details actions taken to optimise the presented pipeline and to remove an artifact that was resulting in the construction of aberrant QSM images.

6.4.1 Pipeline Optimisation

Mask erosion

Signal masking (i.e. the creation of *brain/tissue masks*) is a critical, but often neglected step, in the QSM literature[817]. As previously mentioned, signal masks are required to delineate brain tissue from the surrounding skull. It is important that the region demarcated contains reliable phase signal

since the inclusion of unreliable voxels can significantly influence the quality of subsequent susceptibility estimation. Suboptimal signal masking can introduce errors in background field estimation and mathematical instabilities in the inversion process. This in turn can lead to noise amplification, erroneous susceptibility values, as well as *streaking* and *surface artifacts* appearing throughout the reconstructed QSM image, especially near areas of large susceptibility gradients (e.g. veins), and air- and bone-tissue inferfaces[817,824,825].

Streaking and *surface artifacts*, presenting as light coloured streaking bands and a black and white specked rim around the edge of the brain in the QSM images, respectively, were removed from generated susceptibility maps *via* a process referred to as *mask erosion* (Figure 22). This process involved implementing a function in MATLAB ('ball') that creates a sphere of a designated radius and using it to remove the corresponding number of voxels from the *tissue mask* generated in the *Prep_Data* script. For example, a *ball size* of 3 removes all voxels within 3 voxels of the edge of the *tissue mask*. Subsequent use of the modified *tissue mask* in the QSM pipeline results in the removal of the equivalent number of voxels from the edge of the brain in the reconstructed susceptibility map. Given that this approach effectively results in the removal of data from the surface of the brain, which may be of potential interest to the researcher, a number of iterations were investigated to ensure no more than necessary was removed (see Figure 23). With this caveat in mind, it was ultimately determined that a *ball size* of 3 produced a QSM image of sufficient quality and would be implemented in the *CREST* QSM pipeline.

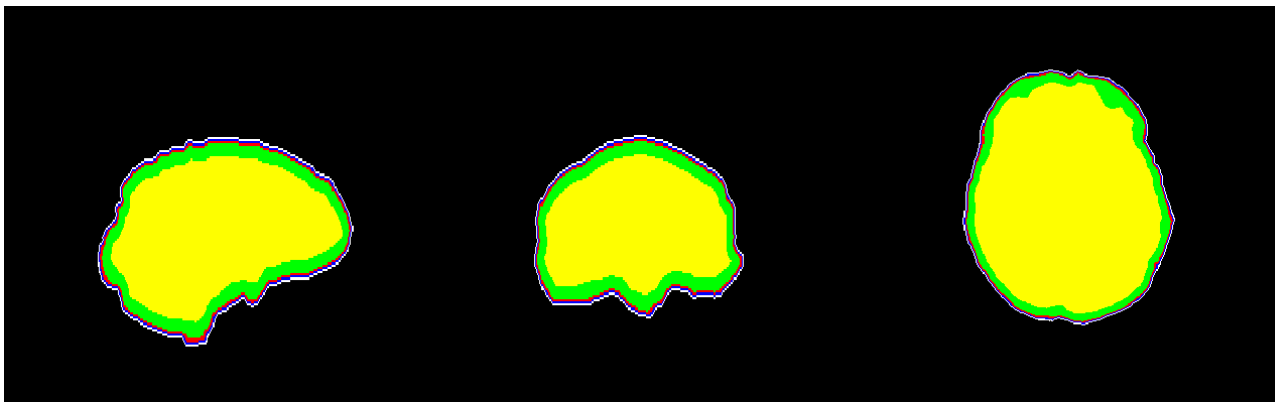


Figure 22. Representative image of generated *Tissue masks* that have been eroded using the *Ball* function. *Note:* White: original *tissue mask*; Blue: *tissue mask* eroded with *Ball size* 1; Red: *tissue mask* eroded with *Ball size* 2; Green: *tissue mask* eroded with *Ball size* 3; Yellow: *tissue mask* eroded with *Ball size* 9.

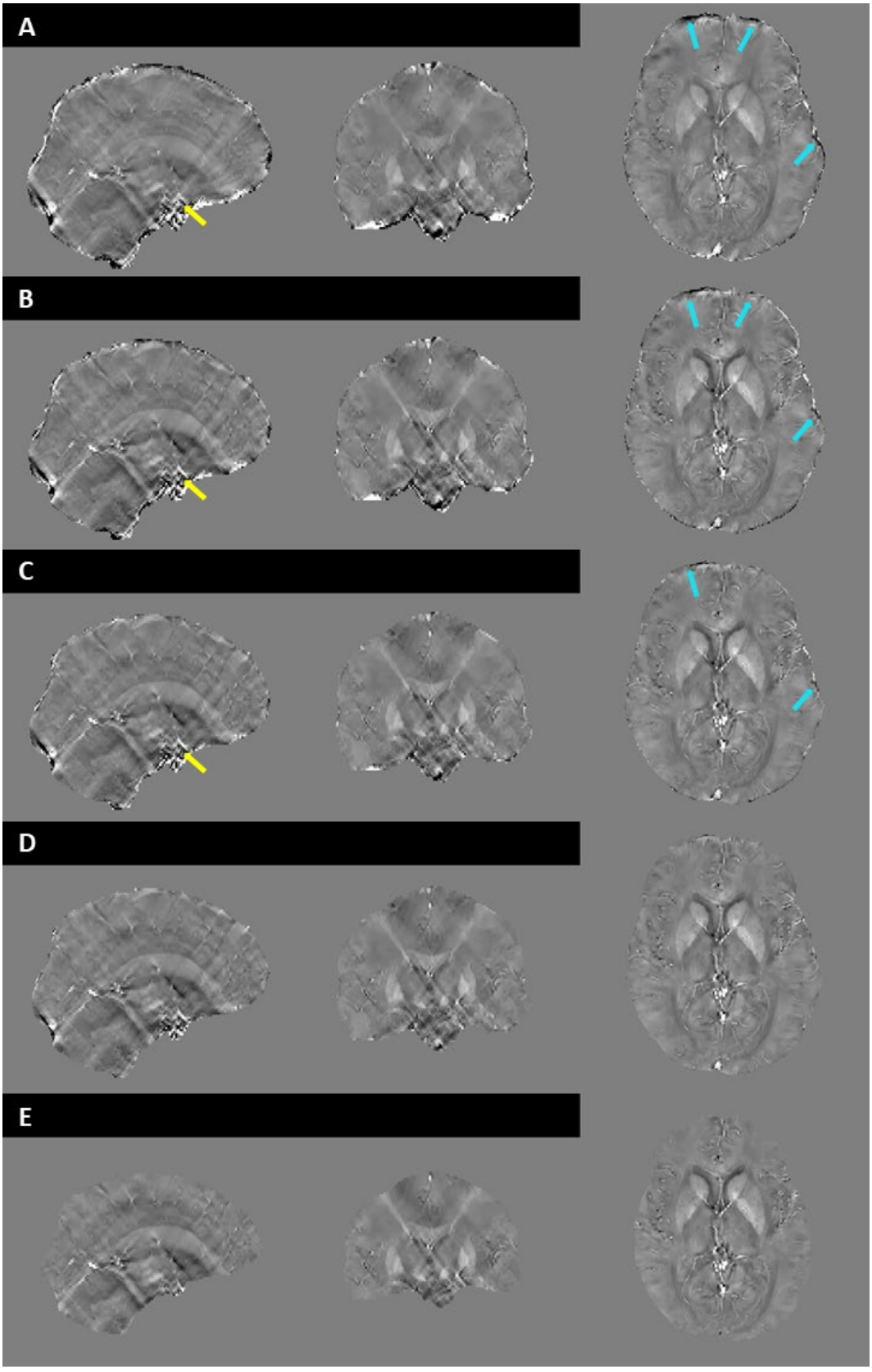


Figure 23. Representative QSM images that have been generated using a selection of eroded *tissue masks*. Use of eroded *tissue masks* can help eliminate streaking artifacts (yellow arrows) and surface artifacts (blue arrows). Given that this process effectively involves the removal of data from the image, it is important to keep this caveat in mind when deciding on the aggressiveness of the erosion applied. *Note: A*: Original QSM image; *B:* QSM image generated with eroded *tissue mask Ball size* 1; *C:* QSM image generated with eroded *tissue mask Ball size* 2; *D:* QSM image generated with eroded *tissue mask Ball size* 3; *E:* QSM image generated with eroded *tissue mask Ball size* 9. λ = 1000 for all QSM images presented.

6.4.2 Troubleshooting

In generating QSM images using the pipeline described, an error was encountered in $n = 5$ scans. As can be seen in Figure 24 below, said QSM images featured a prominent artifact that appeared as vertical and horizontal lines in the sagittal and axial planes, respectively.

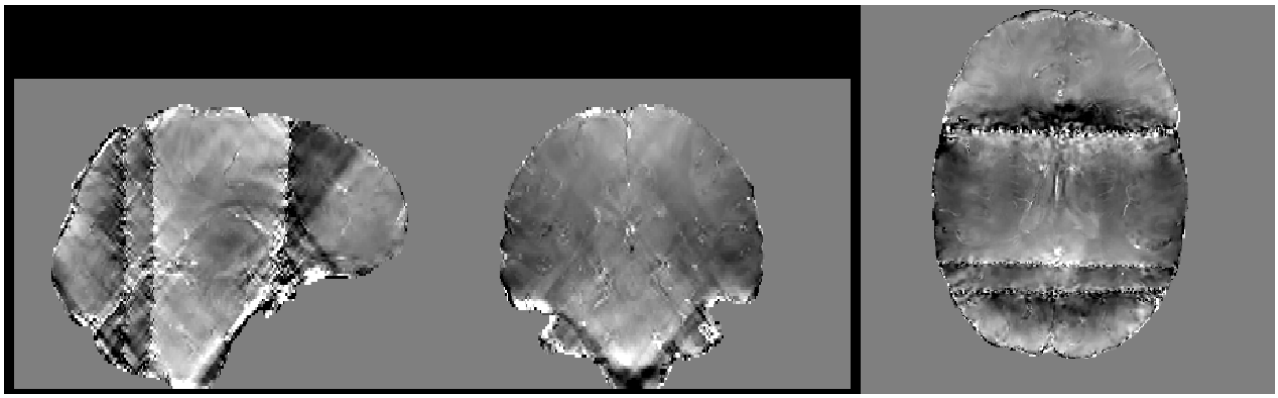


Figure 24. Representative QSM image generated for *CREST 0065* showing artefact. *Note:* $\lambda = 1000$.

It was apparent that these observations indicated the presence of an artefact as *i)* the placement of the observable lines did not correspond to any underlying biological structure or pathology and *ii)* the placement of the lines is meaningful for the SENSitivity Encoding (SENSE) parallel imaging algorithm that is used by Phillips MRI scanners for digital signal processing. Investigation into the other outputs generated by the pipeline revealed that the aberrant lines were not present in the *magnitude* or *R2* decay* images, but could be observed in the *raw* and *unwrapped phase images* (see Figures 25-28 below).

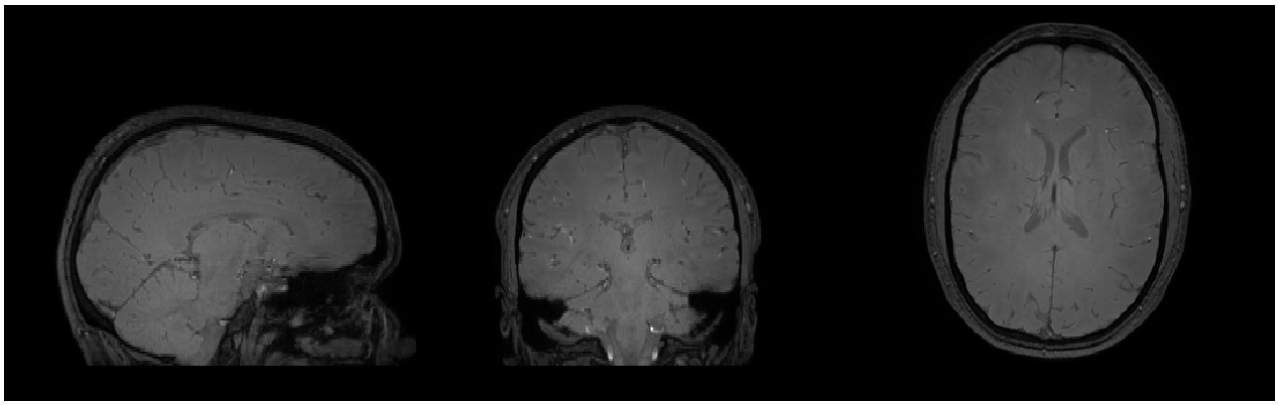


Figure 25. Representative *magnitude image* for *CREST 0065*.

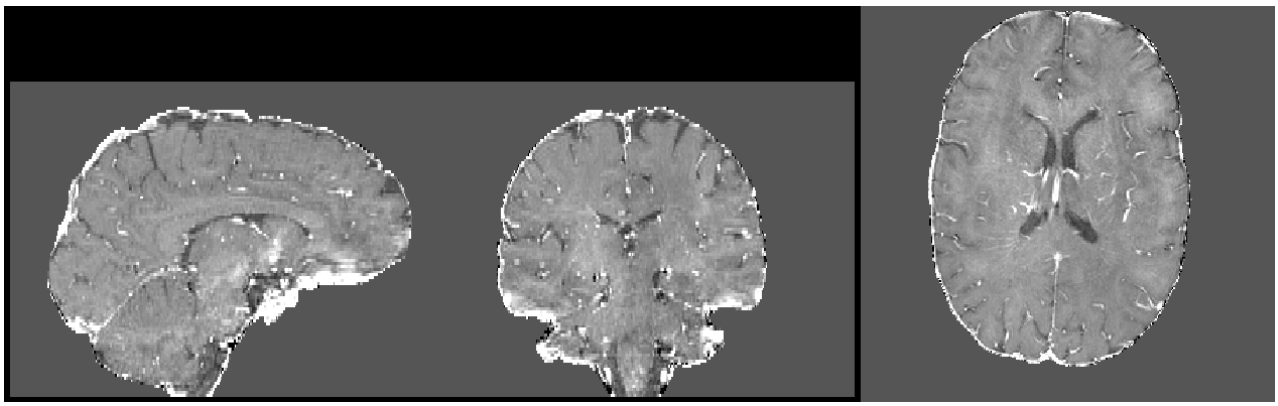


Figure 26. Representative *R2* decay* image for *CREST 0065.*

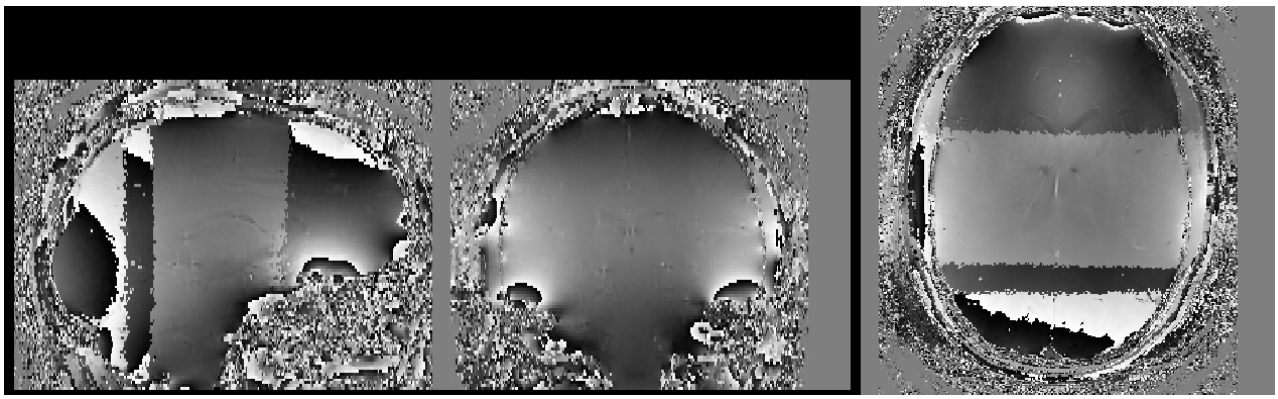


Figure 27. Representative *raw phase* image for *CREST 0065*.

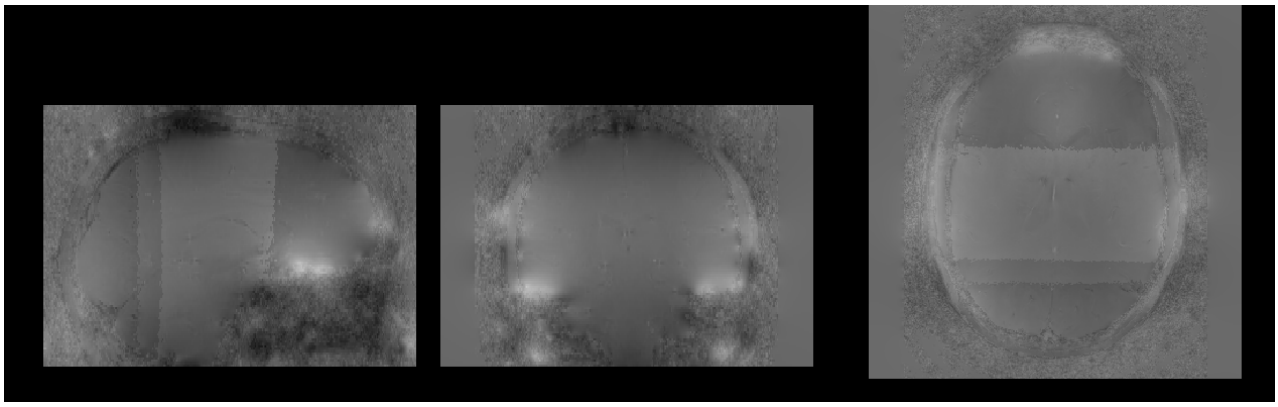


Figure 28. Representative *unwrapped phase* image for *CREST 0065*.

Correspondence with the developers of the MEDI toolbox revealed that this particular artefact is typical to Philips MRI scanners and reflected an error that occurs during the reconstruction of data at the MRI scanner. More specifically, this error results in the phases of individual echoes being adjusted, which result in deviations from linear phase evolution and creates the observable artefact. To compensate for this error and remove the associated artefact, an

additional piece of code corresponding to the *iField Correction* function was sourced from the MEDI toolbox and added to the *Prep_Data* script. The additional function identified, modelled, and removed the source of these artifacts.

```
% Remove Philips Scanner Phase Artefact using iField Correction
[iField] = iField_correction(iField,voxel_size);
```

Figure 29. Code corresponding to the iField Correction function. Following correspondence to the developers of the MEDI Toolbox, this piece of code was added to the *Prep_Data* script of the QSM pipeline as an additional pre-processing step in order to remove the artefact.

The inclusion of this additional pre-processing step resulted in the successful removal of the artefact from all $n = 5$ affected scans and has been permanently integrated into the pipeline.

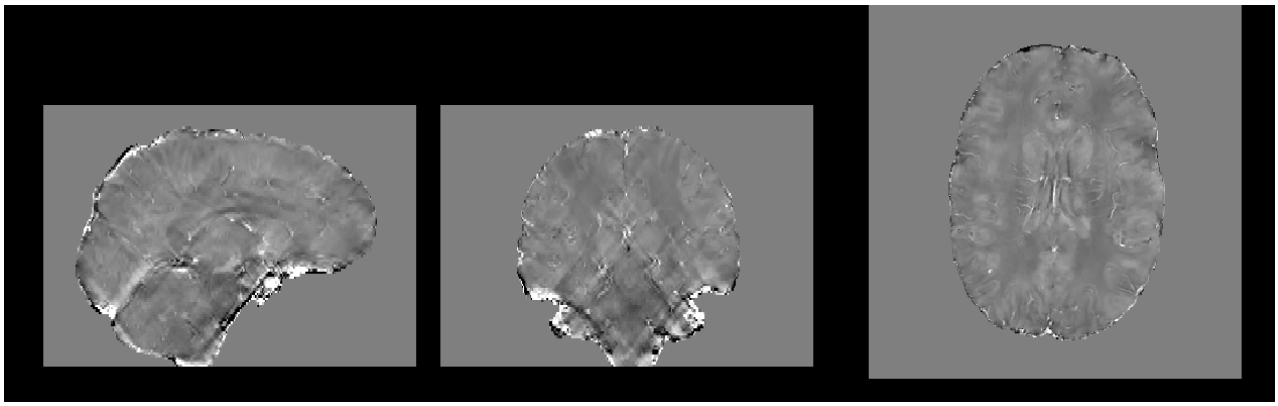


Figure 30. QSM image generated with the inclusion of the iField Correction code in the pipeline for *CREST 0065*. *Note:* No tissue erosion has been performed in this instance. $\lambda = 1000$.

6.5 Example Analysis

Background

Forming the largest part of the diencephalon, the thalami (*sing.* thalamus) are large, bilaterally symmetrical ovoid-shaped GM nuclear complexes that are located deep within the central region of the brain[826,827]. The thalami serve as relay stations for multiple cortical pathways, playing a critical role in a number of fundamental aspects of brain function, including the *i)* integration, processing, and cognition of sensory information; *ii)* regulation of consciousness, specifically arousal, attention and awareness, and *iii)* modulation of motor systems[827].

Within the context of mTBI, the thalami have been identified as important injury sites[828]. Advanced neuroimaging studies have observed changes in thalamic activity, connectivity[829–831], metabolism[832], and perfusion[742,833], as well as microstructural injury[834,835] and decreased tissue

volume[836] following mTBI. Thalamic damage/changes have also been associated with a number of post-mTBI sequelae, including cognitive deficits[830,837–839], fatigue[830,840], headache[830], and hyperesthesia[841], as well as quality of life[842]. Furthermore, investigations into the biomechanical determinants of mTBI have found axons within the thalamus and thalamo-cortical circuitry to be particularly susceptible to deformation by sheer forces that accompany mTBI[843].

Investigations into alterations in thalamic tissue magnetic susceptibility are currently extremely limited across all post-injury stages of mTBI, and reported findings have been mixed[844–848]. However, results reported by Schweser et al., (2017)[847] have shed light on an interesting hypothesis regarding the accumulation of calcium within the thalami at the chronic stages of mTBI, which may be related to the activation of NMDA receptors following injury within this brain structure[60]. In this preliminary investigation, Schweser and colleagues used QSM to ascertain the presence of calcium deposits amongst a cohort of middle-aged, male retired professional contact and non-contact sport athletes, and healthy controls without a history of playing contact sports. An important note is that the authors did not specify how history of concussion was established, if at all, amongst the participants. Micro-calcifications were found within the thalami of 23% of contact sport athletes and 10% of non-contact sport athletes, while none were observed in healthy controls. According to the authors, these findings suggest that deposits of calcium (particularly diffuse, microscopic distributions of calcium) may accumulate in the thalamus following mTBI and persist over time.

Given these findings and the known vulnerability of the thalami to the effects of mTBI, the QSM pipeline described above was utilised to investigate tissue magnetic susceptibility differences in the thalamus amongst individuals with and without a self-reported history of previous mTBI using available data collected as part of *CREST*. More specifically, it was hypothesised that thalamic tissue magnetic susceptibility would be lower amongst individuals with a self-reported history of mTBI relative to individuals who had not previously experienced a mTBI due to the potential presence of diamagnetic calcium deposits.

Methods

CREST Study Design and Participant Recruitment

A detailed description of the *CREST* study design and participant recruitment pathways is presented in Chapter 4 of this thesis. Briefly, *CREST* is recruiting adult participants who have been diagnosed with mTBI by a medical professional within 7 days of their injury. The study comprises of two parts; *Phase I*, which consists of a semi-structured telephone interview conducted within 7 days of injury and *Phase II*, which involves an in-person visit to the *CREST* Research Hub where

participants complete a comprehensive battery of tests, providing outcome measures that include MRI. *Phase II* is also conducted within 7 days of injury; however, the MRI component may be performed up to 9 days following the date of injury due to limitations in scanner availability.

Participants

Relevant demographic and MRI data collected from a total of n = 17 (Female n = 8) participants that were enrolled into *CREST* between August 2019 and early May 2021 were used for the purposes of this example analysis. Amongst this sample of *CREST* participants, n = 11 (Female n = 3) individuals reported a history of previous mTBI, of which n = 2 individuals (both males) experienced a mTBI less than one year prior to their enrolment into the study (i.e. they experienced a previous mTBI shortly before they experienced the mTBI for which they were enrolled into *CREST*). Further details regarding the study sample and history of previous mTBI are reported in Table 1 below.

Table 1. Descriptive statistics for the sample of *CREST* participants for which data was available and used for the purposes of this *Example Analysis*.

	Mean	Standard Deviation	Median	IQR	Mode	Range
Sample Characteristics (n = 17)						
Participant Age *(years)*	38.30	13.04	35.80	20.45	-	20 - 65
∆ Date of mTBI and *CREST* enrolment *(days)*	3.69	1.79	3.88	3.11	2 (n = 5)	1 - 7
∆ *CREST* Enrolment and MRI acquisition *(days)*	3.94	1.35	4	1.50	4 (n = 6)	2 - 5
∆ Date of mTBI and MRI acquisition *(days)*	7.71	1.90	8	2.50	9 (n = 5)	4 -10
History of previous mTBI (n = 11)						
Number of previous mTBI	3	3.09	2	2	1 (n = 5)	1 - 10
Most recent mTBI *(year)*	-	-	-	-	-	1979 - 2020
Time elapsed since most recent mTBI and enrolment into *CREST (years)*	10.18	12.31	6	12	0 (n = 2)* 10 (n = 2)	0 - 40

Note: Time elapsed between individual instances of mTBI for the n = 2 individuals with 0 years between most recent mTBI and enrolment into CREST was 39 and 80 days, respectively. Abbreviations:* ∆: Difference between; IQR: Interquartile range.

MRI Data Acquisition

As is detailed in Chapter 4, *CREST* is collecting MRI data using a number of standardised sequences performed on a 3 Tesla Philips Ingenia Multi Transmit Wide Bore Scanner (Philips Healthcare, Best, The Netherlands) equipped with a 32- channel head coil. Specifications of MRI acquisition parameters are also detailed in Chapter 4. Only QSM and T_1-weighted imaging data have been utilised for the purposes of this example analysis.

Image Analysis

Following MRI data acquisition, raw DICOM data were collected off the MRI scanner and converted to NIfTI format using MRIcroGL v1.2.20190902 (https://www.nitrc.org/projects/mricrogl/) software. More specifically, default parameters were applied, and the Brain Imaging Data Structure (BIDS) sidecar option was selected in order to save the .JSON meta-data necessary for the QSM analysis pipeline. QSM images were generated using the pipeline described above using MATLAB R2019a on Apple macOS Sierra. T_1-weighted images were automatically segmented into cortical and subcortical structure binary masks using FreeSurfer image analysis software (http://surfer.nmr.mgh.harvard.edu/) on Linux (Ubuntu 18.04 Bionic Beaver distribution).

In order to extract the mean tissue magnetic susceptibility from the generated QSM images for the thalami of each individual, a three stage registration process was performed using FMRIB Linear Image Registration Tool (FLIRT[849,850]) to register QSM and FreeSurfer outputs into the same space. To achieve this, the T_2* magnitude images for each participant were first registered to their respective T_1- weighted image *via* FLIRT with trilinear interpretation, 6 degrees of freedom, and mutual information cost function. Registration to the magnitude image was performed due to its superior detail for subcortical structures. Secondly, each participant's QSM image was registered to its respective magnitude image by applying the output matrix from the previous step, thereby transforming the QSM image into T_1 space. Lastly, each participant's FreeSurfer segmentation output was registered to their respective T_1-weighted image, thereby resulting in QSM and FreeSurfer outputs being in common space.

To perform a region of interest analysis, FSLmaths was first used to apply a threshold to the FreeSurfer segmentation output in order to create binary masks for the bilateral thalamus (Figure 31), and the mean tissue magnetic susceptibility value (ppm) within the left and right thalamus was subsequently computed for each participant using FSLstats. Due to the small sample size, it was not possible to conduct analysis at the level of individual thalamic nuclei.

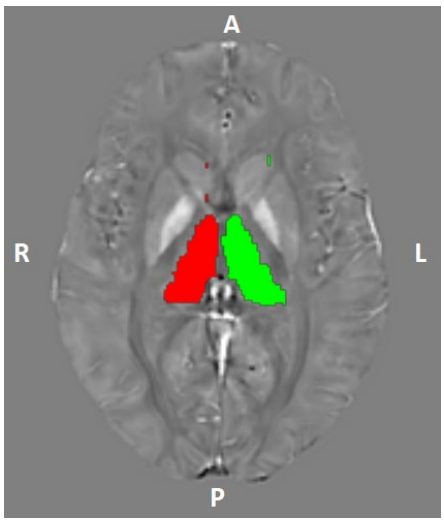


Figure 31. Binary masks generated using FreeSurfer segmentation output and FSLmaths for the left (green) and right (red) thalamus overlayed on a QSM image that has been registered to T_1 space. *Note:* Image is presented in radiological view. Choice of colours used to depict binary masks is arbitrary. Susceptibility map created using tissue erosion *Ball Size* 3 and $\lambda = 1000$. *Abbreviations*: A: anterior; L: left; P: posterior; R: right.

Statistical Analyses

To examine differences in the mean tissue magnetic susceptibility for the left and right thalamus between individuals with and without a self-reported history of previous mTBI, two 2-tailed independent samples *t*-tests were conducted, and Hedge's *g* correction was calculated as a measure of effect size. Statistical analysis was conducted using IBM SPSS Statistics software v27 (Armonk, NY: USA) and $p < 0.05$ was used to indicate statistical significance. Data were graphed using GraphPad Prism software v7 (San Diego, CA: USA).

Results

Registration could not be successfully performed for two individuals (one from each group) due to pre-existing anatomical abnormalities that were unrelated to the mTBI for which they were enrolled into *CREST*. As such, these participants were excluded from the analysis.

Prior to conducting the statistical analysis, data were also screened for extreme outliers according to *Tukey's Outlier Detection Method*, in which data values are considered to be extreme when equaling or exceeding 3 times the inter-quartile range (3xIQR) below the first quartile or above the third quartile. Only one value met these criteria and was subsequently removed from analysis. As such, statistical analysis was performed on a sample size consisting of $n = 9$ (Female $n = 2$) participants with a self-reported history of previous mTBI and $n = 5$ (Female $n = 4$) individuals

without a self-reported a history of previous mTBI. Characteristics of both groups are presented in Table 2 below. The assumption of normally distributed data was evaluated by examining the skew and kurtosis of the data. Results indicated that this assumption was satisfied according to the cut-offs of |2| and |9|[851]. Furthermore, the assumption of homogeneity of variances between groups was examined using Levene's *F* test and was found to be satisfied for both the left (F (*1,12*) = .015, p = .904) and right thalamus (F (*1,12*) = .596, p = .455).

Between-group differences for categorical and continuous demographic and study enrolment variables were examined using Fisher's exact test and a series of 2-tailed *t*-tests, respectively. Results of the analysis did not indicate any statistically significant differences between groups for the variables of sex (*Fisher's exact test (2-sided)* = .091), age (t (*12*) = - .336, p = .742) and number of days elapsed between enrolment into *CREST* and MRI acquisition (t (*12*) = .109, p = .915). A statistically significant difference was observed between groups for the number of days elapsed between participants' date of mTBI and enrolment into *CREST* (t (*12*) = -3.243, p = .007), as well as the number of days elapsed between participants' date of mTBI and MRI acquisition (t (*12*) = -2.675, p = .020).

Table 2. Age, study enrolment characteristics and history of previous mTBI for the sample of *CREST* participants (Total *n* = 14) examined for the purposes of this *Example Analysis*, according to self-reported history of mTBI grouping.

	Mean	Standard Deviation	Median	IQR	Mode	Range
No history of previous mTBI (*n* = 5)						
Participant Age *(years)*	32.82	9.51	35.80	17.25	-	20 - 45
Δ Date of mTBI and *CREST* enrolment *(days)*	1.93	0.76	1.86	1.42	5 (*n* = 2)	2 - 7
Δ Date of mTBI and MRI acquisition *(days)*	6.20	2.39	5	4	9 (*n* = 2)	4 - 10
Δ *CREST* Enrolment and MRI acquisition *(days)*	4.20	2.28	4	3.50	4 (*n* = 2)	2 - 5
History of previous mTBI (*n* = 9)						
Participant Age *(years)*	34.67	10.01	34.70	16.90	-	20 - 51
Δ Date of mTBI and *CREST* enrolment *(days)*	4.45	1.62	4.77	2.07	2 (*n* = 4)	2 - 7
Δ Date of mTBI and MRI acquisition *(days)*	8.67	1.12	9	2	7 (*n* = 3)	7 - 10
Δ *CREST* Enrolment and MRI acquisition *(days)*	4.11	.78	4	1.50	3 (*n* = 3) 4 (*n* = 3)	3 - 5
Number of previous mTBI	3.22	3.38	2	5	1 (*n* = 5)	1 - 10
Most recent mTBI *(year)*	-	-	-	-	-	1979 - 2020
Time elapsed since most recent mTBI and enrolment into *CREST (years)*	11.44	13.38	10	18.50	0 (*n* = 2)* 10 (*n* = 2)	0 - 40

*Note: * Time elapsed between individual instances of mTBI for the n = 2 individuals with 0 years between most recent mTBI and enrolment into CREST was 39 and 80 days, respectively. Abbreviations:* Δ: Difference between. *IQR*: Interquartile range.

Descriptive statistics for the mean tissue magnetic susceptibility of the left and right thalamus for individuals with and without a self-reported history of previous mTBI are presented in Table 3 below.

Table 3. Descriptive statistics for the mean tissue magnetic susceptibility within the bilateral thalamus for individuals with and without a self-reported history of previous mTBI.

	N	Minimum (ppm)	Maximum (ppm)	Mean (ppm)	Standard Deviation	Skewness	Kurtosis
No history of mTBI							
Left Thalamus	5	.008	.020	.014	.004	.282	- .536
Right Thalamus	5	.011	.028	.021	.007	- .304	-1.801
History of previous mTBI							
Left Thalamus	9	.007	.021	.013	.005	.366	-.537
Right Thalamus	9	.005	.028	.016	.009	.165	-1.471

Note: ppm = parts per million

Results of the independent samples *t*-test indicated no statistically significant between-group differences in the mean tissue magnetic susceptibility mTBI within the left (t (*12*) = - .331, p = .746; Hedge's g correction = .173) or right (t (*12*) = -1.074, p = .304; Hedge's g correction = .561) thalamus. Scatterplots depicting the mean tissue magnetic susceptibility of the left and right thalamus for individuals with and without a self-reported history of previous mTBI are presented in Figure 32 below.

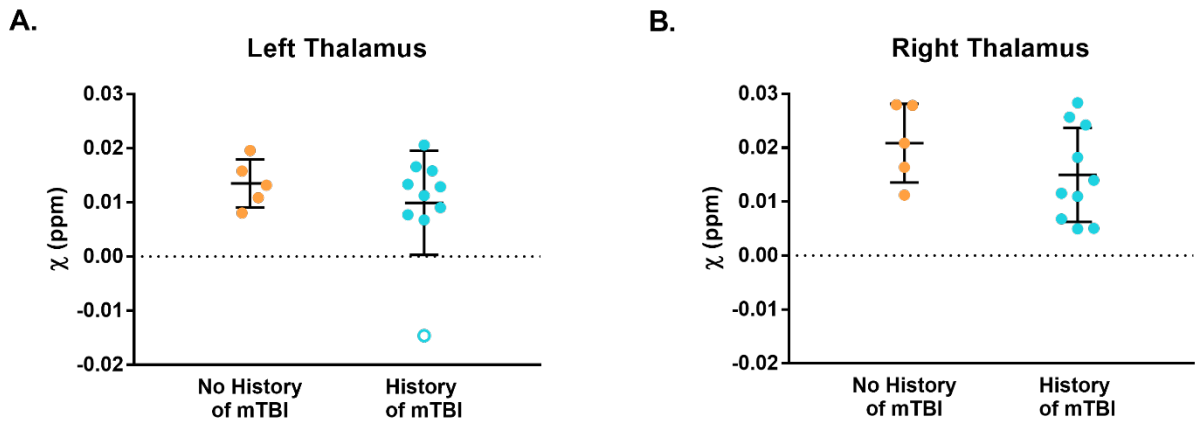


Figure 32. Scatterplots depicting the mean tissue magnetic susceptibility (χ) for the (A) left and (B) right thalamus for individuals with and without a self-reported history of previous mTBI. *Note*: mean and standard deviation shown. Outliers denoted using hollow circles and have been included for illustrative purposes only.

Discussion and Conclusion

The purpose of this example analysis was to demonstrate an application of the QSM pipeline described in this chapter using available data that had been collected as part of *CREST*. Specifically, the pipeline was used to generate susceptibility maps, for which differences in mean tissue magnetic susceptibility in the bilateral thalamus were examined between *CREST* participants with and without a self-reported history of previous mTBI.

Although statistically significant between-group differences were observed for the number of days between days of mTBI and enrolment into *CREST*, as well as MRI acquisition date, these are most likely to be sporadic findings associated with the small sample size and were not considered to bear any significant influence on the other analyses performed in this *Example Analysis*. However, keeping the former in mind, it would be interesting to investigate whether individuals with a history of previous mTBI present to hospital later relative to those without a history of previous mTBI once recruitment for *CREST* is completed.

No statistically significant between-group differences were observed in the mean tissue magnetic susceptibility for either the left or right thalamus. While this finding is perhaps not surprising given the small sample size, it does raise some interesting prospects that warrant further investigation.

For example, the lack of statistically significant differences observed may be related to the analysis having been conducted at a gross anatomical level. As was suggested in the narrative review presented in Chapter 5, it may be more appropriate to perform region of interest analyses at the level of specific nuclei or distinct subregions of anatomical structures of interest given that individual brain structures and their components may be differentially affected by mTBI. This suggestion is supported by the findings reported in the aforementioned study by Schweser and colleagues, in which it was noted that the lesions identified were predominantly located in the medial nuclear group of the thalamus, and similar reports of localized calcium- and iron-related pathological findings following injury have also been described in the preclinical mTBI[852]/TBI[853] literature. Although the ability to investigate mean tissue magnetic susceptibility differences within multiple individual thalamic nuclei in this example analysis was precluded by the limited sample size, investigations performed at the substructural level may be more sensitive to detecting differences in tissue magnetic susceptibility. It may be interesting to conduct future exploratory investigations in a single thalamic nucleus in the current dataset. With additional participants, it would also be interesting to examine whether the tissue magnetic susceptibility of certain thalamic

nuclei correlate with the reporting of specific mTBI-related symptoms and their severity. For example, one could investigate whether there is an association between the susceptibility of the anterior thalamic nuclei and reports of difficulty concentrating and/or performance on cognitive tests of attention. Alternatively, one could also conduct a similar investigation using the average tissue magnetic susceptibility of the right and left thalamus combined. This approach, albeit less sensitive, could reveal a more global level of impairment. Furthermore, different patterns of head injury (e.g. direct injury to the thalamus or afflictions secondary to cortical injury) are expected to differentially affect the thalamus, thus it may also be important to consider mechanism and/or site of injury when conducting such investigations.

While few details were provided by Schweser and colleagues, it was implied that the participants in their study had experienced (multiple) mTBI and were imaged at a chronic time point. In contrast, the majority of individuals with a history of previous mTBI in this sample of *CREST* participants reported having previously experienced only 1 mTBI, most of which had occurred within 10 years of the mTBI for which they were enrolled into the study. Thus, it may be that calcium deposits may only accumulate after a certain number of mTBIs and/or period of time since their occurrence. As such, in addition to controlling for the effects of age, sex, handedness and thalamic volume, future studies with larger sample sizes may also wish to consider investigating whether and how the number and time since previous mTBI/s potentially influence tissue magnetic susceptibility. Likewise, in cases of multiple mTBI, time between individual instances of mTBI may be of significance.

It is important to acknowledge that the interpretation of this investigation's findings are limited by the *CREST* study's research design, which does not involve the recruitment of healthy control participants. Future studies ought to include a healthy and/or non-mTBI orthopedic injury control group in order to help better understand whether, and the extent to which, mean tissue magnetic susceptibility may be altered by most recent mTBI.

In conclusion, this brief report has illustrated an example analysis in which the described QSM pipeline has been applied to investigate a novel hypothesis relating to the presence of persistent calcium deposits in the bilateral thalamus following multiple mTBI, using available data from *CREST*. Although the sample size was too limited to facilitate an in-depth examination of the hypothesis, the investigation has helped identify useful considerations for future studies.

Acknowledgements

I wish to acknowledge and sincerely thank Dr Phillip Ward for providing the original scripts used to generate the susceptibility maps and generously offering his expertise in the development of the described QSM pipeline, as well as Dr Sarah Hellewell for her assistance in scripting and performing the FreeSurfer segmentations for the sample of *CREST* participants examined in the *Example Analysis*. I also wish to thank Dr Michael Bynevelt and the staff at the Radiology Department at Sir Charles Gairdner Hospital for their assistance in data acquisition, as well as the participants who kindly volunteered their time to take part in the *CREST* research project.

6.6 Supplementary Analyses

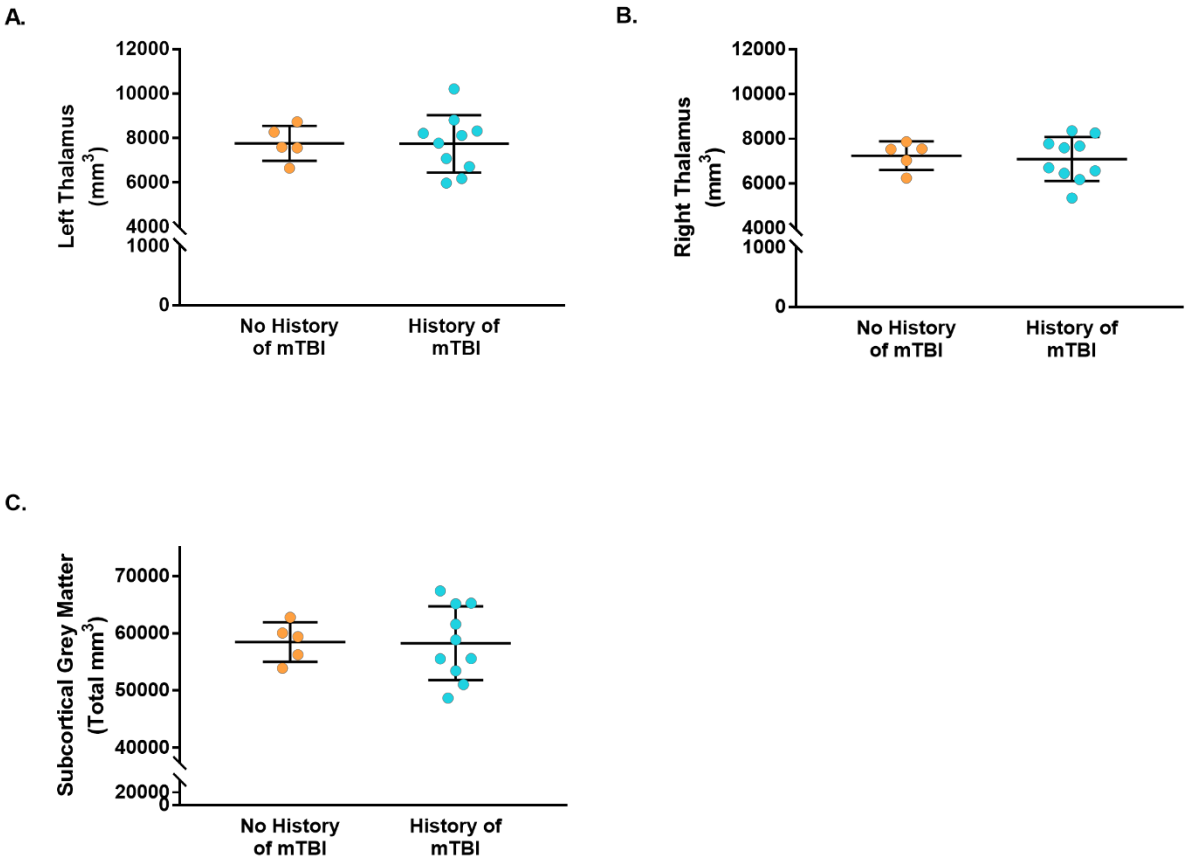
The following section of this thesis presents the results of additional supplementary analyses that were performed on data collected as part of the novel investigation presented in Chapter 6. These analyses compliment the hypothesis examined in the novel investigation.

Specifically, the following was investigated:

- Differences in the size of the left thalamus between individuals with and without a self-reported history of previous mTBI.
- Differences in the size of the right thalamus between individuals with and without a self-reported history of previous mTBI.
- Differences in the total amount of the subcortical grey matter between individuals with and without a self-reported history of previous mTBI.

Supplementary Table 6. Descriptive statistics for the left and right thalami size, as well as total subcortical grey matter for individuals with and without a self-reported history of previous mTBI.

	n	Mean (mm^3)	Standard Deviation	Median	Range	Skewness	Kurtosis	*p*	Test
Left Thalamus									
History of mTBI	10	7736.19	1290.13	7941.10	5977.40 – 10207.90	.392	.046	**.972**	***t-Test***
No History of mTBI	5	7758.70	790.83	7589.80	6648.40 – 8729.20	-.283	-.087		
Right Thalamus									
History of mTBI	10	7091.36	986.55	7150.20	5349 – 8358.50	-.324	-.871	.760	***t-Test***
No History of mTBI	5	7244.04	636.52	7532.20	6239.40 – 7866.70	-1.178	1.039		
Subcortical Grey Matter									
History of mTBI	10	58244.10	6441.12	57207.50	48663 - 67389	.036	-1.349	**.944**	***t-Test***
No History of mTBI	5	58467.60	3458.58	59392	53881 - 62766	-.235	-.875		



Supplementary Figure 6. Scatterplots depicting the size of the (**A**) left thalamus, (**B**) right thalamus and (**C**) total subcortical grey matter for individuals with ($n = 10$) and without ($n = 5$) a self-reported history of previous mTBI. Mean and standard deviation shown. Data were screened for outliers using *Tukey's Outlier Detection Method* prior to undertaking analyses. No outliers were identified.

7 Discussion and Conclusion

7.1 Summary of Findings within the Context of the Literature

mTBI is a complex injury with a multifaceted biological underpinning and heterogeneous clinical presentation. It is increasingly recognised that recovery following this type of injury is diverse and can be influenced by a number of neurobiopsychosocial factors[854]. Most adults who have sustained a mTBI are typically expected to recover within two weeks of injury. However, mounting evidence suggests that this estimate is not always the case. Furthermore, it is becoming apparent that mTBI may be associated with significant long-term effects on health beyond PPCS. Previous reports indicate associations between multiple mTBI and neurodegenerative conditions[24,45,47]. However, a recent study has also found individuals who have experienced mTBI to be at an elevated risk of developing chronic behavioural and health comorbidities, including depression, stroke, epilepsy, as well as risk factors that are associated with cardiovascular diseases such as hypertension and obesity[855]. Furthermore, mTBI has been associated with a number of substantial socioeconomic consequences, including failure to return to work, change in employment status, loss of personal income, difficulty carrying out work-related duties, and productivity decline[856–859], which further highlight the need for research in the area of predicting outcome following mTBI. Given the relative frequency with which mTBI occurs, it is thus imperative that individuals who are at risk of delayed recovery following injury are identified in a timely fashion if we are to help reduce the burden associated with PPCS and improve patient outcomes.

The overarching aim of this PhD thesis was to contribute to the exploration of factors that may be used to predict delayed recovery following mTBI. In reviewing the literature (Chapter 2), it was evident that a significant effort had already been made to identify predictors of PPCS. However, it also became apparent that no single factor is likely to have the capacity to predict PPCS at the level of the individual. Instead, it is anticipated that greater success will be achieved by using a multimodal "suite-based" approach, which incorporates several factors that draw upon the many facets of this injury. However, what factors should be included in such models? The research conducted as part of this PhD thesis endeavoured to shed some light on this exact question.

Commencing with the pilot study presented in Chapter 3, this PhD thesis set out to evaluate the predictive utility of a range of demographics, injury-related characteristics, blood-based biomarkers, neuropsychological and MRI outcome measures, and to identify those most promising for inclusion in future, large scale studies. This investigation found neuropsychological measures pertaining to immediate and delayed memory, attention and executive function, as measured by the *Repeatable Battery for the Assessment of Neuropsychological Status (RBANS®) Update* and the *Trail*

Making Test Form B, to be statistically significant predictors of PPCS, despite the study's limited sample size. These findings are in keeping with previous reports in the literature. More specifically, a positive association between the *Total Score* index of the *Repeatable Battery for the Assessment of Neuropsychological Status Update (RBANS®)* and return to duty has been observed amongst military personnel[860]. Similarly, performance on the two forms of the *Trail Making Test* has been found to predict better functional outcome amongst individuals attending community-based outpatient rehabilitation clinics[861,862]. Nevertheless, these findings of the pilot study are both novel and practical. They are novel since very few studies appear to have previously examined the prognostic capacity of the *Repeatable Battery for the Assessment of Neuropsychological Status (RBANS®) Update* within the context of mTBI recovery amongst community-dwelling individuals within Australia. They are also practical since both of the neuropsychological measures that were found to be predictive of PPCS are convenient to administer with the appropriate training and neuropsychology supervision, thereby facilitating future validation of the reported findings in a variety of clinical and research settings.

While none of the three blood-based biomarkers examined in the pilot study were found to be predictive of PPCS, consistent with findings previously reported in the literature, levels of GFAP were observed to be elevated amongst individuals with mTBI relative to age- and sex-matched healthy control individuals. More importantly, the study was able to contribute normative data for healthy control individuals, which were particularly scarce at the time of the pilot study's inception and initiation.

Logistical difficulties experienced culminated in a limited sample size for the MRI component of the pilot study, which unfortunately prevented the evaluation of the prognostic utility of diffusion imaging for predicting PPCS as was originally intended. However, it was possible to conduct a small-scale investigation comparing individuals with mTBI and healthy controls, exploring differences in diffusion metrics within brain structures known to be implicated in mTBI and the neuropsychological functions assessed by the study's abbreviated test battery. Relative to the age- and sex-matched control group, individuals with mTBI were observed to have a higher FA within the left inferior fronto-occipital fasciculus. A statistically significant association was also observed between the FA within this brain structure and performance on visuospatial-constructional tasks, which is consistent with previous investigations into the functioning of the inferior fronto-occipital fasciculus[863]. Acknowledging the inherent limitations associated with small sample sizes, the pilot study results indicated that neuropsychological tests have the potential to be valuable additions to multivariate models centred on predicting PPCS. Furthermore, the pilot study proved to be a worthwhile venture as it provided many valuable insights into the practicalities of

conducting this type of research on a larger scale, which were taken into careful consideration when conceptualising *CREST*- the research group's next step within this space.

CREST is a large, multi-institutional study currently being conducted within Western Australia, which aims to create a multivariate suite-based predictive model to identify individuals at risk of PPCS. Learning from the pilot study, *CREST* has adopted a broader recruitment strategy that involves hospital-based, GP/allied health and community-based pathways to facilitate greater community involvement in research and capture a wider representation of socio-demographic groups. Furthermore, *CREST* has a significantly expanded battery of outcome measures, which now includes the collection of more detailed demographic details, injury-related characteristics, and relevant aspects of medical history. The neuropsychological assessment battery used in *CREST* also features measures of personality alongside the tests of cognition and measures of mTBI symptomatology and emotional sequelae that were originally used in the pilot study. Blood samples continue to be collected in *CREST* and will be used to investigate a wider range of novel and established blood-based biomarkers. Similarly, neuroimaging has also been retained in *CREST* but has been significantly expanded to include a greater number of MRI sequences as well as qEEG. Lastly, *CREST* has also incorporated an exercise tolerance component, which has traditionally been conducted in adolescent athlete populations and is yet to be investigated within the broader community setting. The protocol for this study, which includes a detailed statistical analysis plan, constitutes Chapter 4 of this thesis. It is anticipated that *CREST*s comprehensive research design will help elucidate the circumstances under which mTBI occur and the experiences of individuals suffering from PPCS within the Western Australian context, as well as identify a suite of factors that can be assessed during the acute stages of injury and may be used to predict delayed recovery following mTBI.

Advanced neuroimaging is a rapidly developing field that has the potential to identify additional factors that may serve as prognostic markers for PPCS. As part of *CREST*, investigators are performing a number of MRI sequences to investigate precisely this, including a SWI sequence that will facilitate the use of a nascent neuroimaging analysis called QSM. Given the novel nature of this technique, this PhD thesis set out to investigate the ways in which QSM had been used more broadly within the context of TBI and to ascertain its potential for use as a prognostic factor. In addition to this, it also endeavoured to summarise relevant aspects of the theoretical underpinnings of this technique in an approachable manner that bridges the gap between biology and neuroimaging fields.

Results of this investigation found that only a handful of QSM studies had been conducted to date, most of which were conducted in mTBI populations, and identified a number of emerging applications in which QSM had been used to better understand iron-related pathophysiological

underpinnings and sequelae of mTBI/TBI. More specifically, the review found that QSM had been used to investigate changes in tissue and venous magnetic susceptibility and alterations in venous structure, as well as identify the presence of microhaemorrhages and calcifications following mTBI/TBI. Furthermore, the review indicated that there is scope for QSM to be used in a prognostic capacity, although with only two studies of this nature having been conducted to date, further research is clearly required in this space. To our knowledge, this investigation was the very first of its kind and resulted in the published review that constitutes Chapter 5 of this thesis.

This PhD thesis originally intended to investigate the predictive utility of QSM using MRI data collected as part of the *CREST* research project. However, these plans were precluded by the ongoing *COVID-19* pandemic, which significantly impacted participant recruitment and select operations of the study. As an alternative, a detailed exposition of the analysis pipeline, which will be used to generate and analyse QSM data being collected for *CREST*, has been presented in Chapter 6 of this thesis. The narrative format and level of detail presented is necessary given the formative stage of this work and the emerging nature of this technology. In addition to this, Chapter 6 also features an example analysis in which the described pipeline was utilised as a proof-of-concept. Using available data from *CREST*, the example analysis comprises a novel investigation to examine the hypothesis that prior mTBI may be associated with alterations in the magnetic susceptibility within the bilateral thalamus. This brain structure was investigated as it is known to be particularly vulnerable to the biomechanical forces of mTBI and has been implicated in the sequelae that accompany injury. Results of this investigation did not identify a statistically significant difference in mean magnetic tissue susceptibility between individuals with and without a self-reported history of previous mTBI. Although this finding is not altogether surprising considering the limited size of the sample examined, the exercise was nonetheless able to successfully demonstrate an application of the developed pipeline. It also provided interesting insights that ought to be considered in future large-scale studies in which this hypothesis may be revisited.

There is no doubt that extraordinary circumstances limited the extent to which this PhD thesis could answer the proposed question regarding what factors ought to feature in multivariate models aiming to predict PPCS. However, the work undertaken has demonstrated the prognostic value of neuropsychological tests and identified that QSM has the potential to deepen our understanding of the pathobiological underpinnings of mTBI/TBI, which in turn may identify additional biomarkers that may be used to prognosticate delayed recovery following mTBI. Furthermore, the *CREST* research project will enrich this field of research by providing novel insight into the circumstances under which mTBI occur within Western Australia and facilitating the creation of a multivariate model to predict PPCS.

7.2 Limitations

Limitations pertaining to the research undertaken as part of this PhD project have been discussed throughout this thesis, however, there are a few limitations that are worthy of being examined in further detail.

7.2.1 Limited Sample Size

The limited sample size is a manifest but unfortunate limitation to the work presented in this PhD thesis, and is especially relevant to the original research presented in Chapters 3 and 6. As previously discussed, a combination of factors contributed to the limited sample sizes of the studies presented in the aforementioned chapters, which included logistical challenges, difficulty recruiting participants, general participant attrition, as well as the unique circumstances relating to the *COVID-19* pandemic. Consequently, neither of the investigations had sufficient power to reliably evaluate the predictive utility of the range of outcome measures surveyed, and/or detect between-group differences. Further research implementing larger sample sizes is clearly warranted, although it is acknowledged that participant recruitment to such studies can prove to be challenging.

The *CREST* research project described in Chapter 4 is attempting to overcome limitations associated with small sample sizes by implementing a heterogeneous recruitment strategy consisting of both hospital and community-based pathways to maximise participant enrolment into the study. This is in contrast to other injury registries and prognostic studies conducted within the mTBI/TBI fields, which typically recruit participants from a single source or demographic cohort[436,864–871]. In using such an approach, it is anticipated that *CREST* will generate a comprehensive data set (specifically, n = 500 and n = 120 individuals for *Phase I* and *Phase II*, respectively) and facilitate future validation studies. Despite this, it is recognised that *CREST* may not be adequately powered to evaluate the prognostic utility of the individual outcome measures being investigated in *Phase II* of the study. Given that, it will only be possible to include a select number of variables in the final multivariate model, and separate regression analyses will need to be performed for each component arm of the study in order to identify and include only those that are most promising.

7.2.2 Not all individuals seek medical attention for their mTBI

It is widely recognised that many individuals who sustain a mTBI do not seek medical care for their injury[29]. While there is currently a paucity of research surrounding individuals' motivations not to seek medical care following mTBI, possible reasons for this may include age, inability to access medical care, lack of awareness of mTBI signs and symptoms, perceived severity of mTBI signs and symptoms, mechanism of injury, and/or complacency[30]. This is a difficult factor to address, however,

it is an important one that needs to be acknowledged due to its significant implications on participant recruitment and generalisability of study findings[872]. Specifically, it results in sample selection biases that effectively render study findings applicable to a very limited subset of the population; individuals who seek medical attention for their injury and/or those willing and interested in participating in research. Reported findings may not generalise to individuals falling outside of this niche. This is especially true for populations that are known to be particularly vulnerable to experiencing mTBI but typically do not, or are unable, to seek medical attention for their injury due to other extraneous factors (e.g. victims of domestic or intimate partner violence, substance abuse problems, and homelessness). While it is not possible for *CREST* to overcome this limitation in its entirety, the study's research design and heterogeneous recruitment strategy is anticipated to help broaden the range of demographic groups (e.g. individuals residing in metropolitan and rural areas, general community members, athlete populations) and mechanisms of injury represented in the study sample (e.g. sports-related mTBI, falls, transport-related accidents, workplace injury, assault). This in turn may improve the generalisability of study findings.

7.2.3 The effect of preanalytical variables on blood-based biomarker data

The ability to obtain valid measures of many protein and other blood-based biomarkers is largely dependent on the quality of the sample being processed[873]. Sample quality has been estimated to account for as much as ~70% of the variability observed in blood-based biomarker analyses, yet this important aspect of biomarker analysis is often overlooked in study design and execution[558,874]. A multitude of variables pertaining to sample collection, processing and quality control have been found to have the potential to adversely affect sample quality[875], and therefore the accuracy, comparability, reliability, and reproducibility of the blood-based biomarker data being collected. It is thus essential that such preanalytical variables are considered and controlled for as much as practical, especially in light of the continued development and increased sensitivity of analytical platforms. Such ultra-high sensitive analysis techniques were used in the pilot study, namely the Quanterix Simoa™ platform, and likewise, *CREST* is intended to ascertain the prognostic utility of a broad range of blood-based biomarkers, using approaches including proteomics, metabolomics, phenomics, and microRNA analyses, which will also require the use of various advanced analytical platforms. While due care has been taken to consider and ensure consistency in said preanalytical variables, given the current lack of well-established uniform standards and quality control evaluation procedures[558,875] it is not possible to completely eliminate or account for the potential effects of preanalytical variables on the data collected for either of these studies.

7.2.4 The temporal release profiles of blood-based biomarkers

Following mTBI, blood-based biomarkers are not released in a uniform fashion; rather, each is associated with its own unique temporal release profile[557]. A number of variables can influence a biomarker's kinetic profile, including the biophysical properties of the marker (e.g. molecular weight, half-life in blood), whether a breach of the blood brain barrier is required for release of the marker into the bloodstream, as well as the anatomical and physiological characteristics of the individual (e.g. glomerular filtration rate, age, sex, and ethnicity)[876,877]. Given the dynamic nature of biomarker release, time of blood sample acquisition becomes an important factor that needs to be taken into consideration when selecting candidate biomarkers to investigate and interpreting data, since it can significantly influence the ability to detect candidate biomarkers. Indeed, variation in time elapsed between mTBI and blood sample acquisition was acknowledged as a potential limiting factor for the pilot study. As has been previously mentioned, the *CREST* research project endeavours to investigate a range of novel and well-established blood-based biomarkers for their ability to predict PPCS. To achieve this, blood samples are being collected from participants within 7 days of their mTBI as part of *Phase II* of the study. While it will be possible to control for variation in time elapsed between mTBI and blood sample acquisition from additional data being acquired as part of *CREST*, the study will nonetheless be limited to investigating only those analytes that are abundant and/or detectable within the days following injury.

7.2.5 QSM remains in a formative stage and its use is currently limited to research settings

QSM is an emerging neuroimaging analysis technique that has the potential to provide unique insights into the pathobiological underpinnings and consequences of TBI, including mTBI. However, due to its nascent nature, QSM remains a relatively resource intensive analytical technique in terms of both data acquisition and analysis, which largely limits its use to research settings. For example, although all major MRI vendors produce SWI sequences that are needed to generate QSM images, additional technical assistance may be required to set up and/or optimise MRI scanners in order to acquire data that will generate high quality susceptibility maps, which in itself can be costly and time-consuming. Furthermore, QSM data analysis requires a level of familiarity with QSM processing and theory, as well as expertise in neuroimaging analysis workflow and available software packages, for which there is currently no uniform standardised methodologies. This in turn can make it difficult to ascertain the comparability and reproducibility of QSM findings, including those that will arise from *CREST*. Nevertheless, QSM remains an active and rapidly evolving field of research. Efforts to optimise and automate the various steps of the QSM processing pipeline are continuously being pursued. Exciting advances have recently been

made with respect to the masking[817], unwrapping[801,804], and inversion[878–880] components. Initiatives to create unified, standardised yet extensible frameworks for QSM image generation and analysis are also underway[881]. Together, these developments will not only generate higher quality susceptibility maps but will also assist in making QSM more accessible to both researchers and clinicians, especially those who wish to utilise this advanced form of neuroimaging analysis but may have little experience with the technique.

7.3 Future Directions

As awareness of mTBI and the complexities of recovery continue to grow, the need to develop multivariate models that have the capacity to identify individuals at risk of poor outcomes is increasingly important. However, there is still much work to do if we are to transform data into future insights about recovery following mTBI. The following section presents a few suggestions regarding potential future directions for the field.

7.3.1 The need to further explore the potential prognostic value of QSM

The review presented in Chapter 5 of this thesis identified a number of ways in which QSM could be used to better understand the pathobiological underpinnings of mTBI/TBI. To recapitulate, it found that QSM can and has been used to examine changes in tissue and venous magnetic susceptibility as well as venous structure, and to detect the presence of microhaemorrhages and calcifications following mTBI/TBI. The review also investigated and summarised available literature and found that amongst the handful of studies that have been conducted to date, only two had investigated the prognostic capacity of QSM. More specifically, one study investigated the prognostic value of venous susceptibility for a sample of individuals presenting to a hospital ED, while the other investigated tissue magnetic susceptibility within the context of sports-related concussion. The results of these studies were promising and encourage further research into the prognostic value of QSM.

Relative to other advanced neuroimaging techniques, which typically provide information on one specific type of finding (e.g. functional MRI is largely limited to providing a current snapshot of brain activity and connectivity *via* the venous blood-oxygen-level-dependent (BOLD) contrast technique[882]), QSM can provide insight on a number of different post-injury changes. Not only does this make QSM highly attractive from a practical stance (i.e. various types of information can be acquired from the one scan, making it more time and cost-effective relative to other advanced neuroimaging analysis techniques), but it also makes QSM a fertile ground for exploring novel prognostic variables. For example, future studies could use QSM to detect microhaemorrhages and investigate the prognostic utility of both the presence of microhaemorrhages as well as number of

microhaemorrhages, either of which could be incorporated into multivariate prediction models as categorical and continuous variables, respectively. An advantage of using QSM over other MRI sequences that can be used to detect microhaemorrhages, such as fluid attenuated inversion recovery (FLAIR), is that it QSM appears to be capable of differentiating between acute and chronic (i.e. historic) microhaemorrhages[883].

7.3.2 Developing prognostic models that account for sex-specific factors

Research into the effect of biological sex on mTBI is in a relative state of infancy, and has been predominantly conducted within the context of sports-related concussion[416]. However, sex-based differences have been observed in both symptom reporting[401,884–889] and outcome[417,888,890–892] following mTBI. Further investigations are needed to validate these observations in adult civilian populations. Moreover, these findings lead to an interesting proposition; perhaps the accuracy of prognostic models could be improved by accounting for sex-specific factors, and in particular, those related to cyclic female hormonal fluctuations.

Reproductive hormones naturally fluctuate over the course of the female menstrual cycle, and hormonal contraceptives can attenuate these fluctuations[893]. Emerging evidence suggests menstrual cycle phase at time of injury may serve as a prognostic factor for outcome following mTBI in women. A seminal study by Wunderle and colleagues[418] found that women who sustained a mTBI during the luteal phase of the menstrual cycle, when progesterone concentration is high, reported worse post-concussion symptoms and quality of life at 1 month following injury, relative to women who were injured during the follicular phase, or those taking oral contraceptives. Multivariate analysis conducted by the authors also confirmed a significant independent effect of menstrual cycle phase on overall quality of life scores as well as somatic symptomatology. Evidence also suggests that hormonal contraception may moderate symptomatic outcomes following mTBI in females. Again, results are preliminary, but a study by Gallagher and colleagues[417] found collegiate female athletes who were using hormonal contraceptives at time of injury reported significantly lower post-concussion symptom severity than peers who were not using hormonal contraceptives, although no association between hormonal contraceptive and length of recovery was observed. Hormonal levels are also known to change over the course of pregnancy[894], however, the effect of hormone levels on outcome during pregnancy following mTBI in humans is yet to be investigated. Emerging evidence from preclinical studies also indicates that an isolated TBI occurring during pregnancy may also be associated with adverse foetal developmental outcomes[895]. Given that pregnant women constitute a uniquely vulnerable population, particularly within the broader context of TBI[896], study into this area of research is warranted.

It is also worth noting that female-specific outcomes following mTBI, such as altered menstrual cycle, as well as broader sexual dysfunction, have largely been overlooked in the literature to date. Changes in the menstrual cycle following mTBI may occur as a results of functional disruption of the neuroendocrine hypothalamic-pituitary-gonadal axis caused by injury[897]. Only one study appears to have been conducted to date in which abnormal menstrual cycles following mTBI have been investigated. In this study, adolescent and young women who had experienced a sport- or recreation-related concussion were found to be almost six times more likely to experience 2 or more abnormal menstrual patterns over the course of 3 months following injury, relative to non-head injury orthopaedic controls[897]. Since abnormal menstrual patterns can have important health implications[898], it is imperative that further investigation is conducted into the potential adverse outcomes of mTBI on menstrual cycles, in both adolescent and adult pre-menopausal women.

The effects of TBI on female sexual functioning have likewise received little attention[899], yet this may be another important outcome that needs to be considered in the broader picture of mTBI recovery. A recent study[900] involving a cohort of n = 89 eumenorrheic females presenting to an ED for concussion or *extremity* (i.e. orthopaedic) injury, found that women who had experienced a concussion to have a 70% increased risk of experiencing sexual dysfunction 6 – 10 weeks following injury, as measured by the *Brain Injury Questionnaire on Sexuality*[901], relative to orthopaedic controls. Furthermore, women who experienced sexual dysfunction following mTBI were also found to score higher on measures of post-concussion symptoms and mood[900]. Despite their preliminary nature, these findings suggest that females may be at an elevated risk of experiencing sexual dysfunction following mTBI, possibly due to disruptions to the hypothalamic-pituitary-gonadal axis resulting from injury. Moreover, they suggest that sexual dysfunction may be associated with symptomatic experience and mood-related sequelae following mTBI.

Overall, these findings suggest that it may be possible to improve the accuracy of prognostic models by incorporating female-specific factors and highlight the need to also consider female-specific outcome measures as part of a more holistic approach to conceptualising mTBI recovery.

7.3.3 The need to investigate the effect and prognostic potential of environmental factors

Environmental toxicants and pollutants are increasingly being recognised for their adverse effects on various aspects of human health[902–908]. Furthermore, exposure to environmental toxins, including herbicides such as glyphosate, has been suggested as a potential risk factor for a number of neurodegenerative diseases[909–912], including chronic traumatic encephalopathy[913]. Despite this, relatively little attention has been given to whether and how environmental factors may contribute to influencing recovery following mTBI. It has recently been suggested that long-term systemic

exposure to environmental toxicants, particularly glyphosate, may have the potential to result in chronic neuroinflammation *via* an indirect route involving an altered microbiome[914]. Evidence suggests that glyphosate can contribute to gut dysbiosis by reducing the amount of beneficial gut bacteria (e.g. Lactobacilli), promoting the growth of pathogenic microorganisms (e.g. Clostridium, Salmonella), and shifting the balance of gut microbiome toward higher levels of Gram-negative bacteria[915]. The latter is particularly significant as higher populations of Gram-negative bacteria naturally increase secretion of lipopolysaccharides; a virulent endotoxin capable of eliciting an innate immune response[913]. Increased levels of circulating lipopolysaccharides are believed to initiate an immunoexcitotoxic response[913,914] within the CNS by activating Toll-Like Receptor 4 (TLR4) expressed on the endothelial cells of the BBB[913]. As such, individuals who have experienced long-term exposure to toxicants, such as glyphosate, may be primed to have a heightened neuroinflammatory response after experiencing mTBI, which in turn could contribute to prolonged recovery. In addition to this, preclinical studies have found glyphosate exposure to be associated with behavioural and cognitive impairments[916], which are possibly underpinned by abnormal dendritic spine development and maturation, as well as alterations in synaptic architecture[917] and serotoninergic, dopaminergic, and noradrenergic neurotransmitter systems[918]. This also raises the questions of whether chronic exposure to environmental toxins may account for or influence the severity of mTBI-related symptoms. The nexus between environmental toxicants and mTBI recovery is certainly fascinating and is one in need of further exploration. It is plausible that environmental factors could potentially serve as additional prognostic factors, which may be of particular relevance to the recovery of individuals who have experienced a mTBI and are known, or are likely, to be at risk of increased exposure to environmental toxicants, including glyphosate (e.g. track and field athletes, agricultural/farming, horticultural, mining and industrial workers).

7.4 Concluding Statement

This PhD thesis, despite its limitations, has made a novel and useful contribution to existing research surrounding the prediction of outcome following mTBI. More specifically, it provided a comprehensive literature review on a broad range of pre-, peri- and post-injury prognostic factors, and identified the predictive potential of two well-established and psychometrically sound neuropsychological measures for PPCS. Further to this, it documented the study protocol for the *CREST* research project, for which data collection is currently underway and will be used to generate a "suite-based" multivariate prediction model upon its completion. Moreover, this PhD thesis reviewed the current state of the literature regarding the emerging applications of QSM, a nascent advanced neuroimaging analysis technique, in the detection of TBI pathology and documented the

pipeline that will be used to generate QSM images for data collected as part of *CREST*. An example analysis was also conducted to investigate a novel hypothesis regarding the accumulation of calcium deposits within the thalamus following mTBI as a demonstration of the application of the described pipeline. Lastly, this PhD thesis has highlighted important directions for future research.

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Appendices

Appendix A: Selected Abstracts

This appendix includes a selection of published and unpublished conference abstracts that resulted from the work presented in this thesis.

Specifically, these are as follows:

Published

1. *14th International Neurotrauma Society Symposium (INTS 2020)* 2021

Unpublished

2. *Symposium of Western Australian Neuroscience* 2018
3. *Symposium of Western Australian Neuroscience* 2019
4. *Curtin University Mark Liveris Student Seminar* 2020

Introducing CREST: The Concussion REcovery STudy

Aleksandra Gozt[1,2], Jacinta Thorne[1], Elizabeth Thomas,[3,4,5], Anoek van Houselt[6], Francesca Buhagiar[7], Gill Cowen[4,8], Alexander Ring[9,10], Daniel Xu[4,5], Suzanne Robinson[5], Melissa Licari[11], Michael Bynevelt[3,12], Carmela Pestell[1,7], Daniel Fatovich[13,14,15], Melinda Fitzgerald[1,2,16]

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Concussion is the most common form of traumatic brain injury in Australia and worldwide. Frequently reported symptoms include headache, neck pain, dizziness, difficulty concentrating as well as disturbances to mood and sleep. While most people recover from the symptoms of concussion within two weeks of injury, approximately 10-20% of individuals will continue to experience ongoing symptoms in a condition known as Persistent Post-Concussion Symptoms (PPCS). Currently, it is not possible to identify which individuals will go on to develop PPCS at the time of concussion diagnosis, and there is no consensus on how to manage patients who experience this condition. Thus the ***C**oncussion **RE**covery **St**udy **(CREST)*** was developed with the aim of identifying individuals that are at an increased risk of developing PPCS using a multivariate, suite-based approach. ***CREST*** is a large, cross-institutional study being conducted in Western Australia, which is recruiting adult participants between 18-65 years of age from both medical and community-based settings who have been diagnosed with concussion. The study includes the creation of a state-wide registry of concussion injury, and data being collected in two stages. In the first stage, data is collected on participant demographics, the nature of their injury, and relevant medical history. In the second stage, eligible participants undergo neuropsychological and exercise tolerance tests, MRI, quantitative electroencephalography, and blood-based biomarkers are quantified. Participant follow-up is conducted *via* telephone interview at 1-, 3-, 6- and 12-months following injury. The primary outcome measure is the presence of PPCS, as measured by the Post-Concussion Symptoms Questionnaire. Multivariate modelling will be used to assess the prognostic value of this 'suite' of factors. It is anticipated that ***CREST*** will help identify individuals at risk of delayed recovery following concussion, as well as the types of symptoms that may prevail in affected individuals. This in turn may help practitioners to better manage patients with PPCS and thereby improve recovery outcomes.

Developing an Evidence-Based Suite for Predicting Outcome following Concussion

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Post-Concussion Syndrome (PCS) is a complex, multi-faceted condition in which the symptoms of concussion continue to persist beyond the timeframe that they are known to typically resolve. Recent estimates indicate that as many as 30% of individuals who sustain a concussion will experience PCS, however there is currently no way of knowing which individuals will go on to develop this debilitating condition. A 'suite' based approach that takes into consideration the physiological, physical as well as the neuropsychological changes (namely, cognition and mood) that are known to ensue following concussion may help facilitate the identification of at-risk individuals. To investigate this concept, a pilot study was conducted in collaboration with Royal Perth Hospital with the aim of identifying, from an array of blood-based biomarker, MRI and neuropsychological outcomes, those that may be of predictive value. More specifically, a cohort of concussion patients were recruited at time of presentation to the Emergency Department (T_0) and again at 28 days (T_1; n=36); age and gender-matched healthy controls were also recruited. Patients were diagnosed with PCS based on their T_1 performance on the Rivermead Post Concussion Questionnaire or scores equalling to, or exceeding, 1.5 standard deviations below the sample mean in any two of the measures featured in the neuropsychological testing battery implemented. Analysis of the neuropsychological data found that, relative to those that recovered, individuals who developed PCS scored lower on the Repeatable Battery for the Assessment of Neuropsychological Status Total Score (RBANS TS) Index at T_0 (t (34) = 2.82; p = 0.008). Follow-up univariate logistic regression further revealed that decreased performance on the RBANS TS at T_0 generated greater odds of PCS (OR: 1.15, p = 0.024), and that a decreased change of five-point on the RBANS TS corresponded to a 97.2% increase in the odds of PCS. Examination of demographic factors found that the odds of developing PCS were almost 5-times greater amongst those individuals who had reported a history of prior concussion (OR: 4.86; p = 0.12) and over 4-times greater in those who had reported being diagnosed with a psychological disorder (OR: 4.36; p = 0.18). Our study did not find evidence of statistically significant differences in concentration of the blood-based biomarkers examined between individuals who developed PCS and those who recovered normally. However, levels of glial fibrillary acidic protein were higher in individuals who sustained a concussion than in controls (ANOVA F (2,60): 12.03; $p < 0.001$). Moreover, tract-based spatial statistical analyses of Diffusion Tensor Imaging data collected from a subset of patients (n=23) indicated that, relative to healthy age and gender-matched controls, individuals with mTBI had lower levels of fractional anisotropy within the left inferior frontal occipital fasciculus (IFOF) at T_0 (t (20.59) = -2.17; p = 0.04). This area of the brain has previously been found to be associated with visual-spatial processing abilities. Consistent with this, decreased levels of fractional anisotropy within the left IFOF were significantly correlated with impaired performance on the RBANS Visual-Constructional subscale (r = 0.55; p = 0.03). In all, these findings indicate that the RBANS TS, history of previous concussion and psychological disorder may be of particular predictive value and warrant further research using a larger sample size so that statistical modelling techniques can be applied to assess the efficacy of a predictive model that features these distinct parameters.

Showcasing CREST: The Concussion REcovery STudy

Aleksandra Gozt[1,2], Jacinta Thorne[1], Elizabeth Thomas[12], Anoek van Houselt[6], Francesca Buhagiar[8], Gill Cowen[13,14], Daniel Xu[12,13], Suzanne Robinson[13], Melissa Licari[3], Michael Bynevelt[7,9], Carmela Pestell[1,8], Daniel Fatovich[5,10,11], Melinda Fitzgerald[1,2,4].

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Concussion is the most common form of traumatic brain injury in Australia and worldwide. Some of the most commonly reported symptoms include headache, neck pain, dizziness, difficulty concentrating as well as disturbances to mood and sleep. While most people recover from the symptoms of concussion within two weeks of sustaining their injury, approximately 10-20% of individuals will continue to experience symptoms beyond this typical recovery time frame. These individuals are said to be suffering from a condition known as Persisting Post-Concussion Symptoms (PCSS). Currently, it is not possible to identify which individuals will go on to develop PPCS at the time of concussion diagnosis, and there is no consensus on how to manage patients that experience this condition. Here we introduce the ***CREST:*** *Concussion* ***RE****covery* ***ST****udy*, a study that aims to identify individuals that are at an increased risk of PPCS. ***CREST*** is a large, cross-institutional study being conducted in Western Australia which is recruiting adult participants from both medical and community-based settings that have been diagnosed with concussion. The study includes the creation of a state-wide registry of concussion injury, and data being collected in two stages. In the first stage, a telephone interview, data is collected on participant's demographics, the nature of their injury, and relevant medical history. In the second stage, eligible participants are invited to attend the Ralph and Patricia Sarich Neuroscience Research Institute, where they are assessed for performance on neuropsychological and exercise tolerance tests, expression of blood-based biomarkers and undergo MRI. Participant follow-up is conducted at 1-, 3-, 6- and 12-months following injury *via* telephone interview. The primary outcome measure is the existence of PPCS, as measured by the Post-Concussion Symptoms Questionnaire, and multivariate modelling will be used to assess the prognostic value of this 'suite' of factors. It is anticipated that ***CREST*** will help identify individuals at risk of delayed recovery following concussion, as well as the types of symptoms that may prevail in affected individuals. This in turn may help practitioners to better manage patients with PPCS and improve recovery outcomes.

Mark Liveris Student Seminar 2020
Abstract

Student Name: Aleksandra Gozt
Student ID: 19322968

Have you completed more than 50% of your PhD/MPhil?
Yes ☒
No ☐

QSM: A Piece of the Concussion Recovery Puzzle?

Presented by:	**Aleksandra Gozt**, School of Pharmacy and Biomedical Science
Course:	Doctor of Philosophy
Supervisor:	Professor Melinda Fitzgerald, School of Public Health
Co-Supervisor:	N/A
A/Supervisor:	A/Prof Carmela Pestell, UWA School of Psychological Science

Concussion is the most common form of traumatic brain injury in Australia. While there are many symptoms of concussion, some of the most frequently reported ones include headache, dizziness, difficulty remembering as well as disturbances to mood and sleep. Most people recover from the symptoms of concussion within two weeks of sustaining their injury, however, approximately 10-20% will experience symptoms for longer. These individuals are said to be suffering from a condition known as Persisting Post-Concussion Symptoms (PPCS). At present, there is no way of identifying which individuals might go on to develop PPCS, nor is there an agreement on how to best go about managing patients that develop this condition. Thus, the **C**oncussion **RE**covery **ST**udy ***(CREST)*** was developed with the overarching aim to identify and evaluate factors that might help healthcare professionals determine whether a person is at risk of delayed recovery following concussion. ***CREST*** is a large, cross-institutional study that is currently recruiting adult participants aged between 18-65 years of age from both medical and community-based settings who have been diagnosed with concussion. The study is examining a broad range of novel and established outcome measures, including demographics, injury-related characteristics, blood samples, cognitive and physical fitness, as well as brain imaging (e.g. MRI and qEEG). Current research suggests that individuals who have previously experienced a concussion are at a greater risk of developing PPCS, and that this may be due to calcium deposits forming in an area of the brain called the thalamus. Quantitative Susceptibility Mapping (QSM) is a novel MRI technique that is particularly sensitive to detecting substances that distort the magnetic field of MRI machines, such as calcium, and presents as an exciting new tool that can be used to examine whether the accumulation of calcium within the thalamus influences an individual's recovery trajectory following concussion.

Appendix B: Poster

This appendix includes a copy of the poster that was presented by the PhD Candidate at the *14th International Neurotrauma Society Symposium (INTS 2020)* that was hosted virtually in 2021 from Melbourne, Victoria, Australia. The poster reflects the work presented in Chapter 4 of this thesis.

Introducing CREST: Concussion Recovery Study

Aleksandra Gozt[1,2], Jacinta Thorne[1], Elizabeth Thomas[3,4,5], Anoek van Houselt[6], Francesca Buhagiar[7], Gill Cowen[4,8], Alexander Ring[9,10], Daniel Xu[4,5], Suzanne Robinson[5], Melissa Licari[11], Michael Bynevelt[3,12], Carmela Pestell[1,7], Daniel Fatovich[13,14,15], Melinda Fitzgerald [1,2]

Background

Concussion

- Concussion is the most common form of traumatic brain injury in Australia and worldwide
- Individuals who have experienced a concussion can report a range of signs and symptoms, which can be broadly categorised into Physical, Cognitive, Emotional/Psychological and Sleep Clusters *(Fig.1)*
- Each concussion presentation is unique: the signs and symptoms experienced after a concussion can differ between people and instances of concussion injury, as can recovery

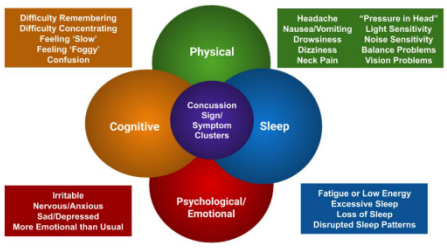


Fig. 1: Concussion sign/symptom clusters.
Adapted from Pardini et al., (2004). Br. J Sports Med. 38(5) 654-664

Persistent Post Concussion Symptoms (PPCS)

- Adults are generally expected to recover from concussion within two weeks of injury. However, 10-20% of individuals continue to experience signs/symptoms beyond this 'typical' recovery timeframe
- Individuals experiencing symptoms for 3 or more months following concussion are said to be experiencing PPCS
- **Currently, it is not possible to identify which individuals will go on to develop PPCS at the time of concussion diagnosis**, nor is there a consensus on how to manage patients who experience this debilitating condition
- **Having the ability to identify individuals at risk of delayed recovery has the potential to help improve patient outcomes**, and is thus highly desirable
- To date, many studies have examined one or a few factors, in hope that they may identify one that is capable of predicting outcome following concussion
- Most studies examine young athletes **or** clinical populations, which limits generalisability of findings
- It is **unlikely that any one individual factor** will be sufficient for determining which individuals may be at risk of poor outcome following concussion
- **Greater success is likely to be achieved by using comprehensive, multivariate "suite-based" approach** *(Fig. 2)*

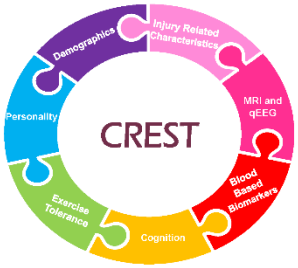


Fig. 2: The multivariate approach of CREST that incorporates pre-, peri-, and post-injury factors is anticipated yield a more accurate prognostic model for PPCS.

The Concussion Recovery Study (CREST)

- CREST is a large, cross-institutional study being conducted in Perth, Western Australia
- The study is being conducted in two stages (*Phase I* and *Phase II*, respectively) and is centred around evaluating a range of existing and novel pre-, peri- and post-injury factors, which may be combined into a multivariate model to **predict** outcome following concussion
- The study is actively enrolling individuals:
  - who are aged 18-65 years
  - that have sustained a concussion injury from any cause (e.g. falls, motor vehicle and transport accidents, assault, sports-related concussion)
  - and have been diagnosed by a medical doctor
  - within 7 days of their concussion

Fig. 3: Map of metropolitan Perth, Western Australia showing location of hospital emergency departments from which participants are being recruited for CREST.

Study Aims

The primary objectives of CREST are:

- To establish a large-scale clinical research database of adults experiencing concussion in Western Australia, in order to observe the typical pattern of recovery from mTBI and determine the incidence of PPCS
- To identify a suite of pre-injury factors and outcome measures during the early presentation period that may be used to predict those at risk of experiencing PPCS compared to those who recover within a typical timeframe

The secondary objective of the CREST study is to:

- Determine the feasibility of recruiting a large cohort of participants with concussion from a variety of sources (e.g., EDs, general practitioners (GPs), and community sporting groups), as this widespread collection of community mTBI data has not previously been conducted to this scale in Australia or internationally to date

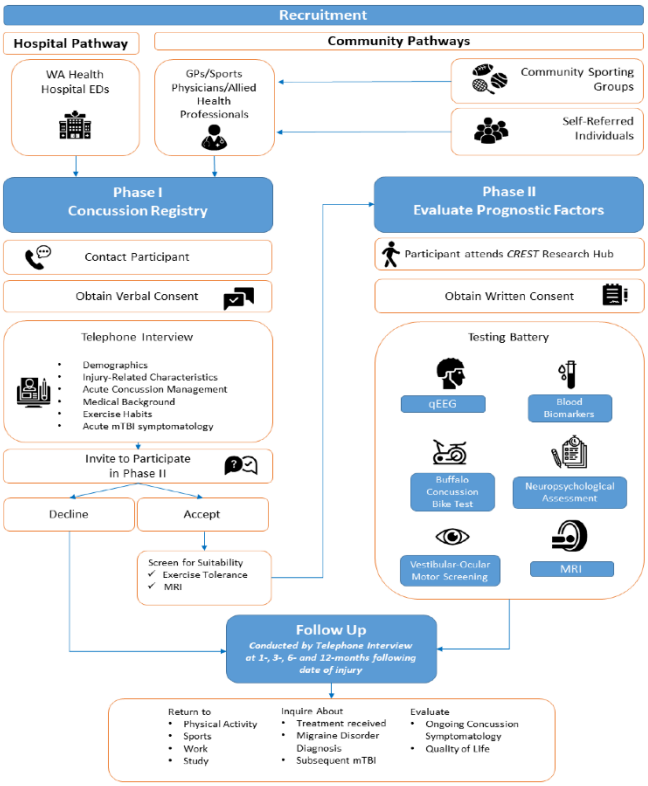
Neuro Trauma 2020
Melbourne, Australia

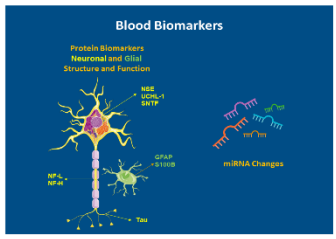
xxx

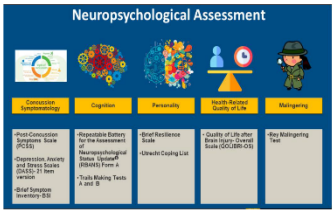
Introducing CREST: Concussion Recovery Study

Aleksandra Gozt[1,2], Jacinta Thorne[1], Elizabeth Thomas[3,4,5], Anoek van Houselt[6], Francesca Buhagiar[7], Gill Cowen[4,8], Alexander Ring[9,10], Daniel Xu[4,5], Suzanne Robinson[5], Melissa Licari[11], Michael Bynevelt[3,12], Carmela Pestell[1,7], Daniel Fatovich[13,14,15], Melinda Fitzgerald [1,2]

Method







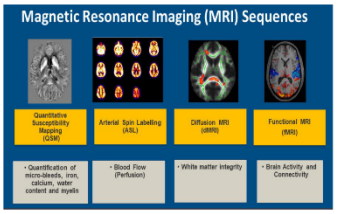


Fig. 4: Flow chart illustrating the CREST study design

Study Design and Procedures

- CREST is recruiting participants from both Medical *(Fig. 3)* and Community-based pathways *(Fig. 4)*
- *Phase I* of the study involves a semi-structured telephone interview during which information is collected about a number of pre- and peri-injury factors that are known or may be associated with concussion recovery
- Eligible participants are invited to participate in Phase II of the study, which comprises a comprehensive, one-off testing session at the CREST Research Hub, designed to assess a range of post-injury factors
- Both *Phase I* and *Phase II* components are conducted within 7 days of a participant sustaining an mTBI
- All participants are followed-up by telephone interview at 1-, 3-, 6- and 12-months following the date of injury
- Longitudinal Follow-Ups assess recovery experiences using validated questionnaires in semi-structured interviews
- Prediction models for PPCS will be built using regression modelling combining *Phase I* and *Phase II* outcome data. Model performance metrics will include traditional measures of calibration and discrimination alongside more novel measures employing reclassification tables and net reclassification improvement

Discussion

Current Study Status

- **CREST is an ongoing study and is actively recruiting participants**
- **To date, n = 103 participants have completed *Phase I* and n = 15 have taken part in *Phase II* testing**
- **Western Australia is a large state, occupying a land mass of almost one-third of Australia. As such, recruitment through the medical pathway has expanded to include hospitals located in regional areas of the State. This will help obtain a more comprehensive picture of concussion incidence within Western Australia**
- **CREST will help expand current knowledge regarding prognostic models for concussion recovery, which are critical for helping direct clinical care and maximising patient outcomes**

Author Affiliations

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This study has Ethics Approval through Royal Perth Hospital Human Research Ethics Committee (#RGS0000003024), Curtin University (HRE2019-0209), Ramsay Health Care (#2009) and St John of God Health Care (#1628).

Appendix C: Ethics and Data Collection

Appendix C includes information related to ethics and data collection procedures. Specifically, the following is presented:

Chapter 3 Pilot Study

- Participant Information Sheet and Consent Form

Chapter 4 *CREST* Protocol

- Participant Information Sheet and Consent Form
- Study Flow Chart Summary
- Concussion Fact Sheet (created by the *CREST* Research Team to raise awareness about mTBI (concussion) after-care and to promote the study).
- Recruitment Posters (created by the PhD Candidate using *Canva* software to promote awareness of the *CREST* research project)

Due to copyright restrictions, copies of all other assessments and questionnaires have not been included.

Participant Information Sheet

Identification of biomarkers that correlate with clinical features and outcomes following concussion

Coordinating Principal Investigator: Professor Melinda Fitzgerald (Curtin University)

Principal Investigators: Prof Daniel Fatovich, Assoc. Prof Carmela Pestell(UWA)

Associate Investigators: Assoc. Prof Glen Arendts
A/Prof Michael Bynevelt
Dr Stephen MacDonald
Dr Swithin Song
Miss Alison Halstrom (UWA)
Mrs Ellen MacDonald

You are being invited to participate in a research project because you have had a concussion. This information sheet explains the study and describes what will be involved should you decide to participate. Please read the information carefully and ask any questions you might have. You may also wish to discuss the study with a relative or friend or your GP.

WHAT IS THE PURPOSE OF THIS STUDY?

Following concussion, the symptoms of most patients resolve within a few days to weeks. However, a small but significant proportion goes on to develop a debilitating post-concussion syndrome, with a range of symptoms including altered thinking, headaches, dizziness and fatigue.

Patients that present to the emergency department with concussion are frequently discharged with no specific follow up, and currently it is not possible to determine who will develop a post-concussion syndrome.

This study aims to identify indicators of whether concussion will develop into post-concussion syndrome. In future, patients showing these indicators would then be identified as likely to benefit from follow-up care and could be targeted for new treatments that are being developed.

This study is designed to do two things:

1. Provide a way of identifying patients with concussion at risk of post-concussion syndrome.

2. Identify blood tests to evaluate the usefulness of any therapeutic strategies we may try in the future.

This study is collaboration between Royal Perth Hospital (RPH) and The University of Western Australia (UWA) and has been funded in part by The National Health and Medical Research Council (NHMRC). It is expected that 50 participants, aged 18 to 50 years, will be recruited

from the Emergency Department at RPH along with 10 age- and sex-matched 'control' participants.

WHAT WILL PARTICIPATION IN THIS STUDY INVOLVE?

If you agree to participate in this study we will first ask you to complete a series of study activities on the first day of your participation ('Visit 1'):

1. Fill in a **questionnaire** that will help us to determine how severe your injury is and provide relevant information about your medical history.

2. Complete a series of **neuropsychological tests** that will help researchers understand how severe your concussion is, and more clearly understand your symptoms. These tests will take approximately 40 to 50 minutes and will be conducted under the direction of A/Prof Carmela Pestell (Director, Robin Winkler Clinic, Clinical Psychologist & Neuropsychologist [MAPS]). This first series of neuropsychological tests will be conducted (when neuropsychology staff are available) at the Emergency Department.

3. A nurse or doctor will **take a sample of your blood (10ml).** Researchers will analyse your blood to see if there any indications of your concussion apparent in your blood sample.

4. **PHASE 2 enrolments only -** Have a **brain scan** by Magnetic Resonance Imaging (MRI). MRI does not involve ionising radiation.

Follow-Up Visit ('Visit 2')
Researchers will contact you to arrange a follow up session 28 days after your first participation day, to be completed at either UWA or RPH. This will involve:

1. At UWA a second 10ml blood sample collection at the Clinipath collection centre. At RPH a 10ml blood sample collection by the Emergency Department research staff.

2. Repeat neuropsychological testing either at the Robin Winkler Clinic, School of Psychology UWA or at Royal Perth Hospital Emergency Department again under the direction of A/Prof Pestell.

If attending UWA you will be met by a researcher who will coordinate the above activities and take you to the Robin Winkler Clinic and Clinipath. If attending RPH you will be met by one of the research staff who will coordinate your blood sample collection and ensure facilitate your meeting with the UWA researcher for your neuropsychological testing.

Although this will end the formal study commitment, if your initial MRI results indicate that your injury is more severe and/or if you are still experiencing symptoms at 28 days after the injury you may be referred for another MRI scan at RPH **(phase 2 participants only)**. Also, if the neuropsychological testing indicates that you still are experiencing significant symptoms or emotional distress, you will be offered a referral for further clinical neuropsychological assessment at the Robin Winkler clinic or the State Head Injury Unit, Sir Charles Gardiner Hospital.

WHAT ARE THE POSSIBLE BENEFITS OF TAKING PART?

We cannot guarantee or promise that you will receive any benefits from this research; however, possible benefits include clear diagnosis of post-concussion syndrome, access to advanced neuropsychological testing including follow up care if required, and access to an additional MRI scan if the first scan was abnormal and significant symptoms persist at the 28 day follow up.

People who receive a concussion in the future may benefit from the study findings due to improved diagnosis and better prediction of ongoing symptoms. A method to identify patients at

risk of developing post-concussion syndrome could also be used to assess the effectiveness of treatment strategies in future clinical trials.

WHAT ARE THE POSSIBLE RISKS AND DISADVANTAGES?

Because this study does not involve a treatment, the risks and disadvantages of taking part are low and are no more than experienced with blood collection and undergoing an MRI.

Blood collection

Having a blood sample taken may cause some discomfort, bruising, minor infection or bleeding. If this happens, it can be easily treated.

MRI Scans (phase 2 participants only)

MRI stands for *Magnetic Resonance Imaging*. A MRI scanner is a machine that uses electromagnetic radiation (radio waves) in a strong magnetic field to take clear pictures of the inside of the body. Electromagnetic radiation is not the same as ionising radiation used, for example, in X-rays. The pictures taken by the machine are called MRI scans.

We will ask you to lie on a table inside the MRI scanner. The scanner will record information about your brain. It is very important that you keep very still during the scanning. When you lie on the table, we will make sure you are in a comfortable position so that you can keep still. The scanner is very noisy and we can give you some earphones to reduce the noise. Some people may experience symptoms of claustrophobia from lying in a confined space. If you do experience discomfort at any time during the scan, you will be able to alert staff by pressing on a call button provided to you.

There are no proven long-term risks related to MRI scans as used in this research project. MRI is considered to be safe when performed at a centre with appropriate procedures. However, the magnetic attraction for some metal objects can pose a safety risk, so it is important that metal objects are not taken into the scanner room.

We will thoroughly examine you to make sure there is no reason for you not to have the scan. You must tell us if you have metal implanted in your body, such as a pacemaker or metal pins.

Information of relevance to your health and clinical care

- Your initial MRI results may indicate that your injury was more severe than first thought. Your treating doctor will have access to this information and will take it into account when deciding upon your best clinical care.

- The MRI scans we are taking are for research purposes. They are not intended to be used like scans taken for a full clinical examination. The scans will not be used to help diagnose, treat or manage a particular condition. A specialist will look at your MRI scans for features relevant to the research project. On rare occasions, the specialist may find an unusual feature that could have a significant risk to your health. If this happens, we will contact you to talk about the findings. We cannot guarantee that we will find any/all unusual features.

- The neuropsychological testing may indicate that you are experiencing significant symptoms or emotional distress. If this happens you will be offered a referral for further clinical neuropsychological assessment and counselling at the Robin Winkler Clinic, School of Psychology UWA This clinic is staffed by post-graduate trainees in clinical psychology and neuropsychology who are being supervised as part of their professional postgraduate training (Masters/PhD), under the supervision of registered supervisors

with the Psychology Board of Australia. RWC is open from 9am to 8pm Monday, Tuesday and Wednesday, and from 9am until 5pm on Thursday and Friday. It is situated on the UWA campus (Myers Street, Crawley). Trainees will typically see an individual client for 12 to 15 individual therapy sessions, although this is flexible and additional sessions can be offered if required.

- If you become upset or distressed as a result of your participation in the study, the study doctor will be able to arrange for counselling or other appropriate support. Any counselling or support will be provided by qualified staff who are not members of the research team. This counselling will be provided free of charge.

WHAT WILL HAPPEN TO MY BLOOD SAMPLES?

Your blood samples will be analysed at UWA for the presence of 'markers' that may indicate the severity of your concussion and help researchers identify useful ways to predict whether symptoms will persist.

Blood samples will be stored at UWA for a maximum of 15 years. The samples will be stored in a re-identifiable format against a study allocated ID. The study ID will be listed against your identifying details contained in an electronic excel log which will be password protected and accessible by research staff only (the 'master log'). This means your sample can only be matched to your name by research staff who have access to the master log.

One of the reasons for storing samples in re-identifiable format is that it allows you to withdraw your samples at any time. You can do this by contacting Assoc. Professor Melinda Fitzgerald on 6488 2353. Samples will be disposed of by incineration in appropriate biohazard containment bags, in accordance with the biohazardous project risk assessment, approved by the UWA Biological Safety Office.

Blood samples may be used to study many indicators of damage; any future study/ies will be restricted to the area of interest covered by this project - concussion and recovery from concussion - and will be approved by a Human Research Ethics Committee (HREC).

WHAT WILL HAPPEN TO INFORMATION ABOUT ME?

Any information obtained for the purpose of this research study that can identify you will be treated as confidential and securely stored. It will be disclosed only with your permission, or as required by law.

Your personal information will be stored de-identified with a study allocated ID. The study ID will be listed against your identifying details contained in an electronic excel log (the 'master log') which will be password protected and accessible by research staff only. The collection of data will be conducted by persons who normally have access to patient records for clinical care or directly related secondary purposes; namely the clinicians and research staff employed by RPH. Non-Health Department employees have signed a declaration of confidentiality.

Your personal information will be stored within a secure filing cabinet in a locked research office designated for research staff only at RPH. These will only be accessed by approved research staff. Electronic databases will be encrypted and protected on a secure network accessible only by authorised research staff. The entries in the database will be only be identifiable with a study ID that can be cross referenced with the master log which will be kept separate.

Your information will only be used for the purpose of this research project and it will only be disclosed with your permission, except as required by law. Blood samples may be used to study many indicators of damage; any future study that links your personal information to data from your blood samples will be restricted to the area of interest covered by this project.

In line with WA Health Guidelines all research data will be retained for 15 years. After that time, all hardcopy data will be shredded into confidential waste. All electronic data will be transferred onto CD and subsequently destroyed.

Information about you may be obtained from your health records held at this and other health services for the purpose of this research. By signing the consent form you agree to the study team accessing health records if they are relevant to your participation in this research study.

Your health records and any information obtained during the research project are subject to inspection (for the purpose of verifying the procedures and the data) by the approved research staff. By signing the Consent Form, you authorise release of, or access to, this confidential information to the relevant study personnel as noted above.

It is anticipated that the results of this study will be published and/or presented in a variety of forums. In any publication and/or presentation, information will be provided in such a way that you cannot be identified, except with your permission.

Information about your participation in this study may be recorded in your health records.

In accordance with the Australia Privacy Act (1998) and other relevant laws, you have the right to request access to your information collected and stored by the research team. You also have the right to request that any information with which you disagree be corrected. Please contact the study team member named at the end of this document if you would like to access your information.

COMPLAINTS AND COMPENSATION

If you suffer any injuries or complications as a result of your participation in this study please contact the study team as soon as possible and you will be assisted with arranging appropriate medical treatment.

STUDY COSTS

There are no costs associated with participating in this study. All tests required as part of the study will be provided to you free of charge. You will be reimbursed for reasonable travel and parking expenses associated with the study visits (e.g., to attend the follow-up visits at UWA or RPH).

No member of the research team will receive personal financial benefit from your involvement in this study (other than their ordinary wages).

DO I HAVE TO PARTICIPATE IN THIS STUDY?

Participation in any research study is voluntary. If you do not want to participate, you do not have to. If you decide to participate and later change your mind, you are free to withdraw from the study at any stage. *You do not have to participate in this study to receive treatment at RPH.*

Your decision whether to take part or not to take part, or to take part and then withdraw, will not affect your routine treatment, your relationship with those treating you or any current or future relationship with RPH or UWA. If you do withdraw from the study personal information already collected will be retained to ensure that the results of the study can be measured properly and the study data is as complete as possible. If you do not want your data retained, please inform the study team at the time of your withdrawal.

If you are interested in the outcome of the study the researchers can provide you with a copy of the research findings once these have been finalised. Please contact Assoc. Professor Melinda Fitzgerald on 6488 2353 to ask for a copy of the study findings.

FURTHER INFORMATION AND WHO TO CONTACT

If you want further information about this project or if you have any medical problems which may be related to your participation (for example, any side effects), please contact: **Assoc. Prof Melinda Fitzgerald on (08) 6488 2353, Monday – Friday, 09:00 to 17;00 or at** lindy.fitzgerald@curtin.edu.au

This study has been approved by the RPH and UWA Human Research Ethics Committees. If you have any concerns about the conduct of the study or your rights as a research participant, please contact Prof Frank van Bockxmeer, Chairman of the RPH Ethics Committee, via (08) 6151 1180 or rph.hrec@health.wa.gov.au and quote the ethics approval number (REG 15-062)

Consent Form

Identification of biomarkers that correlate with clinical features and outcomes following concussion

Coordinating Principal Investigator: A/Prof Melinda Fitzgerald
Principal Investigators: Prof Daniel Fatovich & A/Prof Carmela Pestell
Associate Investigators: A/Prof Glen Arendts; A/Prof Michael Bynevelt; Dr Stephen MacDonald; Dr Swithin Song; Miss Alison Halstrom; Mrs Ellen MacDonald

Declaration by Participant

I have read the Participant Information Sheet or someone has read it to me in a language that I understand. I understand the purposes, procedures and risks of the study.

I give permission for my doctors, other health professionals, hospitals or laboratories outside this hospital to release information to Royal Perth Hospital concerning my disease and treatment for the purposes of this study. I understand that such information will remain confidential.

I consent to the storage and use of blood samples taken from me for use, as described in the relevant section of the Participant Information Sheet, for: (a) This specific research project and (b) Other research projects closely related to this study.

I have had an opportunity to ask questions and I am satisfied with the answers I have received.

I freely agree to participate in this study as described and understand that I am free to withdraw at any time during the study without affecting my future health care.

I understand that I will be given a signed copy of this document to keep.

Name of Participant (please print) _____

Signature _____ Date _____

Declaration by Study Doctor/Senior Researcher†

I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Name of Study Doctor/
Senior Researcher† (please print) _____

Signature _____ Date _____

Note: All parties signing the consent section must date their own signature.

CREST Concussion REcovery STudy

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Participant Information Sheet

A validation study to predict poor outcomes following mild traumatic brain injury

Coordinating Principal Investigator:
Professor Melinda Fitzgerald (Curtin University and the Perron Institute)

Principal Investigators:
Prof Daniel Fatovich (Royal Perth Hospital)
A/Prof Mike Bynevelt (Sir Charles Gairdner Hospital)
Prof Suzanne Robinson (Curtin University)
A/Prof Carmela Pestell (UWA)
Dr Melissa Licari (UWA/Telethon Kids Institute)
A/Prof Daniel Xu (Curtin University/WA Primary Health Alliance)
A/Prof Glenn Arendts (Fiona Stanley Hospital)
Prof Tony Celenza (Sir Charles Gairdner Hospital)
Dr Benjamin Smedley (Rockingham General Hospital)
Dr Ashes Mukherjee (Armadale Health Campus)
Dr Russell Young (Albany Health Campus)
Dr Sjinene Van Schalkwyk (Joondalup Health Campus)
Dr Philip Brooks (St John of God Midland Hospital)
Dr John Iliff (St John of God Murdoch Hospital)
Dr Stephen Honeybul (Sir Charles Gairdner Hospital)
Dr Peter Arthur (UWA)

You are being invited to participate in a research project because you have had a concussion. This information sheet explains the study and describes what will be involved should you decide to participate. Please read the information carefully and ask any questions you might have. You may also wish to discuss the study with a relative or friend or your GP.

WHAT IS THE PURPOSE OF THIS STUDY?

Following concussion, the symptoms of most patients resolve within a few days to weeks. However, a small proportion of people's symptoms do not resolve, leaving them with debilitating persisting post-concussion symptoms including altered thinking, headaches, dizziness and fatigue.

Patients that present to the emergency department with concussion are frequently discharged with no specific follow up, and currently it is not possible to determine who will have persisting post-concussion symptoms.

This study aims to identify indicators of whether concussion will develop into persisting post-concussion symptoms. In future, patients showing these indicators would then be identified as likely to benefit from follow-up care and could be targeted for new treatments that are being developed.

This study has two phases and is designed to do two things:

1. **Phase I**: Understand better the nature of concussion injuries, their management, and burden on the health system

2. **Phase II:** Provide a way of identifying patients with concussion at risk of persisting post-concussion symptoms so that they can be monitored appropriately.

This study is collaboration between Royal Perth Hospital (RPH), Sir Charles Gairdner Hospital, Curtin University, The Perron Institute, The University of Western Australia (UWA) and the Telethon Kids Institute (TKI), and has been funded in part by The Neurotrauma Research Program. It is expected that 500 participants will be recruited into the **Phase I** study, and 120 participants will be recruited into the **Phase II** study.

WHAT WILL PARTICIPATION IN THIS STUDY INVOLVE?

Phase I
If you agree to participate in this study we will first ask you to complete a series of study activities on the first day of your participation

1. A member of the research team will call you by phone or meet with you to answer any questions you have about the study and record your consent to participate.

2. The researcher will then ***collect information*** on the nature of your injury, your symptoms and some more details about your general health. You may also be invited to participate in Phase II of the study.

Phase II
If you meet the inclusion criteria and are able to attend the research centre at QEII Medical Centre you will be invited to participate in an extended set of study activities.

1. You will have a ***quantitative Electroencephalograph (qEEG)*** to measure brain activity. qEEG is non-invasive and no radiation is involved. For this test an electrocap (like a swimming cap), containing electrodes filled with electroconductive gel, will be placed on your head. You will then sit with your eyes open and eyes closed while your brain activity is recorded for approximately 10 minutes. During the eyes closed condition, an eye mask will be placed over your eyes to relax your eyes and minimise eye movement-related data interruptions. This procedure will take approximately 30 minutes to complete in total.

2. You will undergo a ***neuropsychological test*** that will help researchers understand how severe your concussion is, and more clearly understand your symptoms. These tests will take approximately 40 to 50 minutes and will be conducted under the direction of A/Prof Carmela Pestell (Director, Robin Winkler Clinic, Clinical Psychologist & Neuropsychologist).

3. A nurse or qualified researcher will take a 20mL ***sample of your blood*** (this is about four teaspoons). Researchers will analyse your blood to see if there are any indications of your concussion apparent in your blood sample.

4. You will be asked to complete an ***exercise tolerance test*** to assess your tolerance to exercise. This is a stationary exercise bike test, and you will stop the test if any of your concussion symptoms are worse. This test will take a maximum of 30 minutes to complete. Additionally, you will also be asked to complete the Vestibular-Ocular Motor Screening (VOMS) test which involves seven tasks that assess balance and eye movements. The VOMS test will take a maximum of 10 minutes to complete.

5. You will also have a brain scan by ***Magnetic Resonance Imaging (MRI).*** MRI does not involve ionising radiation. This test will take approximately 40 minutes and will depend on the availability of the MRI machine at the time of your visit.

Protocol for conducting CREST Phase II testing under COVID-19 circumstances.

Please note: As part of Curtin University and the Perron Institute's ongoing aim to minimise the risk of spreading COVID-19, and in accordance with the Government of Western Australia and WA Health guidelines, additional measures will be in place during CREST Phase II testing. This will include routine COVID-19 screening questions on arrival at the Sarich Neuroscience Research Institute building, temperature checking, social distancing where possible, increased use of personal protective equipment where appropriate, increased hand hygiene and increased cleaning of the testing environment. These protocol measures will be revised regularly, and modified as necessary in accordance with WA Health guidelines.

Follow-up

For both Phase I and II participants, researchers will contact you by telephone at one-, three- six- and 12-month intervals after your first participation day. You will be asked basic questions about your condition, and any remaining symptoms you may be experiencing.

Although this will end the formal study commitment, if your initial MRI results indicate that your injury is more severe and/or if you are still experiencing symptoms at the three-month follow-up point you may be referred for further medical follow up outside of the study. If the neuropsychological testing indicates that you still are experiencing significant symptoms or emotional distress, you will be offered a referral for further clinical neuropsychological assessment at the Robin Winkler clinic or the State Head Injury Unit, Sir Charles Gardiner Hospital.

WHAT ARE THE POSSIBLE BENEFITS OF TAKING PART?

We cannot guarantee or promise that you will receive any benefits from this research; however, possible benefits include clear diagnosis of persistent post-concussion symptoms, access to advanced neuropsychological testing including follow up care if required, and access to an additional MRI scan if the first scan was abnormal and significant symptoms persist at the three-month follow up.

People who receive a concussion in the future may benefit from the study findings due to better prediction of ongoing symptoms. A method to identify patients at risk of developing post-concussion syndrome could also be used to assess the effectiveness of treatment strategies in future clinical trials.

WHAT ARE THE POSSIBLE RISKS AND DISADVANTAGES?

Because this study does not involve a treatment, the risks and disadvantages of taking part are low and are no more than experienced with blood collection and undergoing an MRI.

WHAT WILL HAPPEN TO INFORMATION ABOUT ME?

Any information obtained for the purpose of this research study that can identify you will be treated as confidential and securely stored. It will be disclosed only with your permission, or as required by law.

Your personal information will be stored in re-identifiable (coded) format with a study allocated ID. The study ID will be listed against your identifying details in a separate electronic excel log (the 'master log') which will be password protected and accessible by research staff only. The collection of data will be conducted by persons who normally have access to patient records for clinical care or directly related secondary purposes; namely the clinicians and research staff employed by RPH. Non-Health Department employees have signed a declaration of confidentiality.

Paper copies of your personal information will be stored within a secure filing cabinet in a locked research office designated for research staff only at RPH. These will only be accessed by approved research staff. Information relevant to the study will be entered into an electronic database called RedCap. RedCap is a central database based at Curtin University that is used for collection and secure storage of research information. The information entered into RedCap will be encrypted and protected on a secure network accessible only by authorised research staff. The entries in the database will only be identifiable with a study ID that can be cross referenced with the master log which will be kept separate.

Your information will only be used for the purpose of this research project and it will only be disclosed with your permission, except as required by law. Blood samples, for participants in Phase II, may be used to study many indicators of damage; any future study that links your personal information to data from your blood samples will be restricted to the area of interest covered by this project.

In line with WA Health Guidelines all research data will be retained for seven years. After that time, all hardcopy data will be shredded into confidential waste. All electronic data will be transferred onto CD and subsequently destroyed.

Information about you may be obtained from your health records held at this and other health services for the purpose of this research. By signing the consent form you agree to the study team accessing health records if they are relevant to your participation in this research study.

Your health records and any information obtained during the research project are subject to inspection (for the purpose of verifying the procedures and the data) by the approved research staff. By signing the Consent Form, you authorise release of, or access to, this confidential information to the relevant study personnel as noted above.

It is anticipated that the results of this study will be published and/or presented in a variety of forums. In any publication and/or presentation, information will be provided in such a way that you cannot be identified, except with your permission.

Information about your participation in this study may be recorded in your health records.

In accordance with the Australia Privacy Act (1998) and other relevant laws, you have the right to request access to your information collected and stored by the research team. You also have the right to request that any information with which you disagree be corrected. Please contact the study team member named at the end of this document if you would like to access your information.

COMPLAINTS AND COMPENSATION

If you suffer any injuries or complications as a result of your participation in this study please contact the study team as soon as possible and you will be assisted with arranging appropriate medical treatment.

Participation in this project does not alter any right to compensation that you may have under statute or common law.

STUDY COSTS

There are no costs associated with participating in this study. All tests required as part of the study will be provided to you free of charge. You will be reimbursed for reasonable travel and parking expenses associated with the study visits (e.g., to attend the follow-up visits at the research centre) upon presentation of an expense receipt.

No member of the research team will receive personal financial benefit from your involvement in this study (other than their ordinary wages).

DO I HAVE TO PARTICIPATE IN THIS STUDY?

Participation in any research study is voluntary. If you do not want to participate, you do not have to. If you decide to participate and later change your mind, you are free to withdraw from the study at any stage. *You do not have to participate in this study to receive treatment.*

Your decision whether to take part or not to take part, or to take part and then withdraw, will not affect your routine treatment, your relationship with those treating you or any current or future relationship with the hospital or research staff. If you do withdraw from the study personal information already collected will be retained to ensure that the results of the study can be measured properly and the study data is as complete as possible. If you do not want your data retained, please inform the study team at the time of your withdrawal.

If you are interested in the outcome of the study the researchers can provide you with a copy of the research findings once these have been finalised. Please contact Assoc. Professor Melinda Fitzgerald on (08) 6457 0514 to ask for a copy of the study findings.

FURTHER INFORMATION AND WHO TO CONTACT

If you want further information about this project or if you have any medical problems which may be related to your participation (for example, any side effects), please contact: **Prof Melinda Fitzgerald on (08) 6457 0514, Monday – Friday, 09:00 to 17:00 or at** lindy.fitzgerald@curtin.edu.au

This project has been granted ethical approval by the Royal Perth Hospital (RPH) Human Research Ethics Committee (RGS0000003024) and Curtin University (HREC2019-0209). If you have any concerns about the conduct of the project or your rights as a research participant, please contact the East Metropolitan Health Service (EMHS) Research Ethics and Governance Unit on (08) 9224 2292 or EMHS.REG@health.wa.gov.au and quote the ethics approval number RGS0000003024.

ADDITIONAL INFORMATION FOR PHASE II PARTICIPANTS

WHAT ARE THE POSSIBLE RISKS ASSOCIATED WITH PHASE II TESTS?

Blood collection

Having a blood sample taken may cause some discomfort, bruising, minor infection or bleeding. If this happens, it can be easily treated.

Physical Tests

A number of the physical tests conducted, including the Buffalo Concussion Bike Test and Vestibular Oculo-Motor Screening (VOMS), are designed to assess whether specific physical activities may provoke your concussion symptoms. Thus, there is a small risk that your symptoms may be exacerbated during these tests. This will be minimised by monitoring your symptoms before and during the tests, and ceasing the test if symptoms increase by three points on a symptom scale. Prior to participation you will also be screened for known pre-existing medical conditions that may compromise your ability to safely complete an exercise test.

MRI Scans

MRI stands for *Magnetic Resonance Imaging*. A MRI scanner is a machine that uses electromagnetic radiation (radio waves) in a strong magnetic field to take clear pictures of the inside of the body. Electromagnetic radiation is not the same as ionising radiation used, for example, in X-rays. The pictures taken by the machine are called MRI scans.

Before the MRI you will be asked to fill out a questionnaire to ensure you tell us if you have any metal implanted in your body, such as a pacemaker or metal pins, which would exclude you from having an MRI.

We will ask you to lie on a table inside the MRI scanner. The scanner will record information about your brain. It is very important that you keep very still during the scanning. When you lie on the table, we will make sure you are in a comfortable position so that you can keep still. The scanner is very noisy and we can give you some earphones to reduce the noise. Some people may experience symptoms of claustrophobia from lying in a confined space. If you do

experience discomfort at any time during the scan, you will be able to alert staff by pressing on a call button provided to you.

There are no proven long-term risks related to MRI scans as used in this research project. MRI is considered to be safe when performed at a centre with appropriate procedures. However, the magnetic attraction for some metal objects can pose a safety risk, so it is important that metal objects are not taken into the scanner room.

We will thoroughly examine you to make sure there is no reason for you not to have the scan. You must tell us if you have metal implanted in your body, such as a pacemaker or metal pins.

INFORMATION OF RELEVANCE TO YOUR HEALTH AND CLINICAL CARE

- Your initial MRI results may indicate that your injury was more severe than first thought. Your treating doctor will have access to this information and will take it into account when deciding upon your best medical care.

- The MRI scans we are taking are for research purposes. They are not intended to be used like scans taken for a full clinical examination. The scans will not be used to help diagnose, treat or manage a particular condition. A specialist will look at your MRI scans for features relevant to the research project. On rare occasions, the specialist may find an unusual feature that could have implications to your health. If this happens, we will contact you to talk about the findings. We cannot guarantee that we will find any/all unusual features.

- If you are experiencing significant symptoms or emotional distress due to your concussion, or become upset as a result of your participation in the study more generally, the research team can assist you to access appropriate clinical care. The kind of care will depend on your individual circumstances, past medical history, and whether you are already linked in with a psychologist, psychiatrist or other medical specialist. This decision will be made in consultation with you and the research team doctor and/or clinical psychologist. In a psychiatric emergency we can assist you to the nearest Emergency Department, or recommend that you contact the Mental Health Emergency Response Line (MHERL) on 1300 555 788 or visit https://www.mhc.wa.gov.au/getting-help/helplines/mental-health-response-line/

WHAT WILL HAPPEN TO MY BLOOD SAMPLES?

Your blood samples will be analysed at the Sarich Neuroscience Research Institute for the presence of 'markers' that may indicate the severity of your concussion and help researchers identify useful ways to predict whether symptoms will persist.

Blood samples will be stored at the Sarich Neuroscience Research Institute for a maximum of 15 years. The samples will be stored in re-identifiable (coded) format against a study allocated ID. The study ID will be listed against your identifying details in a separate electronic excel log (the 'master log') which will be password protected and accessible by research staff only. This means your sample can only be matched to your name by research staff who have access to the master log.

One of the reasons for storing samples in re-identifiable format is that it allows you to withdraw your samples at any time. You can do this by emailing concussionstudy@curtin.edu.au. Samples will be disposed of by incineration in appropriate biohazard containment bags, in accordance with the biohazardous project risk assessment, approved by the UWA Biological Safety Office.

Blood samples may be used to study many indicators of damage; any future study/ies will be restricted to the area of interest covered by this project - concussion and recovery from concussion - as approved by a Human Research Ethics Committee (HREC).

CREST Concussion REcovery STudy

Consent Form

A validation study to predict poor outcomes following mild traumatic brain injury

Principal Investigators:
Prof Melinda Fitzgerald (Curtin University, Perron Institute)
Prof Daniel Fatovich (Royal Perth Hospital)
A/Prof Mike Bynevelt (Sir Charles Gairdner Hospital)
Prof Suzanne Robinson (Curtin University)
A/Prof Carmela Pestell (UWA)
Dr Melissa Licari (UWA/Telethon Kids Institute)
A/Prof Daniel Xu (Curtin University/WA Primary Health Alliance)
A/Prof Glenn Arendts (Fiona Stanley Hospital)
Prof Tony Celenza (Sir Charles Gairdner Hospital)
Dr Benjamin Smedley (Rockingham General Hospital)
Dr Ashes Mukherjee (Armadale Health Campus)
Dr Russell Young (Albany Health Campus)
Dr Sjinene Van Schalkwyk (Joondalup Health Campus)
Dr Philip Brooks (St John of God Midland Hospital)
Dr John Iliff (St John of God Murdoch Hospital)
Dr Stephen Honeybul (Sir Charles Gairdner Hospital)
Dr Peter Arthur (UWA)

Declaration by Participant

I have read the Participant Information Sheet or someone has read it to me in a language that I understand. I understand the purposes, procedures and risks of the study.

I give permission for my doctors, other health professionals, hospitals or laboratories outside this hospital to release information to the Principal Investigators concerning my injury and treatment for the purposes of this study. I understand that such information will remain confidential.

I consent to the storage and use of blood samples taken from me for use, as described in the relevant section of the Participant Information Sheet, for: (a) This specific research project and (b) Other research projects closely related to this study.

I have had an opportunity to ask questions and I am satisfied with the answers I have received.

I freely agree to participate in this study as described and understand that I am free to withdraw at any time during the study without affecting my future health care.

I understand that I will be given a signed copy of this document to keep.

Name of Participant (please print)_____

Signature_____Date_____

Declaration by Study Doctor/Researcher

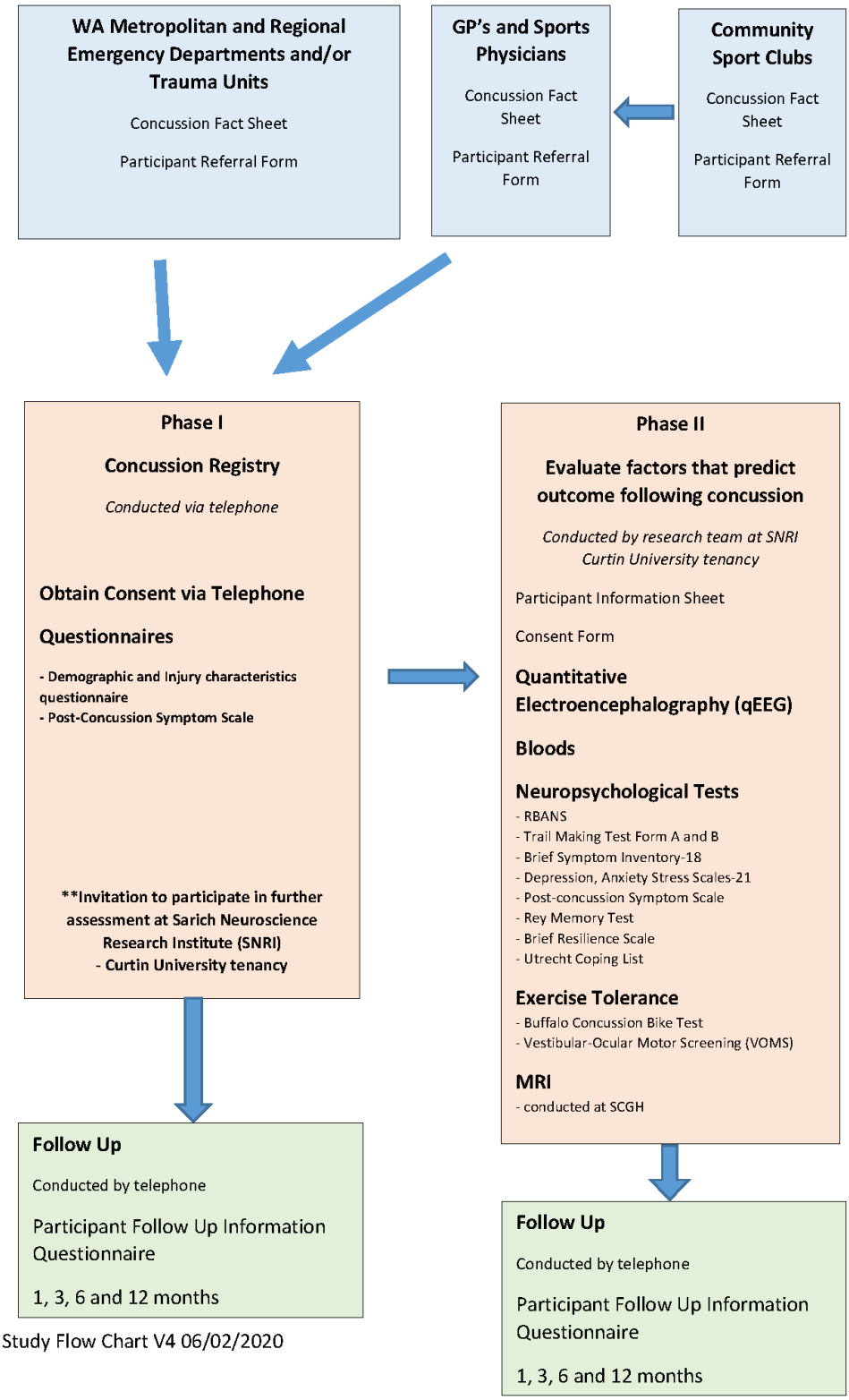
I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Name of Study Doctor/Researcher (please print)_____

Signature_____Date_____

Note: All parties signing the consent section must date their own signature.

CREST Concussion REcovery STudy



Study Flow Chart V4 06/02/2020

CREST Concussion REcovery STudy

Concussion Fact Sheet

What is a concussion?

Concussion is a short-term disturbance of brain function due to either a direct blow to the head or from force transmitted to the head from an impact to another part of the body. A person does not have to lose consciousness ("be knocked out") to have a concussion.

The most common causes of concussion are falls, car accidents, sports injuries and assaults. All people with a suspected concussion should be assessed by a medical doctor to make the diagnosis of concussion.

Symptoms

Concussion can result in a range of symptoms including:

- Headache
- Dizziness
- Nausea or vomiting
- Fogginess
- Sensitivity to light or sound
- Difficulty concentrating
- Difficulty with memory
- Irritability
- Drowsiness or sleep disturbance.

Most people will recover from a concussion within 10-14 days, however approximately 10-20% of people will take a longer time to recover.

Early Management – the first 24-48 hours

Rest	Rest and avoid strenuous activity for the first 24-48 hours after a concussion injury. This may include time off work, school or sport.
Sleeping	It is fine for you to sleep tonight, but you should have a responsible adult check on you every 2-4 hours to make sure you are alright. You should not be left alone initially (for at least 24 hours).
Drinking/Drugs	Avoid drinking alcohol or using recreational drugs for the first 48 hours, as they may change your thinking and worsen your symptoms. It may also make it difficult for other people to tell if your concussion is affecting you or not.
Pain Relief	Do not take prescription drugs, particularly aspirin, strong pain relieving medications, such as codeine, or anti-inflammatory medications as they may mask worsening symptoms that indicates a deterioration of your condition. Only take medication as recommended by your doctor.
Driving	Do not drive a motor vehicle until your symptoms have resolved, or you have been cleared by a medical professional to drive.

Returning to Sport, Work or Study

Physical Activity	After the initial 48 hours of rest, you may gradually increase your physical activity levels ***as long as it does not make your symptoms worse.*** Light exercise such as a quiet walk can be beneficial for your recovery. Start with 5-10 minutes at a time, and slowly build up the time and intensity of activity as tolerated over the next two weeks.

CREST Concussion REcovery STudy

Mental Activity	You may need to "rest your brain" by reducing the length of time spent on activities that involve concentrating such as reading, television and computer or screen use if these activities make your symptoms worse. This will assist your brain's recovery.
Work / Study	Depending upon your work, you may need to take up to one week away from work or study. You can spend short periods of time on activities requiring concentration (10-15 mins each hour) ***as long as it does not make your symptoms worse.***
Return to Sport	If you are returning to playing sport, you ***must*** make an appointment with your GP to be assessed before returning to play. Your sporting organisation may have guidelines regarding returning to sport after a concussion injury. It is important that you have recovered fully from your concussion before returning to sport, as sustaining a second concussion before you are fully recovered may make your symptoms worse.

Medical Emergencies

If you experience any of these signs or symptoms after your concussion, you **MUST** return to hospital or to your local doctor for further evaluation.

- Severe or worsening headache
- Repeated vomiting
- Deteriorating conscious state (drowsiness or difficulty waking up)
- Seizures (convulsions or "fits")
- Increasing confusion, agitation or irritability
- Slurred speech
- Blurred or double vision
- Neck pain and/or weakness or tingling in arms or legs
- Changes in personality or behaviour
- If you have any other concerns or unusual symptoms

In a medical emergency, call an ambulance (000)

INFORMATION ABOUT A RESEARCH PROJECT

CURTIN HEALTH INNOVATION RESEARCH INSTITUTE | Curtin University | perron institute for neurological and translational science

If you have been diagnosed with concussion, you are invited to participate in a Concussion research project. As part of this process, if you have been seen in hospital or have authorised a GP to pass on your details to the research team, you will receive a telephone call within 1-2 days, from a Curtin University researcher who will ask questions about the concussion and invite you to voluntarily participate in an optional observational research study, or participate in further research testing to predict longer lasting symptoms following concussions.

After the initial assessment, we will contact you again after 1, 3, 6 and 12 months to monitor your symptoms and recovery from concussion.

For more information on the study and to discuss participation, you may contact the Research Team on concussionstudy@curtin.edu.au or Mobile 0466 526 849.

Have you recently experienced a concussion injury?

A Research Team from Curtin University & the Perron Institute are investigating which factors may help us to identify people at risk of delayed recovery following concussion.

We are seeking participants

- **Aged 18-65 years**
- **Who have sustained a concussion injury from any cause**
  (eg. falls, transport accidents, sport-related concussions, assault)
- **Have been diagnosed by a medical doctor**
- **Within 7 days of their concussion injury**

The project involves two parts:

Phase I:
Telephone Interview regarding your concussion

Phase II
(eligible subset):
further testing including MRI, qEEG, blood test, exercise bike test & neuropsychological tests

For more information on the study and to discuss participation, please contact the Research Team on

0466 526 849
concussionstudy@curtin.edu.au
www.concussionstudy.com.au

CREST Concussion Recovery Study

CURTIN HEALTH INNOVATION RESEARCH INSTITUTE | Curtin University

This study has Ethics Approval through
Royal Perth Hospital Human Research Ethics Committee (#RGS0000003024),
Curtin University (HRE2019-0209), Ramsay Health Care (#2009) and St John of God Health Care (#1628).

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Appendix D: Other

Appendix D includes other materials relating to this PhD project but are not directly related to the research output. Specifically, the following are presented:

1. **Book chapter**

   Than, M.P., Fatovich, D., Fitzgerald, M., **Gozt. A.,** McKinlay, A., & Snell, D. (2020). The need for traumatic brain injury markers. In Wu, A.H.B., & Peacock, W.F (Eds.), *Biomarkers for Traumatic Brain Injury* (1st ed.)(pp. 9-21) Cambridge, MA: USA: *Academic Press*

2. **Newspaper Article**

   Diving headlong into study. Feature article in Community Newspaper (Perth, Western Australia). January 16th 2020

CHAPTER

2

The need for traumatic brain injury markers

Martin Paul Than[1], *Daniel Fatovich*[2], *Melinda Fitzgerald*[3], *Aleksandra Gozt*[3], *Audrey McKinlay*[4] *and Deborah Snell*[5]

[1]Emergency Department, Christchurch Hospital, Christchurch, New Zealand [2]Royal Perth Hospital, Perth, WA, Australia [3]Curtin University, Bentley, WA, Australia [4]University of Canterbury, Christchurch, New Zealand [5]University of Otago, Christchurch, New Zealand

2.1 Introduction

Traumatic brain injury (TBI), and the subgroup of patients with mild traumatic brain injury (mTBI) represent an important public health system and societal burden. It is estimated that 42 million people worldwide experience an mTBI annually [1]. Males are 1.6 times more likely than females to present to the emergency department (ED) with a TBI [2].

In the United States, which has a population of ~327 million people, approximately 2.8 million people per year are affected by TBI, with 2.5 million of these injuries related to ED visits [3]. Similar burden is experienced in other developed countries; for example, in New Zealand (population ~5 million), a recent population-based incidence study reported a total incidence of TBI of 790 cases per 100,000 person-years [4]. A New Zealand government report states that there are approximately 14,000 people who are treated for TBI each year [5]. In Australia, which has a population of ~25 million, there were over 14,000 hospitalizations for TBI in 2004/2005 [6]. However, mTBI (or concussion) accounts for 70%–85% of TBI [7] and most of these patients do not attend hospital. Indeed, only 10%–25% of people seek medical attention for TBI, so these injuries are substantially underreported. The incidence rates in New Zealand of 790 cases per 100,000 person-years (which is greater than the incidence of cancer) [8] equates to 190,000 to 200,000 cases per year in Australia.

As TBI disproportionally affects young people, chronic disability following TBI is particularly costly both in terms of productive years lost and burden on the healthcare system. A recent Centers for Disease Control and Prevention (CDC) report estimated the

Biomarkers for Traumatic Brain Injury
DOI: https://doi.org/10.1016/B978-0-12-816346-7.00002-6

economic cost of TBI in the United States (including direct and indirect medical costs) to be approximately US$76.5 billion. Severe TBI accounted for approximately 90% of the total TBI medical costs. New Zealand government statistics estimate the annual cost of mTBI to be NZ$83.5 million. The lifetime cost of each TBI in Australia is estimated at AU$2.5 million for moderate and AU$4.8 million for severe injuries. New cases of moderate and severe TBI add more than AU$2 billion in lifetime costs to the Australian healthcare system annually, and the total annual cost of TBI in Australia is AU$8.6 billion [9]. The costs from mTBI are minimally documented and not included in these estimates.

2.2 Context

This chapter will focus upon the need for better tests in the assessment of TBI in three areas:

- The acute assessment of patients in the ED.
- The identification of who will have ongoing morbidity following their TBI.
- The prediction of the extent of such morbidity.

2.3 Probabilities, decision-making, and test thresholds

It is now almost 40 years since Stephen Pauker and Jerome Kassirer (a future editor-in-chief of the New England Journal of Medicine) wrote their article entitled "the threshold approach to clinical decision-making" [10]. The logic explained then remains relevant today. For a specific diagnosis of interest, clinicians are constantly determining and reevaluating the likely probability of that diagnosis. This process starts with the first information the clinician has available from the history (and also even from precontact information such as primary care, ambulance, or nursing records), and is then refined as new information, such as examination findings, laboratory results, and imaging, becomes available. Pauker and Kassirer described that at some point during medical assessment, the disease probability will either (1) pass a lower threshold at which more testing does more harm than good because the disease is very unlikely, or (2) pass above the threshold at which the probability of the disease is already high enough to begin treatment with confidence (Fig. 2.1). These thresholds will vary according to the clinical scenario. The test/treatment threshold concept is applicable to the assessment of patients with TBI.

2.4 Acute assessment of patients with traumatic brain injury in the emergency department

Patients presenting to the ED with TBI can do so via ambulance, primary care physician, or through self-presentation. Ambulance presentations are usually more serious. There is therefore a range of severity from very minor injury through to the comatose patient. In an isolated TBI, the primary ED decision-making focuses upon the identification of those

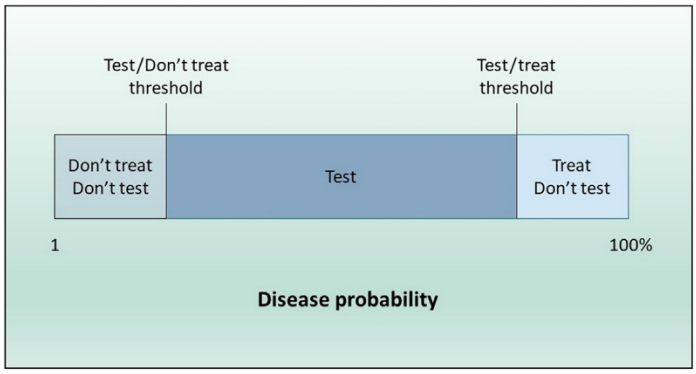

FIGURE 2.1 **Test/treat thresholds.** Medical assessment determines the probability of the disease of interest with each new piece of information making small or large adjustments to this probability. Below the test/no treatment threshold the optimal strategy is for no further testing because the harms outweigh the benefits. Above the test/treatment threshold the probability that the disease is present is enough to begin evidence-based treatment for that disease despite any potential side effects that might occur.

patients with significant intracranial injury, for example, epidural or subdural hematoma. The focus is identifying those patients who require neurosurgical intervention.

In the context of decision thresholds, a very high (near certain) degree of probability of major intracranial injury is required before the patient has neurosurgery. Fortunately, CT scanning is very specific for injury identification (very accurate for rule-in).

The decision of when to request CT scanning is heavily influenced by the fact that missed intracranial injury can be catastrophic. As a result, clinicians are motivated to request a CT scan in order to avoid the risk of missing intracranial injury. This has led to a progressive increase in usage of CT scanning in this context. The use of CT in the ED increased sixfold from 1995 to 2007 and the use of CT has increased at a higher rate in the ED than in other settings [11]. As many as 63% of patients with mTBI in the ED undergo CT scanning [12]. While up to 15% of ED patients with mTBI have an acute finding on CT, <1% require neurosurgical intervention [13,14]. This has also led to the development of clinical decision aids to identify which patients require imaging with the aim of rationalizing usage.

CT scan usage in the ED for TBI varies according to nation, health system, age, and setting [15–17]. Where CT scans are easily available with no resource or funding impediment then there is little downside (other than radiation dosage in children) to scanning large numbers of patients. In systems or settings where access to CT is problematic, then the decision to test must be weighed against other priorities.

In taxation-based health systems (such as New Zealand's National Healthcare System), requests for CT scans for TBI need to be balanced against CT scanning resource availability (particularly out of hours) and large numbers of requests for other clinical problems both from the ED and other hospital areas. The rational use of CT scanning for TBI in the ED has been strongly advocated by the Choosing Wisely movement. Choosing Wisely is a multinational initiative of the American Board of Internal Medicine (ABIM) Foundation which is strongly supported at a government level. It seeks to advance dialogue on

avoiding unnecessary duplication of tests and procedures, and to empower patients to choose care that is supported by evidence, in the context of shared decision-making.

Since the use of CT scanning is still rising despite low numbers of a CT-identified intracranial injury in mTBI, it would be very useful if there was an inexpensive and accurate biomarker that could rule out significant intracranial injury (and therefore the need for CT scanning) with high accuracy. This need is already driving research, and this is described in other chapters.

2.5 Identification of those patients who will have ongoing problems following traumatic brain injury and the prediction of the extent of such problems

The assessment of patients with TBI in the ED has historically focused on the identification (and then onward treatment, especially neurosurgery) of serious intracranial injuries such as cerebral hemorrhage. Once such serious injury is ruled out, then if the patient is reasonably well, ambulant, coherent, and able to look after themselves, they are suitable for discharge with variable degrees of patient information guidance regarding possible ongoing injury symptoms [13].

It is now clear that mTBI is common and has significant impact on patients and society. As a result, research interest has reached fever pitch as more and more implications are discovered in multiple contexts such as the armed forces, contact sport, and workforce employment. The understanding of the pathology and science relating to ongoing symptoms from mTBI is now improving but remains substantially incomplete. This is compounded by the fact that management solutions for ongoing symptoms are poorly developed.

Most people recover fully after a mTBI, but 20% or more can have a delayed recovery due to persisting symptoms, more recently referred to as persisting post-concussion symptoms (PCS). Persisting PCS are known to occur following even the mildest forms of TBI, and there is currently no way of knowing which individuals will go on to develop persistent difficulties.

A range of physiological, structural, and neuropsychological outcomes have been assessed for their capacity to predict outcome following mTBI, most being limited to investigating a single type of measure. The results reported have been mixed [18–21]. Given that PCS is complex and multifactorial, affecting several important aspects of functioning, it is unlikely that any single measure will be able to predict the likelihood of an individual experiencing ongoing symptoms. Current predictors of outcomes following mTBI use algorithms that may be useful for groups of patients but are not helpful at the level of the individual [22–24]. It is thus becoming apparent that a suite of markers is likely to be required to effectively predict poor recovery at the level of the individual. Against this background there is a need for additional tests that can provide guidance in relation to the specific issues below.

1. *Prediction of the severity of injury and the likelihood of ongoing symptoms and disability*

   Some may argue that such a test is of little value if there is not a clear intervention that can then be applied to improve outcomes, but this is not completely true. Clarification of a diagnosis conveys information about injury severity and expected symptoms and can be beneficial for treatment providers, funders, and the patient. From a patient's

perspective, this can be reassuring. Early troubling and unexpected symptoms after mTBI have been associated with increased anxiety and risk for slow recovery [25–27]. Earlier prediction of injury severity and likely associated symptoms can guide return to work planning and expectations. If it were possible to give patients this information, it might be reasonable to provide it. In fact, accurate forewarning of specific problems might prompt an individual to change their career or life path or simply just rearrange their life (if possible), for example, from working full time to part time.

Such information could be valuable to the patient's employer and insurer, although the access to and use of this information would have to be handled with great care given the complexity of clinical issues especially over time, and with stringent safeguards. However, negative consequences of atypical outcomes, especially after mTBI, include unfair labels such as feigned symptoms or malingering; tests capable of determining ongoing injury-related impairment would be useful in this context. Further to this, it is important to recognize that most people recover in a timely fashion following mTBI and undue anxiety about continuing symptoms can contribute to poor outcomes. If a sufficiently accurate and specific predictive test could be developed it may allow unnecessary fears to be allayed.

2. *Identification of those patients who may benefit from specific interventions*

   As previously mentioned, this is a rapidly developing field in which there is much to learn. mTBI covers a broad range of symptoms and likely differing underlying pathophysiology. As such, it is likely that for interventions to be beneficial they will need to be targeted to specific subgroups of symptoms [28]. Tests that can categorize patients into these subgroups can be utilized to focus specific interventions and related resources on the patients for whom they would be most beneficial.

3. *Identification of those patients who would benefit from delayed, structured, or tapered return to usual activities*

   Historically, advice regarding return to work or sport has been variable. Many sports now have organized guidance processes for returning to partial or full participation, including the Zurich graduated return to play program [29,30]. The optimal guidance on returning to work is less clear and will obviously depend on the nature of the work and the extent of symptoms. It would be very useful to have an objective test or tests which provide measurable guidance measures for return to activity.

4. *Identification of patients at risk for development of subsequent mental health issues*

   There is increasing evidence that there is an association between mTBI and development of subsequent mental health problems, including depression and suicide. In fact, there is a growing literature highlighting the impacts of a range of potentially modifiable psychological factors on injury outcomes [31]. It would be important to identify such patients early and potentially initiate early referrals for psychological support and/or specialist mental health monitoring.

5. *Identification of patients who might progress to neurological diseases in association with mTBI*

   The best well-known case of a person with persistent mTBI and who then developed further neurological pathology is Muhammad Ali's progressive development of Parkinson's disease. Multiple mTBI, which can occur during sport, have generally been shown to lead to worse outcomes, increasing the risk of profound long-term consequences, including encephalopathy, mental health issues, and Alzheimer's disease [32,33].

2.6 Issues for children

Early childhood is one of the most common periods for mTBI to occur [3]. Predicting outcomes following mTBI in children is important for informing and reassuring their families and for directing rehabilitation services and return to school and play. Currently, evaluations of mTBI are based on imaging, and parent and child report. This is problematic as many children are injured while they are still preverbal. Biomarkers would also assist in determining whether there was a need for imaging procedures and also possibly in differentiating between children with intentional versus nonintentional injury.

Existing research reports correlations between biomarkers and injury severity measures, global outcomes, and neuroimaging abnormalities. However, methodologies of existing studies vary, making it difficult to combine different studies [34].

For many of the biomarkers currently being investigated in adults, there is little or no data for pediatric populations. This may be due to the difficulties in evaluating biomarkers in a pediatric population; biomarkers are expressed at different levels across the life span [35] and show different patterns in children. For example, S-100B and myelin basic protein have elevated normative concentrations in children [36]. Moreover, neuron-specific enolase (NSE) shows different patterns for children and S-100B is not sensitive for use with children [37].

Goldman and colleagues [35] pointed out that difficulties evaluating biomarkers for use in a pediatric population typically result in biomarkers that are developed in adult populations and then adapted for use in pediatric populations instead of child-specific markers [33]. Goldman and colleagues suggest the following as optimal characteristics of an ideal biomarker for children:

- Noninvasive
- Pediatric-specific
- Results correspond with age-dependent physiologic changes
- Cost-effective
- Well-established pediatric-specific norms

2.7 Issues for women

Some research has suggested that being female has been identified as a risk factor for both the occurrence of a concussion and prolonged recovery from the injury [38]. This increased risk may in part be due to actions of hormones on the brain's inflammatory response. Research has shown that menstrual cycle phase and progesterone concentration at the time of mTBI may affect symptoms [39] and length of time symptoms are present [40].

While hormonal changes may partly explain the higher report of concussive symptoms for females compared to males, more recently the architecture of the female brain has also been implicated. Female axons have been found to be thinner with fewer microtubules than male brains which creates less stability in the brain and more damage to axons when injury occurs. It has been suggested that the extended recovery time for females following concussion may be reflective of longer time taken to repair the axons [41]. It is likely that

both hormonal changes and brain architecture play a role in outcomes following concussion for females [42,43]. The identification of accurate sex-specific biomarkers would provide individualized information about the impact of injury and its impact on recovery, thereby reducing patient stress and uncertainty. Biomarkers would also enable efficient allocation of rehabilitation services to those with prolonged recovery. Currently there is growing information regarding the impact of sex on biomarkers [44].

2.8 Issues for future research to consider

A range of issues hamper progress toward identification of a biomarker or combination of tests capable of detecting brain injury and identifying those with risk for development of persisting symptoms and disability after TBI. These include representativeness of cases captured by research, complexity of mTBI prognosis, and knowledge uptake considerations.

Firstly, many prognostic studies recruit from first healthcare presentation following a TBI, usually at the ED. However, the ED is not the first healthcare contact for many patients, especially for those sustaining mTBI [45]. Diverse recruitment methods and eligibility criteria across the research contribute to samples with diverse injury severity, likely favoring more severe injuries. Many studies and reviews of studies have shown that injury severity and mechanism are not consistently associated with mTBI outcomes. Thus the synthesis of findings, and confidence in resulting prognostic models and their applicability to a diverse mTBI population, remain areas of difficulty.

Secondly, evaluating risk for slow recovery after mTBI is complicated by interacting pre-, peri-, and postinjury factors resulting in individual variability; a biopsychosocial model is probably required [46]. Additional prognostic factors such as preinjury neuropsychological functioning, prior history of mental health symptoms, and previous TBI have all been associated with mTBI outcomes and need to be incorporated into prognostic algorithms.

Finally, many patients do not present to an ED promptly enough (or at all) for some tests to be effective, for example, early enough to have detectable levels of serum biomarkers. This risks problematic missing data and difficulties encouraging clinician uptake. Given the complexity of TBI recovery especially over time, prognostic algorithms or signatures may be better targets than an elusive test (e.g., combinations of clinical data, imaging data, serum biomarkers, and other physiological and/or neurocognitive assessment results) [47].

2.8.1 Summary—the importance of being able to predict post-concussion symptoms

Having the ability to determine which individuals may be at risk of developing PCS has implications for both clinical and research practice. PCS has been associated with disability and high utilization of healthcare services [48–52]. As such, it is useful for clinicians to be able to identify those patients that may be at risk of developing the disorder in

order to optimize patient outcomes, that is, reduce number of missed days at work/school, and to reduce the overall burden on the healthcare system that is attributed to PCS [53]. Furthermore, the ability to predict which patients will have prolonged symptoms would help clinicians and patients by allowing proper anticipatory guidance, determining the need for academic or occupational accommodations, allowing athletic team members and coaches to plan for the prolonged absence of a player, and allowing patients and their coworkers to prepare for prolonged absences from work [54].

The formulation of clinician-friendly tools that can be easily translated into patient care, can empower healthcare providers to identify those patients that are in need of ongoing monitoring and/or whom could benefit from being prescribed specific pharmaceutical agents for recovery or triage to various other forms of early therapeutic intervention [55]. Furthermore, such tools can also be used to bridge the gap between clinical and research settings. For example, clinicians could use them to direct patients towards clinical trials of new therapies that may prevent or ameliorate the effects of PCS. This is particularly important given that research has found TBI to be linked to several chronic neurodegenerative diseases such as Alzheimer's disease and chronic traumatic encephalopathy [56–60]. Thus being able to predict who is at risk of developing PCS can also help researchers identify potential cohorts of people that can be involved in future studies of therapies that aim to prevent or ameliorate the cognitive impairments that characterize these associated diseases. Moreover, being able to identify which individuals are at risk of developing PCS can help increase the statistical power of randomized controlled trials though risk stratification and covariate adjustment [61,62].

2.8.2 Promising areas of predictive biomarker research

The ApoE genotype is the major apolipoprotein within the CNS synthesized by astrocytes and microglia [63]. It is involved in many intra- and extracellular processes in the CNS and has been proposed to affect cellular maintenance and repair [64]. The gene has three common alleles (e2, e3, and e4), coding for three protein isoforms (designated E2, E3, and E4). The APOE-e4 genotype is known to influence cognitive performance and decline. This is possibly because the least active E4-isoform confers a survival disadvantage to injured neurons [65,66]. A meta-analysis by Zhou et al. [67] reported an association between the presence of the APOE-e4 allele and the risk of poor outcome at 6 months after injury. A study by Müller and colleagues [64] found APOE-e4 to be the sole unique predictor of reduced improvement in neuropsychological performance at 6 months postinjury ($b = 0.08$ (95% CI = 0.03–0.14) $P = .006$) [64,67]. These findings suggested that APOE-e4-negative patients improved twice as much as the APOE-e4-positive patients. It is therefore possible that genetic factors such as ApoE genotype may play a moderating role in recovery following TBI.

Whilst there is a strong theoretical and empirical basis that supports the notion of using neuropsychological measures to evaluate the influence of mTBI on cognition, little is known about the ability of neuropsychological test outcomes alone to *predict* nonrecovery. A recent meta-analysis by Allanson et al. [18] found immediate verbal memory ($r = 0.43$; $P < .001$), delayed verbal memory ($r = 0.43$; $P < .001$), visuospatial construction

($r = 0.29$; $P = .001$), set-shifting ($r = -0.31$; $P < .001$), and generativity ($r = 0.44$; $P < .001$) to be correlated with a functional measure of disability experienced following moderate to severe TBI. These findings imply that it may be possible to predict the likelihood of an individual developing chronic symptoms after TBI by considering an individual's performance on neuropsychological tests of these particular cognitive domains when measures are obtained shortly after injury. However, it is increasingly understood that other measures of psychomotor test performance such as those involving visuomotor or oculomotor functions may be superior predictors of PCS and further comparative assessments are needed for validation [68].

Investigation into the extent to which magnetic resonance imaging and, in particular, diffusion tensor imaging scalars can predict PCS is also limited. In Mayer et al. [69] found that a model consisting of only diffusion tensor measures had superior classification accuracy (80% for mTBI patients; 70% for healthy controls), relative to that which featured neuropsychological measures (71.4% for mTBI patients; 60% for healthy controls). Fractional anisotropy measures the degree to which diffusion of water is unidirectional and serves as a proxy measure of white matter integrity. Fractional anisotropy values in the right hemisphere ($F_{2,18} = 6.84$; $P < .01$) predicted variance in attentional deficits [69], while the severity of white matter lesions present at the acute stage of mTBI injury were related to cognitive impairment in the chronic disease stage [70]. Within a PCS-like context, Messé et al. [71] have reported similar findings using a two-step approach. Mean diffusivity quantifies average molecular motion in all directions, and can change with cellular responses to injury such as edema. Average values from six white matter tracts were able to distinguish between patients classified as poor or good outcome with 69% sensitivity and 77% specificity, respectively, if a patient had a 50% chance of developing PCS, which is not clinically useful.

Several blood-based biomarkers indicative of mechanical injury to cells, glutamate excitotoxicity and calcium ionic imbalance, have been evaluated thus far within the context of severe TBI, with those found to be elevated including calcium binding S100 protein β (S100β) [72,73], myelin basic protein (MBP) [74], glial fibrillary acidic protein (GFAP) [75], NSE [76], ubiquitin carboxy-terminal hydrolase 1 (UCHL 1) [77], Tau isoforms [78], and calpain-derived αII-spectrin N-terminal fragment (SNTF) [79]. Whilst several of these biomarkers have also been investigated in respect to mTBI, a consensus is yet to be reached regarding their utility in predicting PCS, particularly given concerns noted earlier about the proportions of mTBI patients who do not present to health services early enough to access such biomarker testing opportunities.

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Diving headlong into study

Kristie Lim

Curtin Health Innovation Research Institute's Aleksandra Gozt and head coach Damien Donaldson. **Picture: Shane Lawrie/BeardySnaps**

PERTH Broncos American Football Club players are tackling the affects of concussion through a university study.

Several players from the Noranda-based club have signed up for Curtin University and the Perron Institute's Concussion Recovery Study.

The study seeks people between 18 and 65 years old who have sustained a concussion and have been diagnosed by a doctor to engage in interviews and testing.

It aims to identify factors which may predict individuals at increased risk of delayed recovery and help deliver better management of treatment.

A state-wide concussion database to document concussions happening in WA will also be established.

Head coach Damien Donaldson said the club joined the study because there had been growing concern in professional American football and other codes around the world about concussion.

"We've had three or four players with suspected concussions who were immediately removed from play," he said.

"They had to get medical clearance before undertaking a return to sport program with our sports trainers.

"There are a large range of symptoms which occur reasonably often but they don't always mean the player has suffered a concussion; that's why it's important they are evaluated by a medical professional."

Donaldson said the club's focus on improving tackling techniques helped with injury prevention, as well as sound warm-up protocols and existing injury management by sport trainers.

Curtin Health Innovation Research Institute researcher Aleksandra Gozt said more information on how concussion injuries happened and where people sought treatment would help researchers influence policy.

"The study came about because although concussion is the most common form of traumatic brain injury in Australia, accounting for approximately 80 per cent of all traumatic brain injuries that occur, little is actually known about its prevalence in WA," she said.

"Furthermore, while most people recover from the symptoms of concussion within two weeks of sustaining their injury, approximately 10-20 per cent of individuals will continue to experience symptoms for longer than two weeks."

Ms Gozt said while there were participants from AFL, equestrian and cycling who had joined, people from any sport or had sustained concussions from accidents, falls or assaults could also join.

"Recovery following concussion can vary amongst different individuals, with some recovering spontaneously while others may experience ongoing symptoms," she said.

"Currently there is no rehabilitation program that works for everyone and many individuals who have suffered a concussion do not have access to a clear recovery pathway.

"For this reason, it is very important for us as researchers to understand what factors may influence a person's recovery."

Recruitment will be finalised by mid-to-late 2020.

Call 0466 526 849, email concussionstudy@curtin.edu.au or visit www.concussionstudy.com.au.

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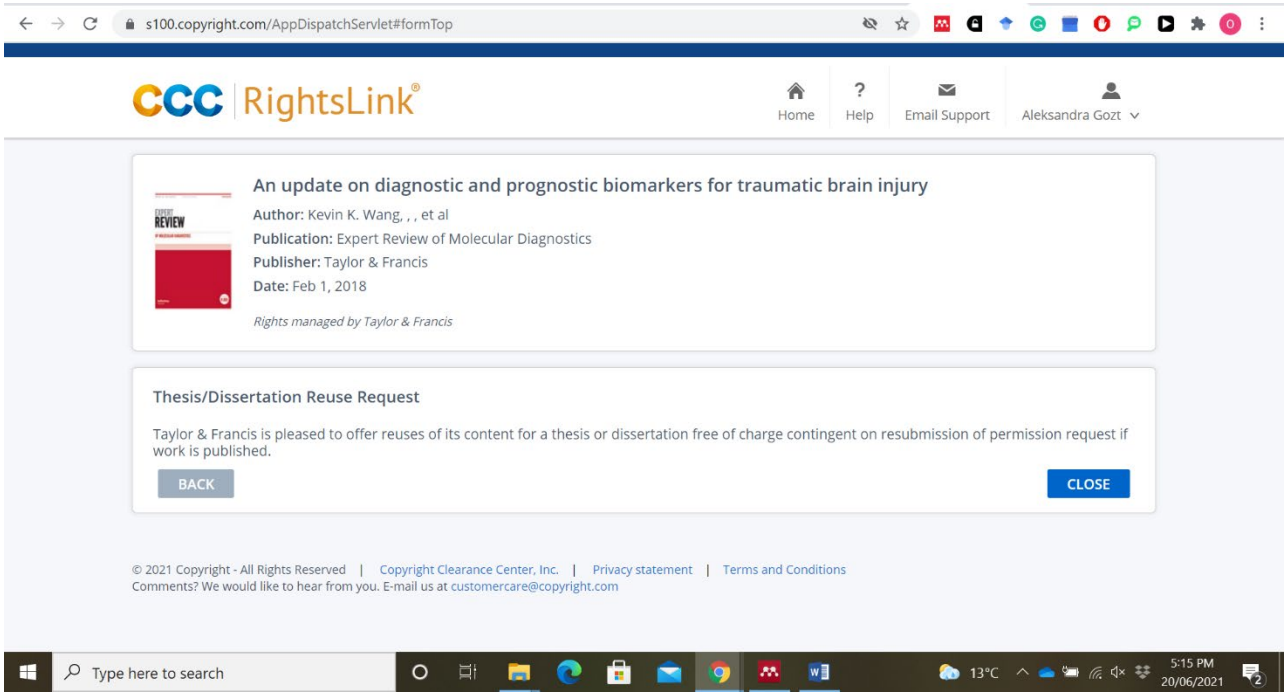
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